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THE STRUCTURE, PHYSICAL AND CHEMICAL PROPERTIES OF THE SOY BEAN PROTEIN GLYCININ

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SUMMARY

The major storage protein of the soybean, glycinin, has been prepared in a homogeneous form and examined by a variety of techniques. It has been found that the protein has a molecular weight of 320 000 and contains two sizes of subunits with different isoelectric points. There are six acidic subunits of ≈ 35 000 and six basic of ≈ 20 000. Analysis revealed three different kinds of acidic subunits and probably three kinds of basic ones also. These twelve subunits are packed in two identical hexagons, placed one on the other, yielding a hollow oblate cylinder of 110 × 110 × 75 Å. Some or all of the subunits are non-spherical resulting in a partial blocking of the central hole. Information about the forces stabilizing the native structure is also discussed.

INTRODUCTION

Interest in the water extractable proteins from soy beans *(Glycine max)* stems from the widespread use of such materials for human and animal nutrition. The major protein component in a water extract has been called glycinin and some of its chemical and physical (hydrodynamic) properties have been studied [1]. Our preliminary studies indicated that glycinin prepared by published procedures [2–4] was not homogeneous since in low ionic strength buffer (< 0.1) at least part of it was capable of dissociating into two equal halves [5]. Since it appeared that the phenomenon was related to the precipitation step in the procedure a new method was developed to remove such material from the preparations thereby providing a homogeneous material suitable for detailed study. Although the more detailed molecular architecture has been speculated upon [6, 7] there is little experimental elucidation at present apart from preliminary electron micrographs [7]. This report describes a detailed study providing such information in terms of subunit arrangements and the forces holding the molecule together and at the same time gives a detailed characterization of glycinin for comparison with previous studies [1, 3, 4, 8].

Materials — Preparation of glycinin

Cold insoluble fraction was prepared by the method of Wolf and Sly [9] from commercial soy flour (Hizyme or 200W grade made by Central Soya, Chicago, U.S.A.). One part of flour was extracted with 5 parts of water at 40 °C for 30 min. The cold insoluble fraction, which was shown to be at least 80% glycinin by ultracentrifugation, was stored at −20 °C. Samples, containing up to 5 g of protein, were prepared for chromatography by dissolving in standard buffer (0.0325 M K_2HPO_4, 0.0026 M KH_2PO_4, 0.4 M NaCl, 0.01 M mercaptoethanol, pH 7.6), or in standard buffer without mercaptoethanol for some experiments, followed by centrifugation at 38 000 × *g* for 30 min and then dialysis against chromatography buffer. 5 × 35 cm columns of hydroxylapatite (Biorad, HTP) were packed and equilibrated essentially as described by Wolf and Sly [10]. A potassium dihydrogen phosphate gradient from 0.03 M to 0.5 M at pH 7.6 was applied at approximately 60 ml/h using an 800 ml Erlenmeyer flask as the mixing chamber and 1 M KH_2PO_4 as the concentrated component. After the proteins had been eluted, the column was washed with 1 M KH_2PO_4, pH 7.6, before re-equilibrating with 0.03 M buffer. The main protein peak from this column which was almost pure glycinin by immunochemical standards contained up to 25% as polymers and also a small amount of polymerized γ-conglycinin, the second major water soluble protein of soy beans. In order to obtain pure glycinin of ≈ 350 000 the glycinin eluted from hydroxylapatite was concentrated by membrane ultrafiltration and applied to a 2.5 × 100 cm column of Sepharose 6B (Pharmacia Ltd) equilibrated with 1 M KH_2PO_4, pH 7.6 containing 0.02% sodium azide. 0.3 g samples yielded optimum resolution and yield. The polymers of both glycinin and γ-conglycinin elute in the void volume followed by the glycinin. That fraction of glycinin dissociating at low ionic strength is polymerized in 1 M KH_2PO_4 and therefore elutes in the void volume. For storage purpose the protein can be freeze dried, after dialysis to remove most of the KH_2PO_4. However, solutions prepared from such material always contained some polymerized glycinin and for critical experiments fresh material was always used.

Acidic and basic subunits were prepared by heating 1% solutions of glycinin in standard buffer to 100 °C for 30 min. An almost quantitative precipitation of basic subunits occurs. This phenomenon will be discussed in more detail elsewhere (Badley, R. A., Barratt, M. D., Flook, A., and Oldani, D., unpublished observations). The basic subunits can be redissolved in standard buffer containing 8 M urea and 0.5% sodium dodecyl sulphate after which they can be dialyzed into water. In this state they tend to reprecipitate. Double distilled water was used and all chemicals were Analar reagent grade.

METHODS

Digestion of protein followed by ninhydrin estimation of ammonia was used to determine the nitrogen content of glycinin [11]. Concentrations were based on dry weight analysis. The amino acid composition was determined on an LKB BioCal BC200 analyzer using a single column three buffer system. Cysteine and methionine were determined according to Moore [12]. Tryptophan was estimated spectrophoto-

metrically, using the contents of tyrosine, phenylalanine and cysteine from the amino acid analysis [13].

