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Biotechnology of Protein and Polysaccharide Gels

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Introduction

Gels are important in biotechnology. There are numerous examples where the fact that a gel is present is central to the particular application.

Many of the most important attributes of foods and the raw materials used to make them are believed to depend on gel structures, and there is an enormous literature devoted to investigations of food macromolecules, with the sort of textures and consumer attributes that can be produced with their aid in mind. Encapsulation, which is employed in pharmaceuticals and has some nutritional applications, also uses gels and the way in which release from them occurs is crucial to the application. Diagnostic tests and some immobilised enzymes also use a matrix to which the active macromolecules are attached, but which are governed by the way in which the gel structures interact with other molecular species. Immunodiffusion methods make use of gels and their special properties.

Another obvious example is the use of gel electrophoresis, which pervades molecular biology to such an extent that it is difficult to believe that the field could ever have developed without it. More than 85% of all the papers in the major biochemical journals contain an example of gel electrophoresis, and it is by far the most widely used technique in the subject. Everywhere, from the isolation and purification of macromolecules such as proteins or DNA to techniques for sequencing, applications depend on the special interactions between a gel matrix and macromolecules transported through it. Forensic tests such as DNA fingerprinting use gel electrophoresis and the details of electrophoretic techniques have been under scrutiny in legal cases in a way that was certainly not envisaged by their inventors. Much can be learned about the way in which macromolecules interact with gel matrices by studying gel electrophoresis.

All this is enough to justify the study of gels and how they form, but there has been a more recent development which brings the focus back to biotechnology in a more direct way. By the use of genetic manipulation it is now possible to alter the

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composition, and hence the structure, of biological macromolecules. Protein sequences can be varied almost at will, while polysaccharides can also be made to requirements, within the limits of naturally occurring enzyme systems. The possibilities of protein manipulation have now become even more extensive, since it is possible to incorporate into the chain some amino acids which do not normally occur in peptides or proteins. These amino acids are not only the numerous ones found in living organisms though never in proteins, but may also include entirely synthetic ones (Ibba, 1996). Finally, the use of transgenic organisms has made the large scale production of such materials at reasonable cost a real possibility in the near future. Potatoes are available which produce starch with structures which lead to gels different from the usual ones, while there are now some reports of proteins which have been specifically engineered to modify their functional properties by site-directed mutagenesis.

These possibilities raise questions which must be asked by those engaged in planning to make modified polysaccharides and proteins. They are, broadly speaking, what do you want these molecules to do, and how should we modify them? Anyone who has spent much time in industrial research will know that this is a question that is rarely, if ever, answered. Indeed the proponents of the 'customer-contractor' principle, popular in industry a few years ago, by which research was to be organised, failed to notice that there was a fatal flaw in the idea. The customer either does not know what he wants, or is unable to define it in meaningful terms, that is to say, terms that mean something to those who will be doing the research. The solution is constant discussion – iteration is a term sometimes used to describe it – between those producing the novel macromolecules and those who are going to use them.

A particular problem with gels is that the modifications to macromolecules that can be achieved by genetic engineering manipulations are described in molecular terms – the presence of an extra reactive thiol group perhaps, or a changed degree of branching in amylopectin – while the consumer attributes are poorly understood in such terms. The latter tend to be described by rheological measurements, often very empirical ones, or even by the outcome of taste panels. In the last analysis all new food products are subject to trial by the public, who may or may not buy them more than once, and the use of taste panels is an attempt to forecast this. Taste panels are not very good at this, and are laborious and expensive. Thus, while they are a perfectly rational approach they are unlikely to be much help in guiding the biotechnologist wondering just how much he should alter the degree of cross linking by site-directed mutagenesis in a protein to be used for meringue manufacture.

This review is an attempt to explore the molecular structure of gels and how far our knowledge of them can be used to make a link between the two extremes of food research, though we will also consider other applications. This has been a slow-moving field, and many of the references given are rather old. They represent significant developments over the last 80 years or so, and in many cases are too old to be in the databases. For more recent references, the papers cited are representative, and should give ample opportunity to enter a database search where far more references would be found than could ever be listed here.

Proteins

DEFINITION OF GELS

It is unexpectedly difficult to define a gel in a way which distinguishes it clearly from other possible states. On superficial examination, very viscous solutions can be indistinguishable from gels. In the past attempts to define gels have mostly been based on descriptions of the process which leads to gelation, leaving the observer to infer what the gel must therefore be.

A particularly influential description of protein gels was put forward by Flory (1941, 1942), and the general concept of gel structure that was derived from it was generally accepted for the next 30 years and is still a commonly held view. It is not so much wrong, as limited, since it is possible that some protein gels do have this structure, though we now know that most of the commercially important ones do not. It is worth quoting Flory extensively.

> Dilute aqueous solutions of certain proteins yield thixotropic gels under suitable conditions . . . the behaviour of egg albumin in aqueous solutions containing acetic acid. The increase in viscosity which follows addition of acetic acid is attributed to gradual uncoiling of the protein chains. In the presence of small amounts of salts the increase in viscosity is greatly accelerated, the viscosity proceeding to infinity with the formation of a thixotropic gel.
>
> Interactions between amide groups are known to be very large probably owing to the formation of hydrogen bonds. If one adopts the view that a small number of hydrogen bonds may be between the dissolved protein chains, the gelation of protein solutions can be readily explained in the light of the network theory. The total number of amide-amide hydrogen bonds must eventually reach an equilibrium value dependent on pH, temperature, protein concentration, solvent medium etc. The proportion of bonds which are intramolecular and therefore do not contribute to increasing the state of aggregation of protein chains will be enhanced by the low protein concentration. It is assumed that at equilibrium the protein chains are completely uncoiled except for the randomly distributed intrachain amide-amide interactions.

For the period this is a remarkably forward looking account, and shows how much understanding there already was of the main features of protein gels. It was interpreted by many (for example Ferry, 1948) to mean that a typical protein gel structure was as indicated in *Figure 1*. Bearing in mind that this is a two dimensional representation of a three dimensional structure, it was still being put forward during the 1970s as the definitive description. Another factor which had some bearing on the development of our ideas on gel structures was gelatin. Gelatin is a breakdown product of collagen, and is widely used in a number of industrial applications. The most important were in the manufacture of photographic plates and films, and as a gelling agent in the food industry. It has minor uses in encapsulation, and more recently in the manufacture of low-fat spreads. It was sufficiently important for a special Research Association to be set up in the UK. Gelatin probably does form gels with a structure like that shown in *Figure 1*(Finer *et al.*, 1975) and at one time a literature search under protein gels would have found numerous papers on gelatin, and little else. We now know that gelatin is so atypical in its behaviour that it is sometimes referred to as 'an honorary carbohydrate' since its gelation and solution behaviour is much closer to that of gel-forming polysaccharides than of any other protein. Even so this all helped to reinforce the general acceptance of *Figure 1* as the stereotypical protein gel structure.

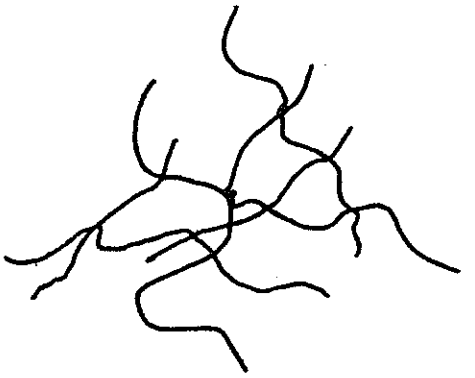

Figure 1. Diagram of gel structures found in gelatin and many polysaccharide gels. The elements forming the mesh are extended chains of the gel forming macromolecule.

It is not too clear now just what was meant by a thixotropic gel since the property of thixotrophy would appear to be impossible to reconcile with current ideas of the gel state. A thixotropic material is one which shows a marked drop in viscosity when it is stirred and which sets again when stirring stops. A familiar example is a thick suspension of starch grains. Stirring an ovalbumin gel will simply result in fragmentation, and while there would be a drop in viscosity it would not re-set when stirring stopped. It is conceivable that a protein gel could be formed which might have such a delicate balance between the intermolecular forces and shear forces that it would show thixotrophy but to the author's knowledge no such gel has ever been made from ovalbumin, though actin can form a gel-like mass which shows thixotrophy. It is considerations of this kind, however, which mean that we cannot say that structures inferred from the Flory account are wrong, though it is now clear that they do not apply to most protein gels.