In order to achieve high sensitivity for N-terminal analysis and to be able to resolve N-terminal isoleucine and leucine a dansyl chloride procedure was adopted. For identification purposes the method of Woods and Wang [14] was found very suitable. For quantitative estimations the method of Zanetta et al. [15] on silica gel plates was used.

Despite optimizing the conditions it was found difficult to achieve greater than 25% yield of total labelled N-terminal residues. Since standards put through the hydrolysis step were recovered in 90% yield it is felt that poor extraction of labelled residues from the brown residue of hydrolyzed protein was responsible for the low overall yield.

Polyacrylamide gel electrophoresis was carried out in 0.6 × 7 cm glass tubes in a Shandon (London, U.K.) apparatus basically according to their manual. For quantitative determinations of molecular weights [36–38] a series of gels ranging in total acrylamide concentration from 3.85 to 9.24% (all containing 2.6% bisacrylamide) was prepared.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis was performed according to Weber and Osborn [16] using 9% gels containing 2.6% bisacrylamide.

The Stokes' radius of glycinin was estimated by gel filtration according to the procedures of Ackers [14].

Ultracentrifugation was carried out in a Beckmann Model E analytical ultracentrifuge equipped with Schlieren and interference optics and an RTIC temperature control unit. Sedimentation velocity measurements were carried out at 20 °C at a speed of 59 780 rev./min. Diffusion coefficients were determined at low speeds (5227 rev./min) using Schlieren optics and the method described by Neurath [18]. Molecular weights were determined by the meniscus depletion sedimentation equilibrium method using interference optics [19, 20].

The measurement of preferential hydration [21] required the use of a synthetic boundary capillary type cell for the sedimentation velocity experiments. Viscosity of the solvent was measured by using a standard U-tube viscometer. Partial specific volumes were determined using an Anton Paar Density Meter (Model DMA 02C) at 20.00 °C [22, 23] and dry weight analysis [24] for protein concentration determination.

For electron microscopy a carbon coated copper electron microscope grid was floated on top of a drop of a glycinin solution in standard buffer (0.2–1 mg/ml) placed on a hydrophobic surface. The grid was then floated in a similar way on top of a drop of the staining solution and left there for several minutes. As negative stain, either a 2% solution of potassium phosphotungstate pH 7.0 or a 4% solution of sodium silicotungstate pH 7.0 (from Taub Laboratories, Emmer Green, Reading, U.K.) was used. Alternatively, the protein solution was dropped on to the grid and rinsed with 3–4 vol. of negative stain. Excess liquid was drained off with a filter paper. Both staining procedures yielded similar results. No improvement was observed when prior to staining the protein was fixed for 20 min in dilute (0.5%) glutaraldehyde solution in standard buffer.

A JEM-7A electron microscope was used operating at 80 kV.

X-ray scattering measurements on solutions (0.5–10%) of glycinin in standard buffer were made using the Kratky camera [25] and CuK_α radiation. The entrance slit width and sample to registration plane separation defining the resolution of the camera were 80 μm and 215 mm, respectively. Other experimental details were as described previously [26].

The geometrical influence of the line collimation system (smearing) was removed (desmearing) using the methods of Lake [27] and Glatter [28].

RESULTS

Criteria of purity of glycinin

As indicated in the introduction rigorous tests are needed for the homogeneity of glycinin. The protein prepared as above yielded a homogeneous single peak in the ultracentrifuge when run in standard buffer and in water. A single band was found on polyacrylamide gel electrophoresis at both low and high ionic strength and on isoelectric focusing in polyacrylamide gels [4]. Polyacrylamide gel electrophoresis in sodium dodecylsulphate showed only two sizes of subunits whilst those characteristic of γ-conglycinin were absent. Gel filtration in standard buffer on Sephadex G200 and Sepharose 4B gave single peaks. Immunoelectrophoresis and double diffusion [29] of glycinin using antiserum against the whole soy bean extract indicated a single

TABLE I

PHYSICO-CHEMICAL PROPERTIES OF GLYCININ

General	
Nitrogen content	16.3% (w/w)
*Extinction coefficient, $E^{1\%}_{280,\ 1\ cm}$	8.1 ± 0.1
*Partial specific volume, $\bar{V}$, at 20 °C	0.730 ± 0.001 ml g^{-1}
*Sedimentation constant, $S^{\circ}_{20,w}$	12.3 S ± 0.1 S
*Diffusion constant, $D^{\circ}_{20,w}$	3.44 ± 0.1 × 10^{-7} $cm^2 \cdot s^{-1}$
*Radius of gyration, R_g	44 Å
*Stokes' radius, r	58.5 Å
Hydration, δ	0.36 g/g
Number of subunits	12
N-terminals	Leu(2), Ile(2), Phe(2), Gly(6)
Molecular Weight	
Amino acid composition	see Table II
**Gel electrophoresis	350 000 ± 35 000
*Gel filtration	302 000 ± 33 000
*Sedimentation equilibrium	317 000 ± 15 000
*Sedimentation-diffusion	322 000 ± 15 000
From subunit sizes	326 000 ± 35 000
Size	
Electron microscopy	100 × 100 × 70 Å (as observed)
	110 × 110 × 80 Å (allowing for a hydrophilic region)
*X-ray scattering	110 × 110 × 75 Å

* In standard buffer.

** Using a Ferguson plot [36–38].

otein component. Detailed discussion of these results will be given elsewhere. From ese tests it is concluded that the glycinin contained less than 1% contamination nsisting either of glycinin polymers or other proteins. Preparations made according published procedures [2–4] always yielded material capable of partially dissociing [5] at low ionic strength (< 0.1). This phenomenon has been further examined id will be discussed in detail elsewhere.

A summary of the results is shown in Table I and only specific comments will made where their derivation is not clear.

N-terminal analysis of glycinin shows four different amino acids present. eucine, isoleucine and phenylalanine always appear in approximately equal molar oportions together with three or four molar equivalents of glycine. Due to the latively low recovery of total N-terminals it was not possible to unequivocally ow whether the ratio of glycine to each of the other three residues was 3:1 or 4:1. owever, analysis of the isolated acidic and basic fractions [30] showed that the idic fraction contained only leucine, isoleucine and phenylalanine as N-terminals. contrast, the basic fraction contained only glycine. Since glycinin has been found contain equal numbers of acidic and basic subunits by comparing the subunit olecular weights to the weight fraction precipitated upon heating, it is concluded at the molecule contains the following N-terminals; 6 glycine, 2 leucine, 2 isoucine, 2 phenylalanine.

The subunits of glycinin were determined by polyacrylamide gel electrophoresis sodium dodecyl sulphate to be of only two sizes. These have previously been referred as the acidic and basic subunits from their behaviour upon isoelectric focussing [30]. olecular weights of 19 600 (basic) and 34 800 (acidic) were found which are marinally lower than previously reported [30]. Calibrated scanning of the gels indicated at the two types were present in glycinin in equal molar proportions.

The Stokes' radius of glycinin determined by gel filtration can be used to make estimate of its molecular weight by combining the Svedberg equation and Stokes' aw. Thus:

$$f_r = \frac{6\pi\eta\, Nr\, S^0_{20}}{(1 - \overline{V}\varrho)}$$

here η is the solvent viscosity, ϱ the solvent density, N Avogadro's number, $\overline{V}$ the rotein partial specific volume at 20 °C and S^0_{20} the protein sedimentation coefficient solvent at 20 °C and infinite dilution. The following values have been used. $S^0_{20} = 1.3 \cdot 10^{-13}$ s, $\overline{V} = 0.730$, $\varrho = 1.018$ g ml^{-1}, $\eta = 1.034 \cdot 10^{-2}$ poise, $N = 6.023 \cdot 10^{23}$ nd $r = 58.5 \cdot 10^{-8}$ cm (the average of determinations at 25 °C and 4 °C). A molecular eight of 302 000 ± 33 000 has been calculated using S^0_{20} rather than $S^0_{20,w}$ as was one by Koshiyama [4].

The sedimentation coefficient extrapolated to standard conditions (zero concntration at 20 °C in water), $S^0_{20,w}$, was found to be 12.3 S from a linear least squares nalysis. This showed that the concentration dependence of $S_{20,w}$ obeyed the following elationship. $S_{20,w} = 12.31 - 0.078\,C$, where C is the protein concentration (mg·l^{-1}) and $S_{20,w}$ is expressed in Svedberg units. The molecular weight determined by eniscus depletion sedimentation equilibrium at 20 °C and 10 583 rev./min at a proin concentration of 0.55 mg·ml^{-1} is 317 000. The diffusion coefficient, was found