Gel structures involving close-packed arrays of spherical particles have been suggested for both proteins and polysaccharides and appear nowadays to be described as particle gels rather than thixotropic gels. Certainly suspensions of colloidal particles, also known as colloidal fluids, can show complex pseudo phase behaviour and systems which approximate to gels (Pusey *et al.*, 1986). Dickinson (1990) has also used this description for the predominantly spherical aggregate systems found in yoghurt and cheese, and extended fractal theory to the cluster formation.His assertion that gels are a special sort of colloidal system represents a rather different standpoint from those who assert that gels are giant molecules and shows how difficult any clear-cut definition of gels in molecular terms can be. Both kinds of structures can exist and whether or not they are both given the description of gels remains a matter for discussion.

The reason that Figure 1-type gels cannot apply universally is a fundamental misconception about the structure of the 'uncoiled' or 'unfolded' protein, which is not inherent in Flory's description but is in descriptions such as *Figure 1*. At this point it is necessary to specify the kind of protein involved. Globular proteins, a term which includes virtually all enzymes, as well as nearly all the important industrial proteins such as wheat glutens, oilseed and legume proteins like legumin and vicillin, dairy

materials such as whey proteins and egg albumin, have a particular kind of structure. Caseins are not quite so highly structured but in practice behave in the same way. They are more or less spherical (and in hydrodynamic terms that means that they have an axial ratio between one and five) and have a precisely defined structure in the native state. It is only fair to point out that in 1942 many in the field did not believe this, and terms such as 'colloidal solution' which is an inherent contradiction but carries all the overtones of a reluctance to accept that proteins could be sharply defined in molecular terms, were widely used. Other kinds of proteins are also important – the filamentous proteins of muscle like myosin and actin, and such materials as silk fibroin and wool can be involved in structure formation and gelation, though usually only after heavy manipulation. Gelatin and elastin have already been mentioned. Glycoproteins such as orosomucoid are different again. They all require special consideration, and in what follows it is assumed that the protein is a relatively structured globular one.

THE STRUCTURE OF THE UNFOLDED STATE

The terms unfolded, uncoiled, denatured and disrupted have all been used to describe what happens to proteins when they lose their native structure. The native structure is the one they have *in vivo* and can retain on careful isolation. In the case of enzymes and some other biologically active proteins it is the one in which they show activity. Most of the commercially important proteins have no enzymatic activity. They are largely storage materials, as in the seed proteins, or have a nutritional function as in milk, and as a result attracted rather less interest and even now many of the structures have not been determined.

The general phenomena of protein gelation are not particularly well known. In general, if a solution of a globular protein is heated it first becomes turbid. This means that the particles have become of a size comparable to the wavelength of the light used to observe them, and since this is between 250 nm and 450 nm, and globular proteins typically have a dominant dimension of about 5–8 nm, they must have aggregated. Further heating leads to one of two results – large lumps separate out (the process of coagulation), or the whole volume becomes occupied by an elastic mass which does not fall out of the test tube when it is inverted. This is a gel. It may be opaque or transparent, which is again a function of the size of the particles and holes in the structure. For a food manufacturer the difference is very important, and for example in the manufacture of yoghurt a key part of the formulation is to find the conditions under which a gel-like structure can be obtained with the minimum amount of protein, while avoiding coagulation.

The great majority of globular proteins will form a gel if they are heated at a concentration of 15%–20% between pH 4 and pH 8.They tend to coagulate at concentrations less than this. A test used in industry for proteins to be used in filament spinning (see below) was simply to make a slurry of the protein in water at 20% and autoclave it in a tin can. Commercial proteins such as soy isolates (or ground-nut protein when it was available, before aflatoxin removed it from the market) wheat gluten and caseins all form gels under these conditions, sometimes quite transparent ones. Occasional samples failed to gel, and these were usually unusable in later processes. The precise reason for this remains unknown, but may be related to the loss of the ability to form covalent links. Although gelation occurred on heating, the gel

remained stable on cooling and there was no reversion. The process, which does not even require the protein to be in solution is a notable one.

In marked contrast gelatin disperses on heating, the solution gels on cooling, but is thermolabile and the gel melts when heated. Also concentrations as low as 2% can gel. It is not unreasonable to suppose that the mechanisms in the two cases are different.

If heating is carried out on very dilute solutions, aggregation does not occur and the change in the shape of the molecule can be followed by a variety of methods, of which circular dichroism and other optical methods are the most useful. In general the viscosity and the space occupied by the molecule rises, and the side chain-side chain interactions which are responsible for the native structure are disrupted.

Similar changes occur in solvents such as aqueous solutions of urea at high concentrations – at least 8 M – and what has become the standard medium, 6 M guanidine hydrochloride solution. These solutions have the advantage that much higher concentrations can be studied since they also prevent aggregation, and while gelation can occur it does so through covalent bonding usually via the formation of interchain disulphide links. It is usual therefore to include a reagent which can prevent this. The chemistry is complex, but frequently involves the formation of mixed disulphides between the reagent, for example mercaptoethanol, and the protein. Guanidine hydrochloride reliably breaks all non-covalent bonds.

The structure of the molecule in this solvent was examined by Tanford and co-workers (1967) in a series of classic papers. They showed that the protein approximates to the random coil. The random coil is a fairly precisely defined structure applicable to synthetic homopolymers, and since proteins are not homopolymers, containing as they do more than 20 different units, they can never do more than approximate to it. The random coil is defined by a particular number distribution of the end to end distance in the chain. This is measured as the crow flies, and can vary between zero, when the ends are in contact, to the total length of the chain extended as nearly in a straight line as the bond angles permit. It can also be used to define an average radius of gyration for the molecule. The most important conclusion is that while all possible

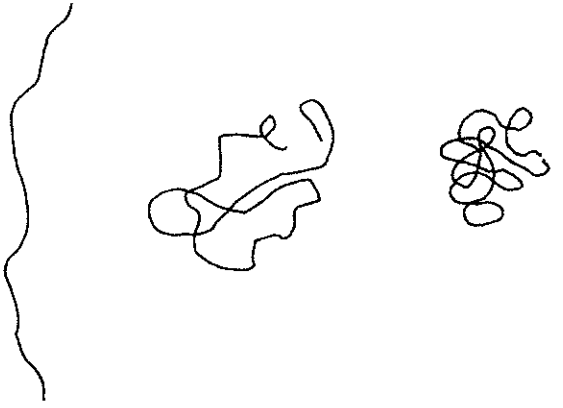

Figure 2. Configurations found in proteins in the random coil form. At the left is shown a relatively extended chain, at the right a compact sphere with the ends in contact. Most of the molecules will have a configuration similar to that shown in the centre, and will be more or less spherical in shape.

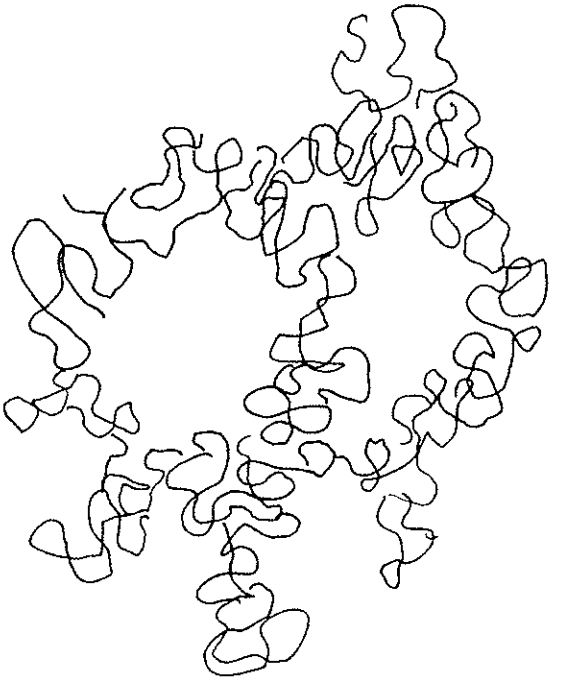

Figure 3. Gel mesh structures formed by randomised macromolecules such as random coil proteins and polysaccharides.

configurations will be present most of the molecules will be like that illustrated in *Figure 2*. They will be, in hydrodynamic terms, spherical. If intrachain disulphides are present the molecule will probably be more compact. The reality of this is reinforced by the observation that myosin, for example, which has a high axial ratio in the native state – around 50 – drops sharply to around 3 in denaturing conditions (Sharp and Offer, 1992).