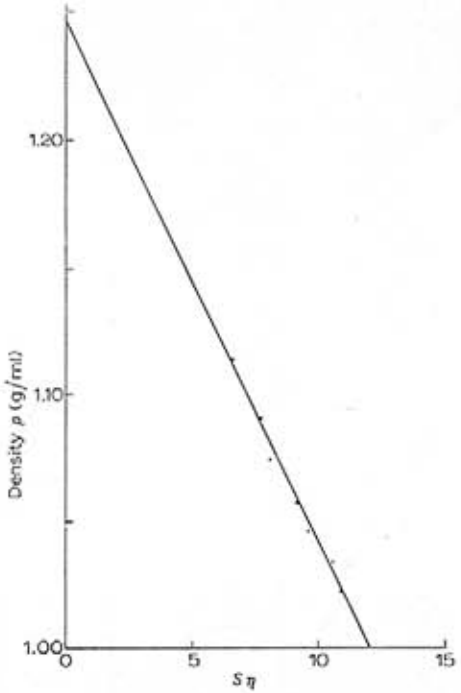


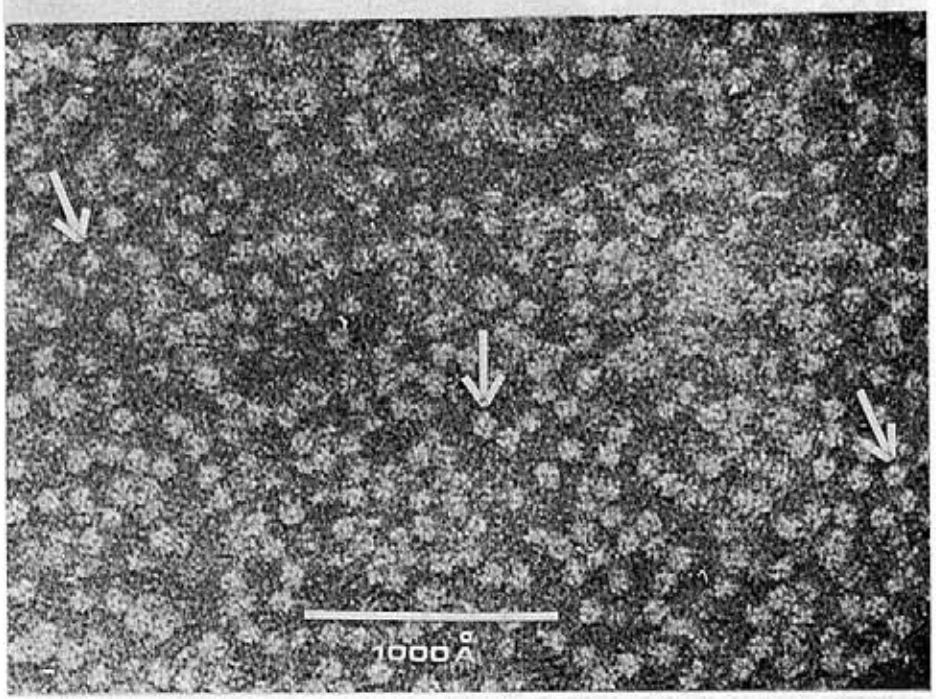
Fig. 1. Change in solution density (ϱ) with the product of sedimentation coefficient (S) and viscosity (η) for solutions of glycinin (4.7 mg·ml^{-1}) in sodium chloride, pH 7.5, of varying concentrations.

to be independent of glycinin concentration (4–12 mg·ml^{-1}) within the standard error of the determinations (± 3%). Using the values of $S^0_{20,w}$ and $D^0_{20,w}$ in the Svedberg equation [20] a molecular weight of 322 000 ± 15 000 was obtained.

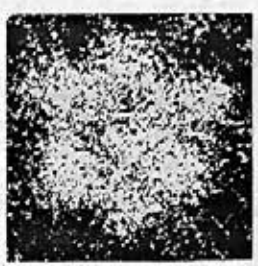
Fig. 1 shows the dependence between $S\eta$ and ϱ where S is the measured sedimentation coefficient of glycinin in a series of NaCl solutions at pH 7.5 and η and ϱ are the viscosity and density of the medium. From the value of ϱ at $S\eta = 0$ the preferential hydration can be determined [21].

Fig. 2a is an electron micrograph of a negatively stained preparation of glycinin. Many polygonal structures, particularly pentagonal and hexagonal are visible. There are also trilaminar structures (see arrows) in which two layers of protein are separated by a layer of stain. Fig. 2a also shows patches of aggregated glycinin molecules which probably result from the negative staining procedure. In electron micrographs which were taken at an optimum under-focus setting of the objective lens to enhance the contrast of the protein molecule in the stain layer it is clear that glycinin consists of subunits. Further information concerning the subunit structure of glycinin was obtained by using a technique similar to that described by Valentine et al. [31]. The protein molecules trapped in the stain layer are present in all possible orientations. Images of molecules having similar orientation were selected from a pool of about 100 electron micrographs such as those shown in Fig. 2a. The images chosen were enlarged to a magnification of 4 million and by superpositioning of 4–5 images representing molecules of a specific orientation the electron micrographs in Fig. 2b to 2d were produced. Fig. 2b represents one typical orientation. It is identified as a

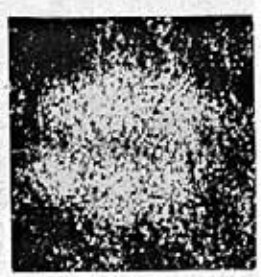
FIGURE 2



2a



2b



100 Å

2c

2d

Fig. 2. Electron micrographs of glycinin. (a), Typical view of negatively stained material; (b), Top view, image obtained by superpositioning 6 different views from 2a; (c) and (d), Superpositioned images of side views.

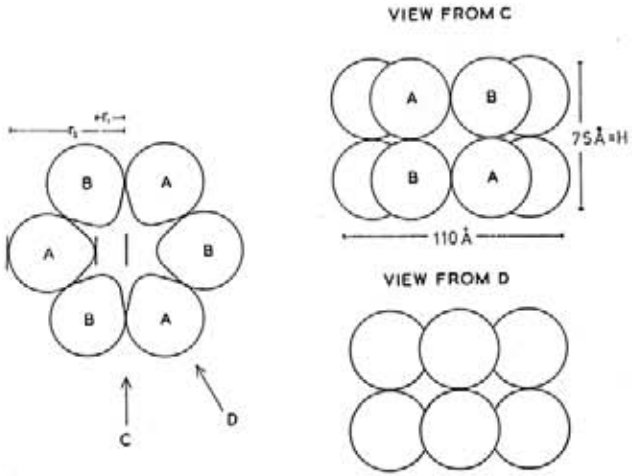


Fig. 3. Schematic representation of the native glycinin molecule based on the data given in this paper.

glycinin molecule resting on its flat face and viewed from the top. The subunits appear to be packed in an approximately hexagonal arrangement with a suggestion of a stain filled hole in the centre. The trilaminar structure in Fig. 2c is identified as the molecule standing on edge showing two rows of four subunits. The two layers of subunits are separated by a layer of stain. The structure shown in Fig. 2d is also identified as a molecule standing on edge showing two rows of subunits each being represented by spots. We interpret this in terms of two different views of the molecule. In Fig. 2c it

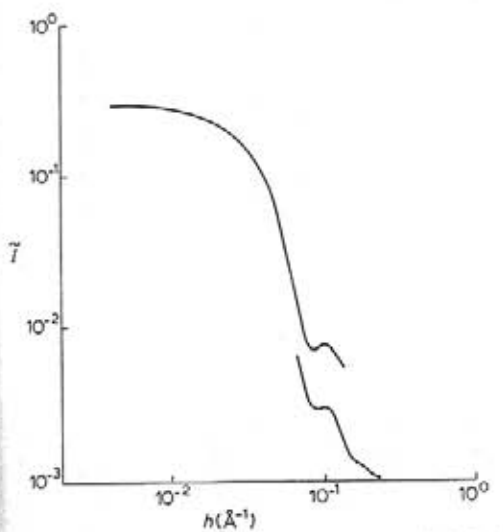


Fig. 4. Small angle X-ray scattering curve of glycinin in standard buffer. The curve is the experimental curve obtained with a line collimated primary beam (smeared). Intensity $\tilde{I}$ is in arbitrary units; abscissa, the variable $h = (4\pi/\lambda) \sin\theta$ where 2θ = scattering angle and λ = x-ray wavelength (1.54 Å, CuK_α). The insert is the scattering from a concentrated (10%) solution of glycinin in standard buffer.

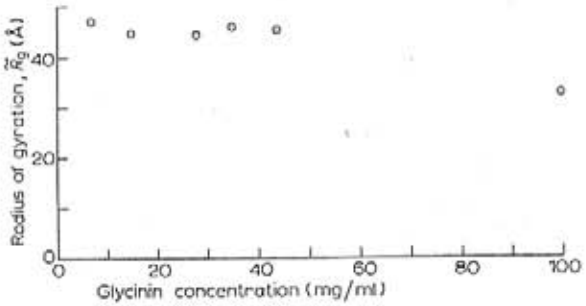


Fig. 5. Apparent Radius of gyration ($\bar{R}_g$) as a function of concentration $\bar{R}_g$ is in Å, concentration is in mg protein/ml.

is viewed along a diameter passing through subunit centres and in Fig. 2d the diameter passes between subunits. A significant proportion of images showing molecules standing on edge appeared to show 4 subunits in one of the rows rather than three.

The high-magnification pictures were used to measure the dimensions of glycinin and its subunits. The measurements are summarized in Fig. 3 which is a schematic representation of the glycinin (quaternary) structure as derived from the electron microscopy, X-ray scattering and other techniques (see discussion).

Small angle X-ray scattering measurements using solutions of glycinin in the concentration range 0.5–5%, yielded the characteristic scattering curve shown in Fig. 4. The inset of Fig. 4, shows the scattering obtained at higher angles ($h > 0.57 \cdot 10^{-1} \cdot \text{Å}^{-1}$) from a concentrated ($\approx 10\%$) solution of glycinin. This curve shows two well resolved subsidiary maxima at $h = 0.104$ Å^{-1} and 0.169 Å^{-1}.

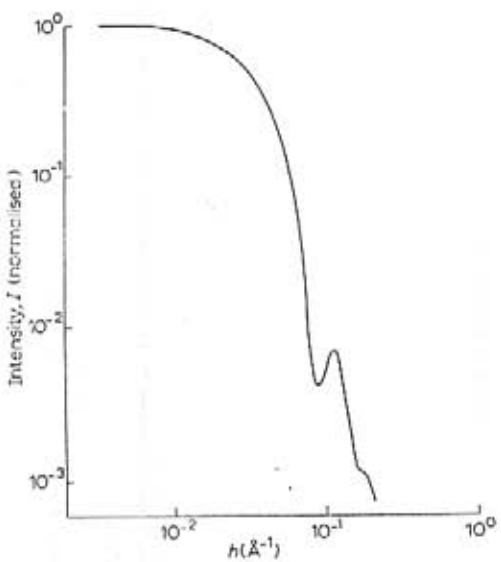


Fig. 6. Scattering curve for glycinin obtained by combining scattering measured in dilute solution with that measured at higher concentration, after removal of the effect of collimation geometry (desmearing).