It is impossible to build structures like that suggested in *Figure 1* from predominantly spherical particles, and the assumption that 'unfolded' proteins are extended chains is unwarranted. This also has implications for the behaviour of protein solutions on extrusion through dies in spinning processes, since the same belief that unfolded proteins are extended chains led to suggestions about shear forces bringing about alignment and orientation of the molecules which does not in fact seem to occur. Spheres, of course, cannot be oriented by shearing. Assembling particles like these to form a gel leads to structures like those shown in *Figure 3*.

THE STRUCTURE OF GLOBULAR PROTEIN GELS

The only direct evidence on the structure of globular protein gels comes from electron micrographs (Tombs, 1974a). Typical images are shown in *Figure 4*. Although this one was derived from ground-nut protein, it is closely similar to the images obtained from many other proteins such as soy which gel in the same way. All the legume storage protein body proteins, which are closely related in general structure, produce

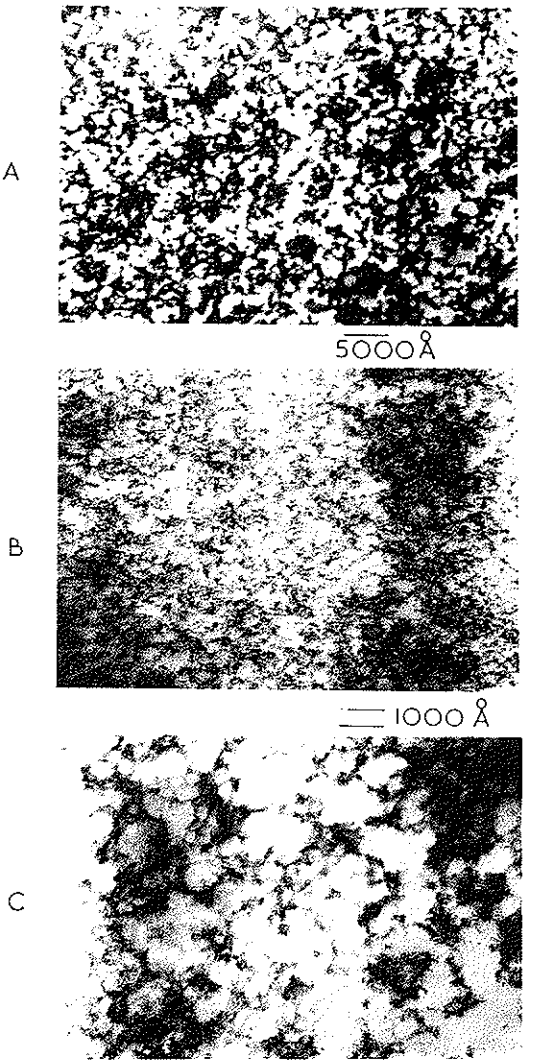

Figure 4. Electron micrographs of typical protein gel structures. All structures are embedded, sectioned and stained with uranyl acetate. **A.** At the top, an arachin gel at 15% protein made by reducing the pH from 12 to 4, the setting conditions used in textile filament spinning. **B.** An arachin gel at 15% protein made by heating at 110°C at pH5 in 4% sodium chloride solution. **C.** Bovine serum albumin at 2% protein, in 8 M urea, where the interchain links are mostly covalent disulphides. From Tombs, 1974.

gels based on aggregates of the main protein. These are typically built of hexameric units, where the six subunits may all be different though similar, and where polymers containing up to 240 subunits, of about 30, 000 molecular weight each can form reversibly depending on the ionic strength, pH, and protein concentration (Tombs *et al.*, 1974). On heating, such systems form random aggregates leading to an open structure like that shown in *Figure 3* and *Figure 4*. Calculation suggests that in order to form such structures one would need concentrations of between 15% and 20%, to produce gels with dimensions similar to those seen in electron micrographs (Tombs, 1974).

Calculations have been based on two quantitative treatments. For a regular rectangular array, the pore size p is given by:

$$p = 1.5d/\sqrt{c}$$

providing that the thickness of the strands d is small compared with p. c is the concentration of the gel forming agent in suitable units (Raymond and Nakamichi, 1962). Note that p is not the maximum dimension of the pore, but the diameter of the largest sphere that could be put through it. This treatment came from attempts to give a quantitative treatment of gel electrophoresis based on the idea that there is a distribution of pore sizes in gels, and that the mobility of migrating particles will be determined by the proportion of pores that they can get through (Tombs, 1965). Ogston (1958) meanwhile derived the expression

$$(p)^2 = 0.25/l$$

for a random array of fibres which were assumed to have length l, but not thickness. This is reasonable since, if one imagines the regular array randomised, some pores would become smaller and some larger, so that the mean p of a randomised gel would not actually differ much from p. In fact for the gels of main interest here, the strand thickness is comparable to the pore size, and more elaborate calculations can be made to allow for this. The basis of all predictions of the pore size from the concentration is to calculate the total length of strand that could be formed from the protein, assuming different aggregation mechanisms. A cubic centimetre of gel contains of the order of 10^9 km strand and constitutes a giant molecule.

OTHER GELS FROM PROTEINS

To summarise the types of gels that can be formed is not easy since each protein really needs to be considered in the light of the interactions that may be specific to it. However we can distinguish the following groups, which will include the great majority of the types of gel likely to be encountered:

(1) Heat-stable gels, generally formed by heating concentrated solutions. They are irreversible, opaque and difficult to reproduce routinely and reliably. They contain a variable amount of covalent bonding formed by aerobic oxidation or possibly interchange of disulphides.
(2) Thermolabile gels, generally formed by cooling hot solutions of low concentra-

tion (i.e., less than 5%). They are reversible, contain high proportions of hydrogen bonds, are transparent and liable to syneresis. Gelatin is by far the best known.

(3) Low pH gels. Sometimes reversible, and may in some cases be sols stabilised by charge interactions. They coagulate on heating. They tend to have low concentrations because aggregation or polymerisation is not random, leading to greater strand lengths from a given weight of protein. A good example is insulin.

(4) Gels induced by unfolding reagents such as urea. These can be varied. Bovine serum albumin will gel in 8 M urea at about 3% protein, but the gel will melt on heating followed, as the temperature rises, by the formation of a second irreversible gelation. At low temperature the bonding is mostly hydrogen bonds, while at higher temperatures it involves covalent disulphide bonds.

(5) Gelation induced by proteolysis. Limited proteolysis can produce alterations to the way in which the molecules interact, which can lead to aggregation and, in suitable cases, gelation. The most important commercial example of this type of gel is the formation of cheese by the aggregation of casein micelles, following proteolysis of the kappa casein component. There are others involved in blood clotting mechanisms.

AGGREGATION PROCESSES

It is clear that the process of aggregation is central to the process of gelation, since the fundamental structural units of the gel, the pieces of strand that form the network, are formed by the interaction of smaller, more or less spherical particles. The result, sometimes known as the string of beads model, depends entirely on the nature of the aggregation, and the interchain links.

The first, and until recently the only, substantial treatment of aggregation was due to Smoluchowski (1916) in papers that are probably more often cited than read. He applied a statistical mechanical approach to both the kinetics of aggregation and to predict the number distribution of the size of the aggregates after any time t. This is more interesting in the present context and states

$$\frac{n_t = n_0(bn_0t)^{a-1}}{(1 + \beta n_0 t)^{a+1}}$$

where n_t is the number of particles after time t, n_0 the initial number, a is the number average degree of aggregation and β is a constant.

Figure 5 shows a test of this relationship based on observations of albumin aggregation with estimates of particle size obtained by electron microscopy (Tombs, 1970). Smoluchowski actually applied his equations to the aggregation of colloidal sols, and obtained much experimental evidence of their validity. He assumed that all contacts between particles during aggregation had an equal probability of leading to adhesion, which probably is the case for colloidal sols. The deviations from prediction in *Figure 5* are because this is not true for albumin, where many of the interchain bonds are covalent disulphides. It was not until 1949 that Kleczkowski (1949) applied Smoluchowski theory to the aggregation of serum albumin, and mixtures of albumin and mosaic virus. He showed by semi-empirical measurements of turbidity that the theory did indeed apply to this sort of aggregation. Barbu and Joly (1953) were others

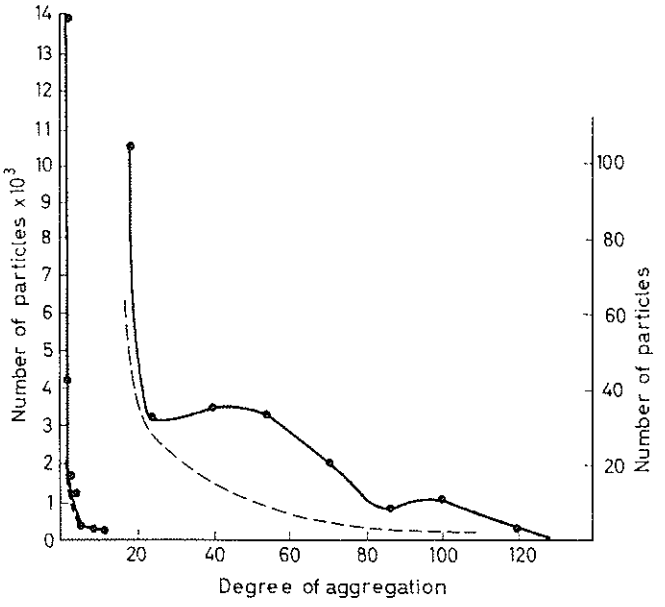

Figure 5. A test of Smoluchowski aggregation theory. It shows the number average degree of aggregation of albumin after heating at 0.9% at 69 C for 5 min. The broken line is the prediction of theory (see text) while the filled line indicates the results obtained from electron micrographs. The scale at the right applies to higher degree of aggregation. There is deviation with an excess of aggregates at about 50 and 100 initial particles in the clump. From Tombs, 1970.

who studied protein aggregation. A Czechoslovakian group published a long series of papers on the aggregation of serum albumin, and were pioneers in the use of electron microscopy to examine the aggregates (Bartl *et al.*, 1963).

More recently, there has been interest in whether the aggregation is a fractal process (Mandelbrot and Evertsz, 1990). A fractal process is one where the relation which defines the connection between the number of units in the aggregate and its dominant dimension,

$$N(R) = (R/a)^d$$

(where N is the mass of an aggregate of size R, and a is the diameter of the monomer units. d is the fractal dimension) is true. Photon correlation light scattering is for theoretical reasons well suited to following fractal processes. Although most work has been done on colloidal sols (e.g., Weitz *et al.*, 1985), some measurements on proteins, using this technique, have shown that the aggregation of casein and antibodies is broadly fractal in nature.(Horne, 1987). The value of the fractal dimension can be used to characterise different types of aggregation, for example where the aggregates are compact or more random in structure (Rarity, 1989).

Detailed predictions of gel structures have been made, based on various aggregation models, and by using computer-based calculations have led to accurate predictions of the appearance of gel structures in electron microscopy. Early attempts were stimulated by the availability of electron micrographs of protein gels (Tombs, 1974a), and made it clear that a string of beads aggregation model could account for the gelation

behaviour of globular proteins. Results obtained by Clark *et al.* (1981) and Clark and Lee-Tufnell, 1985), in particular, demonstrate that the model can account very well for the gelation of a variety of proteins.

Biotechnology: structure forming and spinning processes

Filamentous proteins, such as wool or especially silk, have always had a higher value than amorphous ones, and there has been a commercial incentive to try to turn the one into the other for nearly two centuries. Indeed, long before it was known that gelatin and silk were both proteins there were attempts to make substitutes for silk from gelatin (Wormell, 1955). During the period 1920 to 1950 a substantial industry came into existence based on making a wool substitute from casein and other proteins. This involved forming filaments by a spinning process. It was discovered that many proteins, as well as casein, could be used to form filaments. Soy, ground-nut, and cotton seed protein, amongst those commercially available, and many others such as albumin derived from slaughter house wastes, lupin and potato protein, which were not available but could be regarded as potential sources, were used to form filaments (Young and Lawrie, 1975). The process involved making a strongly alkaline solution of the protein – pH 11 to 12 was usual, containing about 20% protein – followed by extrusion into an acid fixing bath, usually acetic acid, with added sodium chloride. For textiles cross linkers such as formaldehyde or glutaraldehyde were used to improve stability. They probably reacted with the amino groups in the side chains of lysine. A similar process which involves anular extrusion is used to make sausage casings from collagen, but textile filaments used platinum spinnerets involving up to 20, 000 holes. There were disadvantages to this process – the very alkaline solution was unstable. A strong smell of ammonia indicated that amide group hydrolysis was taking place, while the solution tended to gel if left standing so that interruptions to the process tended to result in pipelines full of gelled protein. Also the filaments contained substantial amounts of acetic acid and sodium acetate, which had to be removed. Despite this a substantial industry came into existence, but was eliminated by competition from synthetics such as nylon and terylene.

Even so, in the years immediately after the 1939 to 1945 war, when meat rationing was in force in the UK, these filaments were seen as possible ingredients for an imitation meat product. In particular Boyer (1954) developed a process in the USA for forming filaments from soy protein, while in the UK a similar process based on ground-nut protein was developed. After ground-nut protein became unobtainable, a novel process based on soy protein was invented (Tombs, 1972). It avoided the need for a high pH by using a mesophase form of the protein, which had the requisite high concentration, and a low viscosity suitable for extrusion. The filaments were set in hot water. The disadvantage of this process was that the mesophase could only be formed from native protein, and most soy protein is denatured during isolation. Soy protein is extracted from the meal remaining after lipid has been extracted from the beans, usually by hot hexane. Contrary to first impressions, the damage to the protein occurs, not from this, but from the practice of using steam injection to remove the last traces of hexane from the meal. If this is avoided native protein can be obtained.

The product formulation involved aligning the filaments to simulate the fibrous structure of cooked meat, and binding them together with another material, typically

egg albumin since it is readily available in hundred-ton quantities, though carbohydrate agents can also be used, colouring and flavouring them. Although some products reached the market, for a variety of reasons they were not very successful. The commercial interest has never entirely disappeared, and now appears to be undergoing a revival, possibly because of a rise in vegetarianism. It has yet to be established, however, that vegetarians want an imitation meat, as opposed to an acceptable main meal centre, which is not quite the same thing.

Although the alkaline and the mesophase process can be used on many different proteins, including virtually all the plant protein body storage proteins, (excluding monocotyledons – which can be used, but in a different way) one of the factors limiting their use is the stability of the filaments (Wright and Bumstead, 1984). For obvious reasons, the stabilising cross linkers used in textile filaments cannot be used, and in practice the level of available cysteine side chains able to form interchain disulphides seems to be critical. Soy is comparatively rich in such groups, while ground-nut is poor, and this is reflected in their stability towards, for example, autoclaving. It is rather disconcerting to find that carefully formulated structured imitation meat dissolves in tomato sauce, on canning, when made from some proteins but not others. The role of SH in soy protein gels has been investigated extensively (Shimada and Cheftel, 1988; Doi and Kitabatake, 1989) and cultivar variation appreciated (Kinsella, 1979; Nakamura *et al.*, 1984).