The region of the curves at angles corresponding to $h < 0.25 \cdot 10^{-1}$ Å^{-1} was analysed according to the treatment of Guinier [32] to give the apparent radius of gyration [$\bar{R}_g$] of the scattering particle. The extrapolation of the radius of gyration to "infinite dilution" is shown in Fig. 5. As can be seen from this figure the effects of interparticle correlations which normally artificially reduce the value of the radius of gyration as the concentration increases are extremely small in the case of glycinin.

The scattering curve obtained from a 10% solution of glycinin was combined with the curve characteristic of the more dilute samples and the composite scattering curve desmeared. The result is shown in Fig. 6. This curve which represents the scattering from an isolated glycinin particle yields a value of 44 Å for the radius of gyration.

Utilising the results of electron microscopy and the data derived from the chemical analyses we have attempted to derive more detailed information on the gross morphology and the geometrical arrangement of the subunits of glycinin.

In Fig. 7 the experimental scattering curve is compared with the shape scattering curves calculated for oblate hollow cylinders. This model, representing a low

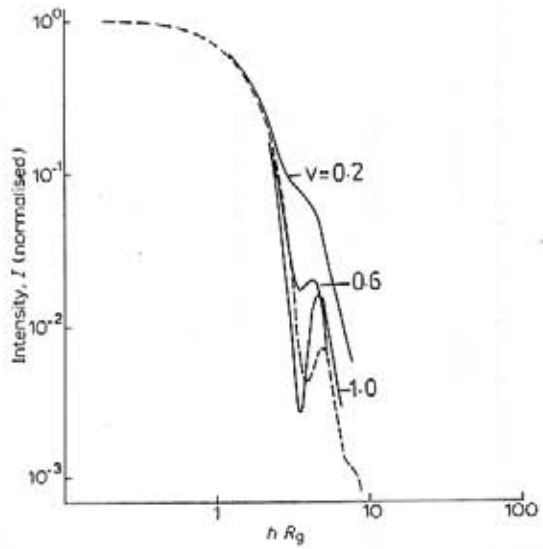


Fig. 7. Experimental scattering curve from glycinin (dashed line) compared with the scattering calculated for a simple oblate hollow cylinder; ratio of inner to outer radius, $r_1 : r_2$ is 0.3, ν = axial ratio $H/_{2r_2}$. The abscissa is the dimensionless variable hR_g and the intensities have been normalized [$I(0) = 1$].

resolution view of the morphology observed by electron microscopy ignoring the subunit structure, gives reasonable agreement for $hR_g < 2.5$ between the experimental curve and that for a cylinder of axial ratio 0.7, inner radius to outer radius 0.3. These values for the model parameters are in good agreement with those derived from the overall dimensions determined by electron microscopy. At higher angles, in the region of the subsidiary maxima, the experimental scattering curve does not agree with the curves calculated for these simple models.

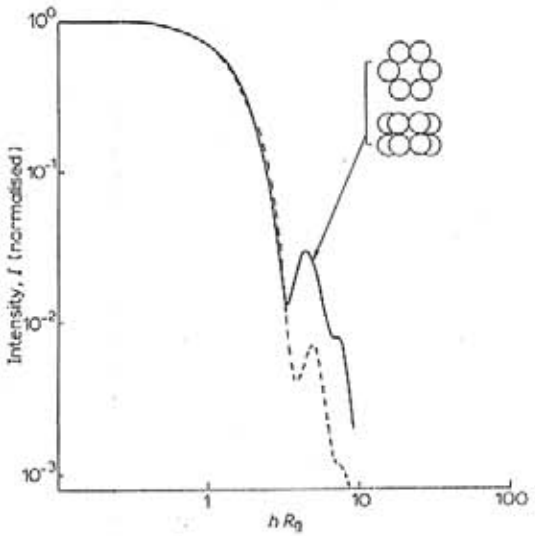


Fig. 8. Experimental scattering curve from glycinin (dashed line) compared with scattering calculated for model composed of 12 subunits in hexagonal packing (see text for details).

Fig. 8 shows the experimental scattering curve compared with that calculated for a particle composed of 12 identical subunits arranged in a structure consisting of two hexagonal rings. Although the scattering curve for this model exhibits subsidiary maxima at the same positions and of the same overall form as those observed experimentally, the calculated and experimental curves do not agree in the relative intensities of the subsidiary maxima. Calculations based on similar models with ellipsoidal rather than spherical subunits show a complex behaviour of the position and intensity of the subsidiary maxima. Thus without prior information regarding the shape of the individual subunits, which might be expected to be considerably more complex than simple spheres or ellipsoids and also non-identical, we have not attempted a further refinement of the model scattering curves.