It is clear from electron micrographs that the filaments obtained by extrusion are cylindrical gels and there is no evidence of molecular orientation. It is for this reason that they are best referred to as filaments rather than fibres, since this implies a similarity to biological fibres, such as wool and silk, which do show molecular orientation. The lack of orientation seems to have no effect on their utility.

MODIFICATION OF GEL FORMING PROTEINS BY GENETIC ENGINEERING

Previous experience suggests that a single thiol group can have a significant effect on the covalent cross linking important to the properties of protein gel based products, and since site directed mutagenesis can be used to influence this, there is a possibility that proteins with different properties in use may appear. This could happen either as the result of a deliberate attempt to improve cross linking, or by accident as a result of, for example, an attempt to improve nutritional value.

Mankind and his domestic animals derive a large part of their protein intake from the storage proteins of seeds, and these have been the target of genetic engineering for a number of years, with the objective of improving the amino acid content. The main limiting amino acids are methionine, cysteine and tryptophan, and most work has been directed to improving the methionine content (see Hefford, Chapter 7 in this volume). In ground-nut, for example, the storage protein arachin contains a single methionine residue in each sub unit chain, and a single mutation would double the content, and considerably improve the economic value (Tombs and Lowe, 1967). A search for adventitious mutants with such a mutation failed to find any, though it was successful in *Phaseolus*, but more recent methods of mutagenesis should lead to a re-examination of this possibility. Methionine is limiting in the most used legume, soy beans, and more work has been done on this plant than any other legume (Scott *et al.*, 1991). Problems that might occur, and to some extent have already been recognised are:

(1) a deficiency in the amino acid synthesising capacity of the plant
(2) failure of multi sub unit proteins to assemble properly after modification
(3) failure of deposition in protein bodies
(4) failure to mobilise properly on germination.

All these are factors that relate to the metabolic situation within the living plant. There may be further consequences in use. For example heating has been suspected of reducing the digestibility of the methionine content of the common bean *Phaseolus vulgaris* as a result of aggregation (Genovese *et al.*, 1996) and tighter structures as a result of genetic manipulation will certainly influence this factor.

Many of the attempts to improve nutritional value in seeds have involved classical breeding methods to change the balance between protein body and non-protein body proteins in the cell. Reducing the protein bodies usually improves the amino acid balance, since cytoplasmic proteins are richer in the limiting amino acids. They are also better at gel formation. There are, however, limits to how far this can be progressed by breeding methods.

Another approach would be to bring about the over-production of a cysteine-rich protein, such as metallothionine, by suitable insertion of promoters. Although these proteins do exist in plants it might be desirable to introduce a heterologous gene, but all such attempts will almost certainly come up against the metabolic limitations listed above.

Less direct effects may result from the availability of enzymes as a result of biotechnology developments. One such is the potential use of transglutaminase. This enzyme can form covalent cross linked gels from proteins by linking glutamate and lysine side chains, and its possible use in food manufacture has been appreciated for some time. Unfortunately, the enzyme was not available, though a microbial source has now been found (Matsumura *et al.*, 1995). The reaction is similar to that involved in the old plastein reaction in which partial hydrolysates are cross linked by adding proteases.

There is current interest in finding a non-animal source of a gelatin or some protein which would behave like gelatin. This is mainly aimed at Asian vegetarian confectionery markets. It could be met by the expression of suitable segments of the cDNA for collagen in some suitable plant, and would yield a gelatin of unusual homogeneity which might find application beyond simply confectionery. For example, low-fat spread technology uses gelatin, and could therefore be objected to by vegetarians, but there may be a case for better characterised gelatins in this application (Clewlow *et al.*, 1995). An alternative approach would be to use extensin, a component of the cell wall with elastin like properties.

A pioneering study explicitly aimed at increasing the cysteine content of a protein with a view to improving its properties in application appeared in 1991 (Dale *et al.*, 1991). The serine codon differs from that for cysteine by only one residue, in the example shown in *Figure 7,* where there is TCT and AGC as against TGT and TGC, which makes the conversion of a serine residue to a cysteine relatively easy. Fortunately serines are fairly common residues, while cysteine is relatively rare in most proteins.

The pea protein vicillin has been expressed in yeast by standard methods, and it has offered an opportunity to test the idea that extra cysteines would lead to aggregation.

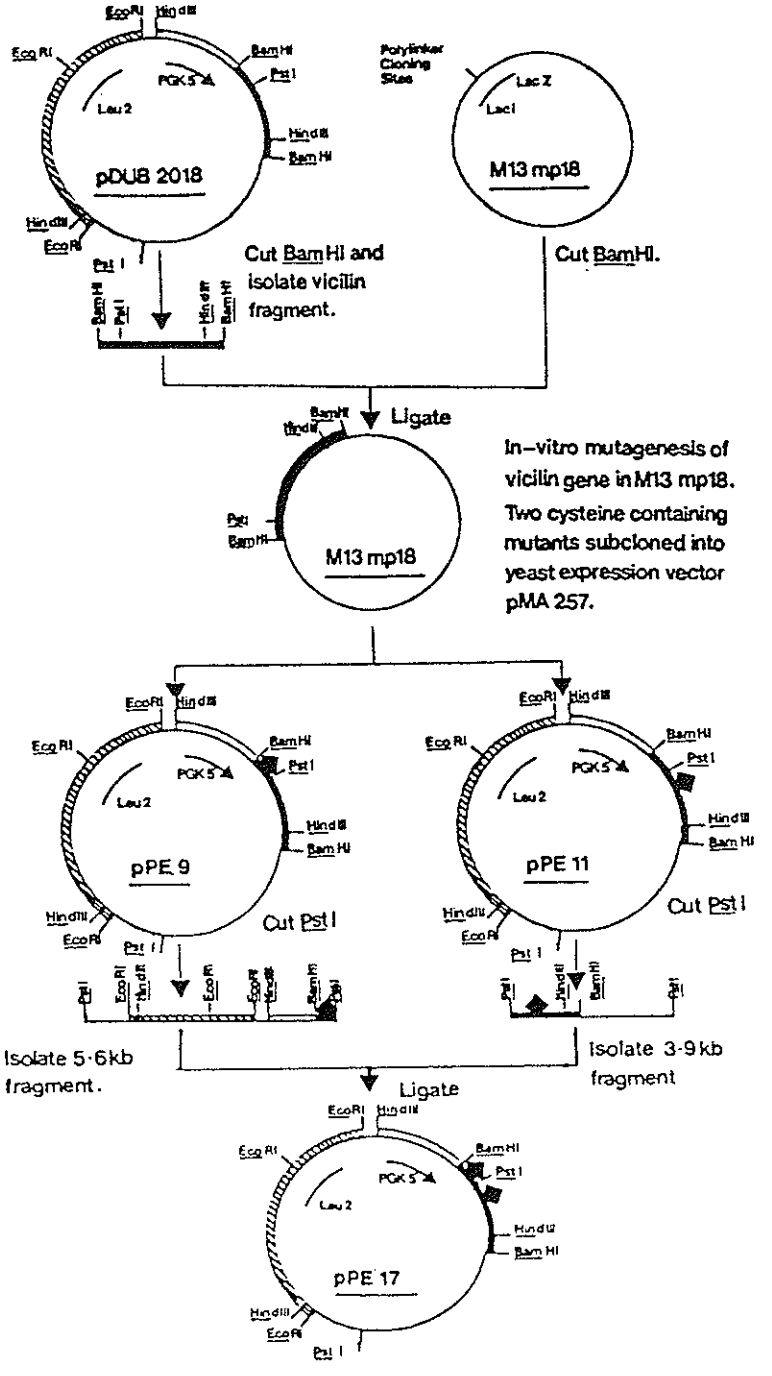

Figure 6. Cloning of vicillin cDNA, insertion into the M13 plasmid, mutation and cloning into pMA257. The intention is to produce the changes shown in *Figure 7* to the sequence of the expressed protein. From Dale *et al.*, 1991.

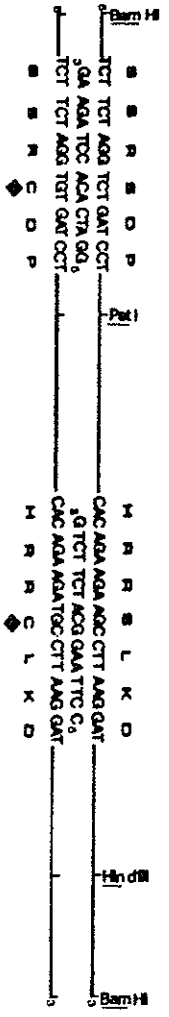

Figure 7. Mutagenesis oligonucleotides used to make pPE 17 (see *Figure 6*). The result is to convert a serine into a cysteine. From Dale *et al.*, 1991..

Figure 6 and *Figure 7* show the changes made to two of the serine residues. As revealed by gel electrophoresis, the cysteine containing peptide formed up into covalently linked trimers on expression within the yeast. Isolated single chains however showed a greater ability to aggregate, this time into large generalised particles.

This is broadly what would have been predicted, but the usefulness or otherwise of the modified protein will have to wait for much larger amounts to become available. This does show, however, that the appropriate alterations to protein body proteins are feasible. Many of these developments are taking place in commercial secrecy. It can be predicted, however, that legume proteins with enhanced cysteine contents will have superior filament forming properties as well as superior nutritional value. There may well be problems, too, with their metabolism and in digestibility.

A separate issue is whether computer simulations of aggregation processes could be used to forecast the effects of inserting an extra cross linking group. Although considerable advances have been made in this respect (e.g., Barker and Grimson, 1989) it remains to be put to experimental test.

Polysaccharide gels

Polysaccharides have found very wide use in industry, particularly in the food industry as emulsifiers, stabilisers and gel forming agents. They also have numerous other industrial uses, as glues for example, and pastes, which do not depend on their ability to form gels, and will not be considered here. Their role as emulsifiers and stabilisers depends on their ability to interact with the surface of droplets, or particles in colloidal sols, and prevent their coalescence. This too does not depend on their gel forming ability.

They do, even so, form gels of a variety of types which are just as varied as those formed by proteins. The nature of the interchain links is fairly well understood, though in some cases the details are still disputed, and a difference from protein gels is that covalent interchain links are rarely involved. The most important difference, however, is in the shape of the molecules, since most gel forming carbohydrates form flexible rods, and not the spherical random coil particles typical of globular proteins.

This leads, at once, to the prediction that on the whole polysaccharides are likely to form gels at lower concentrations than proteins and this is true. Indeed coagulation is not a phenomenon that one associates with polysaccharides since if they do not gel they are more likely to form viscous solutions, though in some cases gel like particles might separate out. Agar and the closely similar gellan are both able to form gels when solutions are cooled to about 40 C, and these gels melt again at about 70 C. This wide hysteresis is made use of in some applications such as growth media. Most polysaccharides gel through hydrogen bonds, often with the formation of helical structures, and side to side association of the chains. This is the mechanism with neutral materials such as gums, mainly galactomannans, and esterified pectins. Charged polysaccharides such as alginates, and low ester, high carboxyl pectins gel on addition of cations such as calcium, an effect which is made full use of in some food processes. In every case, however, the concentration requirements and the general parameters of the gel are determined by the fact that they are all fairly flexible rods in shape, and correspond to the type of gel illustrated in *Figure 1*. This does not, of course, mean that they do not show subtle variations in viscosity or elasticity, often enhanced by mixing different polysaccharides together, which have been very extensively investigated (for example, Harding *et al.*, 1995). The major exception to this rule is starch which, in some cases, forms gels rather more like the type shown in *Figure 3*.

STARCH GELS

Starch, a mixture of amylose and amylopectin, occurs as grains, generally alongside the protein bodies already mentioned, in the seeds of plants. The grains are highly ordered, with radial symmetry, the details and the ratio of amylopectin to amylose varying between species. Many mutants are known with high proportions of both amylose and amylopectin. On germination they are degraded by a collection of

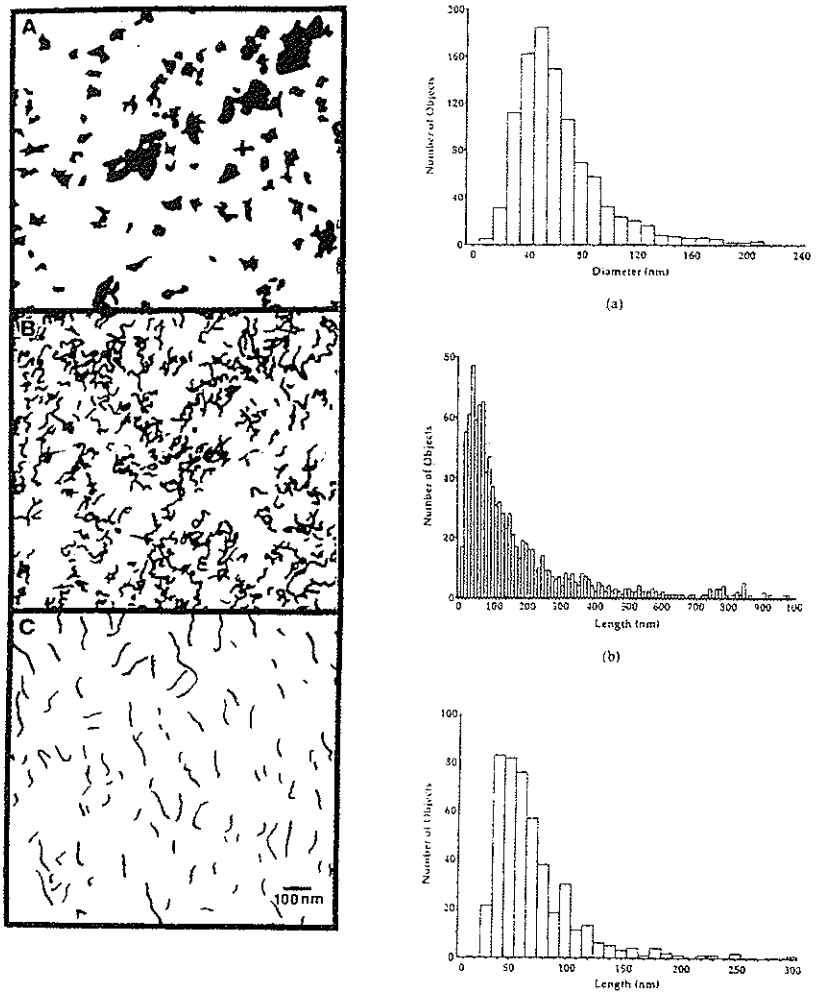

Figure 8. Size distribution of starch particles made by heating starch grains in water. These particles are the gel forming elements that produce gels in more concentrated mixtures. At the left the electron microscope images of selected particles, at the right the number distribution. **A**. Amylopectin patches. **B**. Asymmetric amylopectin. **C**. Pseudohelical amylose. Reprinted from M.L. Fishman, P. Cooke, B. White and W. Damert, Size distribution of amylase and amylopectin solubilised from corn starch granules. In: Carbohydrate Polymers **26,** © 1995, 245–253, with kind permission from Elsevier Science Ltd, The Boulevard, Langford Lane, Kidlington, OX5 1GB, UK

enzymes which are able to attack the grain although the starch is not in solution and there are severe steric restrictions to access. In processes which depend on the breakdown of starch by added enzymes this is a major consideration, and it is usual to heat the grains. This results in the disruption of the grain and the formation of gel structures which are more easily attacked.

Some results of electron microscopy of starch disrupted in this way are shown in *Figure 8* (Fishman *et al.*, 1995). This study was concerned with differences between

high amylose and high amylopectin variants of maize starch. As might be expected, the amylopectin tended to produce low axial ratio particles, and the amylose longer rod like ones in these images. The gels formed from these two types of particle would clearly be different, though it should be remembered that in solution amylose is more spherical (Morris, 1990). There is no information as yet on whether this is of any significance to subsequent processing. It may find some applications in relation to staling of bread, which involves changes to the starch gel structures. Although high amylose and high amylopectin starches have been available for some time no distinct uses for them have been described. This may change soon (Bruinenberg *et al.*, 1995). A number of attempts have been made to alter the type of starch made by potatoes, a major source of industrial starch, particularly in Europe. The proportion of highly branched amylopectin has been increased by the insertion of an *E. coli* glycogen synthase gene (Shewmaker *et al.*, 1994), while antisense RNA techniques have been used to suppress amylose content (Kuipers et al 1994) and it seems that transformed potatoes could shortly be grown on a large scale. Paper coatings are envisaged as one possible outlet for modified starches, but for the moment this looks like a technology in search of an application. Some food applications are under investigation under conditions of commercial secrecy.