DISCUSSION

One of the reasons for performing the present study was the finding that glycinin prepared by several published procedures [2–4] was shown not to be homogeneous under certain conditions. The dissociation is most readily observed when samples are run on polyacrylamide gels in a conventional Tris-glycine buffer of ≈ 0.04 M. Then, material, homogeneous by ultracentrifugation, immunochemistry and gel filtration in standard buffer and isoelectric focusing in water, shows a faster running component which is the half molecule of glycinin [5]. In fact the dissociation of the protein at low ionic strength has been considered a property of the native molecule [1, 5]. Since it is possible to prepare glycinin which does not show such behaviour but in which it can be induced by various treatments (this will be discussed elsewhere) it is felt it is not a property of the native protein. The preparative procedure described allows the removal of the offending fraction together with γ-conglycinin since both polymerize in 1 M KH_2PO_4, pH 7.6. Use of this solvent allows easy and rapid separation of the various fractions without relying on either lengthy [2, 4] or difficult [3] column procedures. The present preparation can be seen to be very similar in many of its properties to those already referred to. Its characterization is summarized in Tables I and II.

The nitrogen content and partial specific volume are in quite good agreement with a previous result [8] and a previous calculation [4]. The extinction coefficient is particularly sensitive to trace quantities of yellow coloured, low molecular weight compounds which extract with the protein. The value of 8.1 agrees well with that of Koshiyama [4] but is lower than other values [8]. The amino acid composition is similar to that reported by Catsimpoolas [33] except for serine, glutamate, valine and methionine. There are at least four different types of polypeptide chain present. The distribution of the 12 N-terminals found differs from a previous report [3] although Okubo et al. [34] do indicate that the acidic subunit fraction contains no N-terminal glycine. They and others [3] do not indicate that both leucine and isoleucine appear as N-terminals in this fraction. The reason is thought to lie in a lack of resolution for leucine and isoleucine in the methods used. Incomplete recovery of N-terminals, which experimentally seems to yield higher ratios of glycine to the remaining residues may explain the different ratios reported [3]. Urea gel electrophoresis [35] and isoelectric focusing [6] suggest that there are in fact three different kinds of basic subunits which means, therefore, six different polypeptide chains in all. Previous estimates [4, 8] of the sedimentation coefficient agree well with the present work. The diffusion constant, determined ultracentrifugally, has not been reported

TABLE II

AMINO ACID COMPOSITION OF GLYCIN

Residue	g/100 g protein*	Gram residues/320 000 g protein
Asp	13.10	365
Thr	3.37	107
Ser	4.16	153
Glu	18.03	447
Pro	5.40	178
Gly	3.97	223
Ala	3.55	160
Val	5.08	165
Ile	4.69	133
Leu	7.17	203
Tyr	4.05	80
Phe	5.73	124
His	2.22	52
Lys	4.88	122
Arg	7.75	159
½ Cys	1.44	42
Met	1.84	45
NH_2	2.86	541
Trp**	—	27

* Corrected data time course hydrolysate for four samples at 24, 41, 48 and 72 h.
** Value obtained spectrophotometrically.

previously. The molecular weight of glycinin has been determined by several unrelated methods. Table I contains the results. The agreement between the values obtained from the combined sedimentation-diffusion method and from the sedimentation-equilibrium method is excellent. The other three techniques which are not expected to be quite as precise agree within the estimated experimental errors. Earlier estimates [8] by sedimentation equilibrium and light scattering, yielded molecular weights for glycinin higher than in the present study, 363 000 and 345 000, respectively. Both methods are rather sensitive to aggregated material. However Koshiyama [4] obtained values close to those reported here but was not able to use experimental values for diffusion constant and partial specific volume.

The probable arrangement of the subunits in the native 11S glycinin molecule has been deduced from the X-ray scattering and electron microscope studies together with the molecular weight and subunit information.

The axial ratio determined by small angle X-ray scattering (0.67) and substantiated by electron microscopy is quantitatively consistent with a structure consisting of two hexagonal rings of subunits. Such structures have been speculated upon previously and preliminary electron micrographs indicate ring structures are present [6, 7]. The subsidiary maxima observed in the X-ray scattering curve may occur for two reasons. Either they reflect the symmetry of the scattering particle in the shape scattering function or they are due to pronounced correlations between subunits within the scattering particle. The positional agreement and the qualitative agreement in shape between the subsidiary maxima in the experimental curve and the maxima in the curve calculated for the hexagonal structure based on spherical subunits demonstrates that this model is an adequate description of the intersubunit distances. However, the lack of absolute agreement in the relative intensities illustrates that the assumption of an identical spherical shape for the subunits is an over-simplification. Making this assumption, however, and using the value $R_g = 44$ Å we calculate a value of 18.6 Å for the average radius (R) of the subunits. This value in turn gives the overall dimensions shown in Table I. Assuming the molecular weight of the subunits to be the average of those determined for the acidic and basic subunits (27 500) and using the value 0.73 ml/g determined for the partial specific volume we calculate the average subunit radius to be 20 Å, in excellent agreement with that derived by X-ray techniques.

Calculations of the scattering from subunit structures based on 10 (pentagonal arrangement) and 14 subunits (hexagonal arrangement with additional subunits occupying positions at the centre of the hexagons) indicate a complete quantitative and qualitative disagreement with the experimental scattering.

The interpretation of electron micrographs in structural terms is well known to present difficulties. The quality of the information so derived can be enhanced by parallel studies using other methods. The micrographs shown in Fig. 2 are thought to give a reliable picture of the native molecule in view of the excellent agreement about the overall shape of glycinin, in terms of diameter and axial ratio, as described in Table I.