ELECTROPHORESIS AND DIFFUSION IN POLYSACCHARIDE GELS

The structure of starch gels became of academic interest when Smithies introduced starch gel electrophoresis and became interested in the relationship between molecular size and mobility(Smithies, 1962). In this work, which related the mobility of a series of proteins to the number of amino acid residues in the chain and therefore the molecular weight, the proteins were unfolded in 8 M urea to produce random coils. Starch gels and the use of urea have been completely replaced by polyacrylamide gels and SDS in what is now a very widely used method, but the results are similar to those originally found by using starch gels. This was not the first use of polysaccharides as a medium for electrophoresis since agar, and agarose, had already been used (Uriel and Grabar, 1956). Agarose was preferred to agar since it is uncharged, while agaropectin appears to carry a small number of sulphated charged groups, which gave rise to electroendosmosis. This is undesirable. In agar gels, at around 1% concentration, the mobility of proteins in electrophoresis is more or less identical to that in the Tiselius-free solution apparatus. The agar nevertheless prevents broadening of the zones during electrophoresis, and must therefore be doing this by preventing convection currents due to heating caused by passage of the current. It can hardly be due to restricted diffusion since the mobility is unchanged. This is borne out by work which made use of the ability of antibodies to diffuse through agar gels, and a variety of ingenious arrangements can be used in analytical methods (see, for example, Allison and Humphrey, 1960; Grabar and Williams, 1955). Although these methods have largely been replaced by others that rely on surface adsorption of antibodies, the ability of proteins to diffuse freely through low concentration polysaccharide gels like agar remains of practical importance.

In sharp contrast to agarose, the mobility of proteins through starch gels in electrophoresis is not the same as in free solution. It is lower, and the extent of retardation is related to the size of the protein molecule. The starch gels used are much

more concentrated – typically 15% – and were made from hydrolysed potato starch by heating a suspension which set to a gel on cooling. Although the formulation was arrived at empirically, we can see that this is exactly the sort of result which would be expected from a gel made up predominantly from spherical particles. Applying the same sort of calculation as that for globular protein gels does suggest pore sizes of magnitude comparable to the diameter of globular proteins.

PECTINS AND THEIR ENZYMATIC MODIFICATION

Pectins are the most important polysaccharide gel forming agents in terms of tonnage and value, and are widely used in jam manufacture. Sugar mixed with pectin is sold, and still used by housewives in many countries. Pectins have other uses as emulsifiers and are finding new uses in low fat spread manufacture.

The impact of biotechnology is likely to be on the supply of enzymes used to modify their properties, rather than on the source materials. As is well known, the use of antisense RNA to suppress pectinase activity has lead to the appearance of tomatoes with better keeping properties, and superior methods for making tomato puree. This was the first significant introduction of genetically engineered products into the supermarket, but it has little importance for gel systems. It is possible that the same techniques could be used to help to prevent unwanted breakdown of pectins in the source materials but there is no need for this at present.

There are four enzymes which act on pectins. Pectin lyases cleave bonds between methylgalacturonate residues and pectate lyases split between the galacturonate residues that lack the methyl group. They are really groups of enzymes with preferences for the ends or the middles of the chains, endo and exo action, and widely distributed in all those organisms that live on plants, and need to break down their cell walls. The structure of pectate lyase has been determined (Pickersgill *et al.*, 1995) with a view to protein engineering, though there are at present no applications for these enzymes.

Pectins form two different types of gel. The esterified chains form thermolabile gels depending mainly on hydrogen bonds for the interchain links, which form slowly on cooling. The other type, which is formed by the carboxyl group rich de-esterified chains, is induced by adding calcium ions. Variable de-esterification results in a range of gel forming ability, and gel strength.

The pectate hydrolases which split the chain at the glycosidic bond are the ones of interest in fruit ripening, and can cause trouble during pectin isolation. It is generally desirable to keep the molecular weight of the isolated pectin as high as possible and these enzymes reduce it. However, one major source, apples, have no pectate hydrolase activity and molecular weight reduction is more due to chemical hydrolysis than enzymes (May, 1990).

The most important enzyme is pectin esterase which removes methyl groups from the carboxyl groups and changes the level of charged groups. This has an effect, as pointed out, on the gelation behaviour. Both the esterase and the pectate hydrolase (polygalacturonidase) are added during isolation from apples to control the properties of the isolate. They are from fungal sources and might be improved by transgenic methods. This is because there are situations where enzymatic removal of methyl groups is required while avoiding chain splitting and molecular weight reduction.

Fungal enzymes tend to occur as mixtures of several of the enzymes that attack pectin, and it is not easy to obtain enzymes completely free of the other activities. A range of pectins with variable distribution of methylation along the chain are potentially available and could probably find uses.

Pectins are branched, with arabinogalactan side chains of variable frequency, depending on the source. There must be enzymes able to remove these side chains but they have not so far been investigated, nor has their effect on gelation behaviour. The sole report suggests that the side chains themselves may interact to bring about gelation in apricot juice. Pectins also have occasional rhamnose residues in the main chain, which must also have an influence on the overall shape of the molecule, and probably also interfere with side to side chain interactions in gelation.

ALGINATES

Alginates (mannuronic and guluronic acid polymers from brown algae, with carboxyl groups)and caraggeenans (sulphated galactans of red algae) are similar to pectates in having charged groups and forming gels when calcium ions are added. Electron microscopy shows that their gel structure is of the type shown in *Figure 1* (Hermansson, 1995). Their production is still at the hunter-gatherer stage of development since they are harvested directly from the sea wherever they can be found.Two different carrageenans are recognised – ι and κ – with different gel forming properties. It is difficult to obtain the one free from the other, even when they are isolated from cultivated sea weeds with different ratios of the two types. The presence of small amounts of κ carrageenan can modify the gelation properties of the other to such an extent that it might be worthwhile trying to find some way of eliminating it. There is little scope for genetic engineering at this stage, while the weed is essentially gathered where it grows in the sea but if it should be cultivated in a way that would permit segregation and control of the type growing, then modification of the source seaweed would probably be effective. Farming is just beginning in Canada and in the Pacific and may do something to offset the way in which the crop can fluctuate widely from year to year. Much the same applies to alginates where there are species differences in the detailed structure and gel forming ability.

Furcellaran, an alginate obtained from the floating weed *Furcellaria fastigiate*, and which was used, has now disappeared because the weed has become scarce. It has been replaced by a carrageenan, a case history which shows how insecure the supply of some of these gelling agents can be (Morris, 1987).

Alginates and carrageenans have found some interesting uses, along with pectates, in the manufacture of fruit products. Fruit is an important component of many food products. It is the most expensive ingredient of yoghurts, as sold, and fruit pies, as well as ice creams. The manufacturing problem is that all these applications demand pieces of fruit, recognisable slices for preference, and in making these there are inevitably waste smaller pieces. It has been found that alginates can form gels with rather fruit-like textures, and a technology which mixes fruit puree with alginates and pectates, employing sometimes extrusion, and uses calcium to set the gels, has appeared.

GALACTOMANNANS: MODIFICATION OF GUMS BY ENZYMES

Gums have been used for a long time as adhesives, as their name suggests, but more recently have found other uses in food formulation, uses which depend on their gel forming ability. The interchain links are side-to-side association of the mannan main chains, and are hindered by the presence of galactose residues which are unevenly distributed along the chain. The number and location of these residues varies between species, and affects their utility in a significant way. Like seaweed, the sources of gums are not secure. The trees, for the most part, grow in parts of the world liable to agricultural and political change, which can lead to sudden fluctuations in supply. This naturally has effects on the price and availability of individual gums. Manufacturers have therefore sought to find ways of substituting one gum for another in an attempt to stabilise the situation, as well as to develop new combinations. The idea of removing galactose side chains to modify properties is not very new (McCleary, 1979; McCleary *et al.*, 1985) but was one of those ideas that could not be used because the required enzyme, an α galactosidase was not available. It has now been made available as a result of transgenic production, and provides one of the best examples of the way in which biotechnology has improved the feasibility of a process.