The choice of images for the superpositioning technique presented some problems. Within a single field of view many different orientations of the glycinin are present. Only those molecules with long axes parallel or perpendicular to the optical axis of the microscope were likely to be useful and hence these images were selected. The presence of a significant number of apparently pentagonal images is thought to illustrate the orientation problem. Three dimensional models suggest that only a slight tilt of a top view of glycinin is necessary to produce such an image. Furthermore, superpositioning of these views shows that they possess hexagonal symmetry. The side views of glycinin (Figs 2c and 2d) illustrate 2 different orientations of the molecule. In 2d the viewing direction is along a diameter passing through subunits whilst in 2c it passes in between.

In Figs 2b to 2d the negative stain appears to penetrate between the two layers of subunits but not between the subunits of a single hexagon. The stain can interact with hydrophilic areas, such as the outside of a protein, and the dark area between the two layers is thought to be such a region. A layer of ≈ 5 Å thickness around each hexagon is suggested and this has been taken into account in estimating the dimensions shown in Table I. The agreement with the X-ray scattering results and comparison of the molecular weight derived from such dimensions with the values from ultracentrifugation support this idea. The presence of hydrophilic groups between the two protein layers suggests that they are held together by electrostatic and/or hydrogen bonding forces. In contrast, the lack of stain between the subunits within a layer suggests mainly hydrophobic forces are involved. The ability of glycinin, after very mild treatments, to undergo an ionic strength dependent cleavage between the two layers which does not affect intersubunit bonds within a layer supports this assignment. The molecular weights of the subunits indicate that their expected diameters are too similar to allow differentiation in the micrographs. Therefore we can say nothing directly about the relative arrangement of the acidic and basic subunits. However, their amino acid compositions [30] show the basic subunits to be the more hydrophobic. This appears to be manifested in their preferential precipitation during heat denaturation. Thus an alternating arrangement of acidic and basic subunits [6] around each ring is thought to be the most likely geometry but the stability being primarily due to hydrophobic rather than ionic interactions.

The electron micrographs of glutamine synthetase by Valentine [31] show a clearly stained hole in the middle of a hexagonal ring. Although the diameter of glycinin is less (100 Å compared to 140 Å) such a feature might be expected to be visible here also. The micrographs indicate that this is not the case since many of those images showing a stained centre are not hexagons. Together with X-ray scattering curves this observation suggests that some or all of the glycinin subunits are not spherical thereby resulting in the blocking of part of the expected central hole.

The structure of 11S glycinin, incorporating the features discussed above, is illustrated in Fig. 3.

NOTE ADDED IN PROOF

Preliminary results indicate the presence of one or more disulphide links between pairs of acidic and basic subunits within each ring. This is the first direct evidence for the alternating arrangement proposed by ourselves and others [6, 7].

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STUDIES ON THE SUBUNITS OF MYOSIN FROM MUSCLE LAYER OF *ASCARIS LUMBRICOIDES SUUM*

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SUMMARY

1. A purified preparation of *Ascaris* myosin was obtained from the muscle layer of *Ascaris lumbricoides suum*, using gel filtration and ion-exchange chromatography.

2. *Ascaris* myosin whether purified or unpurified, had almost the same ability for ATP-splitting and superprecipitation.

3. *Ascaris* myosin and rabbit skeletal myosin were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. A significant difference in the number of light chains between both myosins was found. *Ascaris* myosin was found to have one heavy chain and two distinct light chain components (LC_{1-A} and LC_{2-A}), having molecular weights of 18 000 and 16 000, respectively. These light chains correspond in molecular weight to the light chain 2 (LC_{2-S}) and light chain 3 (LC_{3-S}) in rabbit skeletal myosin.

4. LC_{1-A} could be liberated from the *Ascaris* myosin molecule reacted with 5,5′-dithio-bis(2-nitrobenzoic acid) (Nbs_2) with recovery of ATPase activity by addition of dithiothreitol.

These properties are equivalent to those of the LC_{2-S} in rabbit skeletal myosin, although *Ascaris* myosin when treated with Nbs_2-urea lost its ATPase activity.

INTRODUCTION

The present authors reported in the previous paper [1] that *Ascaris* myosin was prepared from muscle layer of *Ascaris lumbricoides suum*, a swine intestinal nematode, and that some properties of *Ascaris* myosin differed from those of rabbit skeletal myosin. The ATPase activities were 3 to 4 times lower than those of rabbit skeletal myosin. The maximum level of superprecipitation was about 4 times higher

Abbreviations: *Ascaris* myosin, myosin A from muscle layer of *Ascaris lumbricoides suum*; LC_{1-A} and LC_{2-A}, light chains of *Ascaris* myosin in increasing order of electrophoretic mobility; LC_{1-S}, LC_{2-S} and LC_{3-S}, light chains of rabbit skeletal myosin in increasing order of electrophoretic mobility; Nbs_2, 5,5′,dithio-bis(2-nitrobenzoic acid).