Guar gum is one of the more reliably available gums, but locust bean gum, which is similar to it, is not. However locust bean gum has specially useful properties because it has only 22%–24% galactose as opposed to the 38%–40% in guar. This means that the gels are more elastic, and in some products this difference is vital. Bacterial galactosidases were found in laboratory trials to be able to modify the properties but the most effective enzyme was an α galactosidase found in the beans of *Cyamopsis tetragonoloba*, the guar tree, itself. It is doubtful if this could ever have been made into a commercial source of the enzyme, but in any case it suffered from the further difficulty that extracts of the seed also contained a mannanase and a mannoside mannohydrolase, which split the main chain.

Transgenic production of galactosidase was therefore doubly desirable since it should both provide a secure supply of the enzyme and remove the undesirable associated activities. This has been achieved in yeast (Overbeeke *et al.*, 1989) and the enzyme successfully applied to modification of guar gum (Bulpin *et al.*, 1990).

In a further development the galactosidase has been produced as a fused product with a surface binding protein found in the yeast itself. The outcome is the galactosidase firmly anchored to the surface of the yeast. (Schreuder *et al.*, 1996). It is not clear whether the enzyme immobilised in this way would be effective, since at present it is used on a gel of the substrate at high guar levels. It is remarkable how often the enzymes that attack polysaccharides seem to be most effective when the substrate is present in the form of a gel. No doubt the pores are large enough to admit the enzyme, and the activity itself results in fairly high diffusion rates.

Other possible routes to a modified guar gum include, of course, direct manipulation within the seed of the enzyme systems that are responsible for the degree of branching. The problem here is that genetic engineering of trees is very difficult because of the long time taken for the transformed plant to grow and produce seeds. It might be several years before the results of any attempt at transformation could be assessed, and even longer before successful attempts could be turned into a producing plantation. Another route to polysaccharide production is by plant tissue culture.

Modified plants can be induced to secrete polysaccharides in tissue culture, and experiments are currently under way on the 100-l scale: the resulting material must however be expensive.

Bacterial polysaccharides

Gelling agents derived from bacteria have made steady progress ever since the first one, xanthan, was introduced about 50 years ago. Current production is near 10, 000 tons a year. It is made by *Xanthomonas campestris*, in a process that was originally devised as a way of making use of surplus maize starch as a growth medium. Others have followed including curdlan, gellan, pullulan and numerous similar polysaccharides that have not been put into commercial production. In fact bacteria can be used to make nearly all the gel-forming polysaccharides, including alginates. The sole exception appears to be pectins.

Genetic manipulation of bacteria is much better understood than that of higher organisms and because of the fast reproduction times, much quicker to yield results, in marked contrast to gum producing trees. For this reason, manipulation of the enzyme systems responsible for polysaccharide synthesis, either suppression or insertion of extra activities, is more likely to be tried (Griffin *et al.*, 1996). Some academic work has been done to explore the possibilities, especially the use of glycosyl transferases. These enzymes, which transfer hexose residues from donors such as sucrose to growing chains can be used to form oligosaccharides, and have the advantage that unlike most polysaccharide biosynthetic systems they do not employ nucleotide derivatives.

Dextrans, which are linear chains of glucose residues, with very limited branching, are secreted by *B. macerans*, and are familiar in laboratories in the form of chemically cross linked gels. The way in which they interact with other solutes has been a subject of considerable interest, and is known to lead to incompatible phase separation with other polymers, including proteins. What is less well known is that dextrans cross linked into a gel can still bring about phase separation (Ogston and Silpananta, 1970; Ogston and Preston, 1973). Incompatible phase separation depends on the volume occupied by the molecules and this changes little when they are linked up to form a gel. Cross linked dextran beads placed in a sufficiently concentrated solution of a globular protein will show droplet formation within the matrix, if the protein is able to penetrate it. This is a laboratory curiosity at present but might find application in drug-delivery systems and in storage methods for therapeutic proteins.

Cyclodextrins have also attracted much attention, but not as gel formers. Cross linking gives gels with interesting porosities, which again may have value as sequestering agents and in drug delivery.

Conclusions

There are some interesting parallels between the main protein and polysaccharide gel forming agents. Oil seed meals originally appeared as by-products of lipid production, and still have that status. Although they are now being challenged by oil palm, soy beans are the largest single source of lipids for human consumption, which are used for margarine manufacture and for cooking oils. Other oilseeds such as ground-nut and

rapeseed are also important in international trade. The meal, that is to say the residues left after lipid removal, would be produced in any case and in some cases represents a disposal problem. Cotton seed meal for example, although it contains protein at least as valuable as other seeds, has limited use because it also contains gossypol. Cotton seed is, of course, grown primarily as a source of cotton textile fibres. In the case of soy, enough uses have been developed for the meal during the last 50 years to make the value of the meal roughly equal to the value of the lipid. By far the major use is as animal feed, and the amount that has been diverted to human food use in the form of protein isolates is a small proportion of the total. Human food grade protein isolates are relatively expensive – about five times as expensive as the same amount of protein in the form of meal – which has limited their use as gel forming agents. At least one of the reasons for the commercial failure of imitation meat products based on spun filaments was that they were no cheaper than the real thing. The public generally expects substitutes to be cheaper and, unless there is some other reason for preferring them, will not buy them. It is possible that the rise of vegetarianism may provide the necessary motive. The protein level in meal, at 40%–50%, is high enough for gel formation and a range of products made by extrusion directly from meal have maintained a small market presence, and are relatively cheap. The introduction of improved gelling properties as a result of genetic engineering might have some impact here, since the gel formation in such mixtures of polysaccharide (mainly cellulose) and protein is marginal and could usefully be increased. Direct suppression, perhaps by antisense technology, could also help to make such protein sources as cotton seed meal easier to use by removing undesirable components. Since nearly all these sources are by-products of other raw material manufacture, they are unlikely to get any cheaper, but could be made more useful.

Since the overwhelming commercial interest is as a cattle food, where the limiting amino acid content is all-important, it is not suprising that interest has concentrated on this aspect. There may however be unexpected effects on other properties as well, and if cysteine is manipulated this is virtually certain.

Notably enough, pectin, commercially the most important polysaccharide gel former, is also made from a by-product. In Northern Europe the source is apple pomace, the residue left after extraction of juice for cider manufacture. Further South, the source is the peel of citrus fruits, which is otherwise burned as fuel or incorporated into animal feed. In both cases, after extraction of the pectin there is still a residue to be got rid of, mainly as a soil conditioner.

The impact of genetic engineering on pectin is well known, and represents the first application of antisense methods to suppress an enzyme. During ripening, the pectin in the cell wall is degraded. This has no direct application to pectin isolation as yet, but could potentially be used to control the size of pectin chains in the source material. It is difficult to see a commercial case for doing this in the foreseeable future.

The use of enzymes made by transgenic production is likely to increase. The example cited here, of galactosidase with required specificity, illustrates a process that could not have been operated without such a supply, and there will probably be many others in the future. The enzymes involved in degradation of starch gels have been obtained from microbial sources for a long time, and these will be continuously improved.

The outlook for polysaccharides made with modified properties by altering the metabolism of the source organism is less clear, though much progress has been made

with potatoes. For the time being, this is more difficult than modifying them by *in vitro* processes because of the lengthy and expensive research required and the difficulty of breeding large numbers of transgenic plants. The raw material would have to be of exceptional value to justify this, and there are no applications in bulk use in view at present. The same applies to polysaccharides made *in vitro* by the enzyme systems currently available, in small pharmaceutical-scale operations. The overall impression is of capabilities looking for applications, for both protein and polysaccharide gel forming agents.

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