mycelium from 24 h, reaching a maximum at 3–4 days then declining by day seven. Electron micrographs of the mycelium at later times was complicated by the state of the mycelium, but few granules could be seen. The granules, which were 100–400 Å in diameter, appeared to be polysaccharide on the basis of periodic acid–thiocarbohydrazide–silver proteinate staining [2]. Large amounts of lipid material were also present.

Glycogen was extracted from mycelial samples of *A. niger* taken at daily intervals and estimated colorimetrically. The glycogen content of the mycelium increased up to the sixth day, then declined. The amount of glycogen measured suggests that the polysaccharide granules were glycogen.

Glycogen synthetase activity was determined by measuring the amount of uridine diphosphate (UDP) formed from UDP glucose in the presence of glycogen and glucose 6-phosphate. UDP was estimated by the colorimetric determination of the pyruvate produced from phosphoenolpyruvate in the pyruvate kinase reaction. Glycogen synthetase activity was detected in all samples, reaching an apparent peak of activity on day seven, then declining until it returned the initial level on day ten (Table 1). The glycogen phosphorylase level was measured by the release of inorganic phosphate from glucose 1-phosphate when glycogen was synthesized. Phosphate was determined colorimetrically. Phosphorylase activity appeared to increase with time, although not linearly, with the greatest increase in activity on days nine and ten.

The observed levels of the two enzymes were broadly consistent with the levels of glycogen observed at different times. Assuming that the synthetase is the major synthetic enzyme and that breakdown is due to phosphorylase, then the pattern of increasing synthetase activity over the first 7 days followed by a fall, with the level of phosphorylase rising in the latter stages would explain the observed glycogen levels. The late increase in phosphorylase activity coincides with the depletion of the carbon sources in the medium, and this is reflected in a lower internal concentration of glucose 6-phosphate. Glycogen synthesis in yeast depends in part on a glucose-6-phosphate-dependent enzyme, while the two forms of phosphorylase are both inhibited by glucose 6-phosphate [3]. The observations made in *A. niger* are consistent with such a mechanism.

Table 1. *Glycogen content and related enzyme activity*

Glycogen contents are given as μg/g wet weight. Enzyme activities were measured in crude extracts prepared by bead milling 1 g wet weight mycelium with 2 ml of Tris buffer, pH 7.4.

Day	Glycogen content (μg/g)	Glycogen synthetase (units)	Glycogen phosphorylase (units)
2	200	0.4	0.2
3	1600	0.42	0.45
4	3500	0.44	0.46
5	4000	0.50	0.36
6	5400	0.64	0.70
7	3000	0.98	0.56
8	3000	0.78	0.60
9	2000	0.52	1.27
10	2000	0.40	0.93

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Interactions of a model block copolymer drug delivery system with two serum proteins and myoglobin

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It is generally recognized that block copolymers prepared in dilute solution in selective solvents undergo a reversible association to form micelles [1]. Many of these block copolymers form micelles in aqueous solvents [2]. Consequently, it is not inconceivable that block copolymers have the potential to be engineered for use as carrier systems for targeting drugs. A previous study determined the hydrodynamic properties of a three-block copolymer poly(oxyethylene/isoprene/oxyethylene) utilizing analytical ultracentrifugation as the principal technique [3]. The aim of the present study was to investigate the possible (non-favourable) interactions of two plasma proteins (human serum albumin, haemoglobin) and myoglobin with this particular block copolymer.

Sedimentation velocity experiments (Fig. 1) were performed using an MSE Centriscan 75 analytical ultracentrifuge, employing both scanning absorption and schlieren optics. All (apparent) sedimentation coefficients ($s_{20,w}$) were determined at 20.0°C and rotor speeds of ~45 000 rev./min. Standard MSE 10 mm pathlength single-sector cells were employed and loaded into a multi-hole rotor (to ensure identical run conditions). [By comparison of the resulting absorption profiles (for 'single-species' controls and mixtures) an estimation of the extent of interaction is possible (see [4])]. The solvent used throughout these experiments was a phosphate/chloride buffer, pH 6.8, I 0.10. Mixtures of various concentrations of block copolymer and protein were investigated, to establish any influence concentration may have upon interaction.

The following strategy was used: (i) the formation of any aggregate was monitored by following the sedimentation of any possible turbidity at low speeds; (ii) the presence of micelles in the mixture cell was determined and consistency with the control cell species was ensured; (iii) the heights of the absorption profiles were compared and sedimentation coefficients for the protein in both mixture and control cell were calculated.

We observed: (i) no detectable turbidity in each case (hence no apparent supramolecular aggregate formation); (ii)

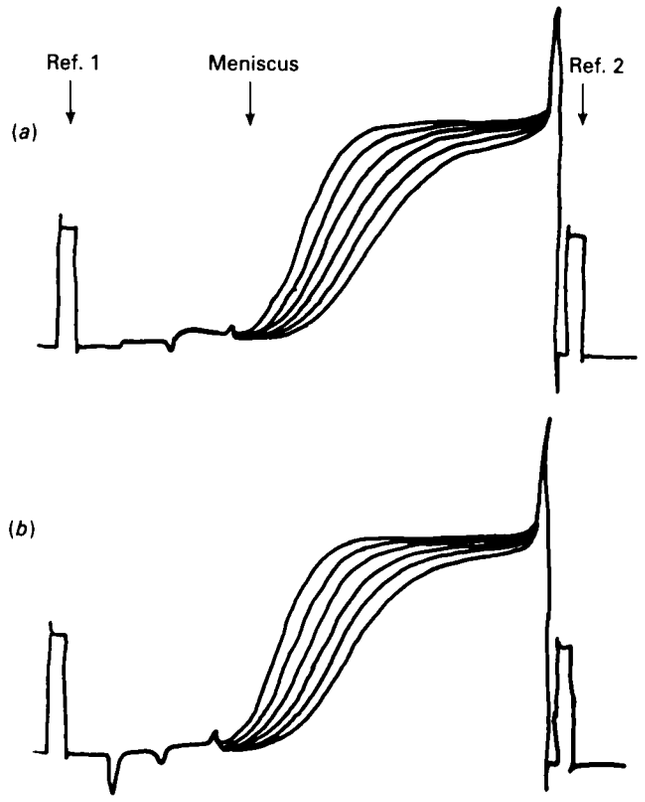

Fig. 1. *Scanning absorption profiles from a sedimentation velocity experiment on the MSE Centriscan*

Rotor speed 45000 rev./min, temperature 20.0°C. Interval between scans 7 min and sedimentation from left to right. (*a*) Haemoglobin control (concentration 2.0 mg/ml). (*b*) Haemoglobin–copolymer mixture (haemoglobin concentration 2.0 mg/ml and block copolymer concentration 2.0 mg/ml). Scan wavelength in both cases was 280 nm.

comparable quantities of micelles were present in control and mixture cells (as judged from the form of schlieren boundaries, notwithstanding Johnston–Ogston [5] effects; (iii) the apparent sedimentation coefficients and profile heights of the proteins in the mixture and control cells were similar, within the limits of experimental error.

If there had been any appreciable interaction between protein and micelle, one would expect a reduction in the concentration of protein sedimenting in the mixture cell, reflected in a reduction of the height of the absorption profiles: these phenomena were not evident. Furthermore, there was no evidence of any species both absorbing at 280 nm and sedimenting with a rate comparable to that of the micelles. It would be unwise at this stage to interpret these observations in more quantitative terms, owing to the presence of different micellar forms and the Johnston–Ogston effects and related phenomena [5].

It would appear from these observations that there is no appreciable interaction of these plasma proteins with the micelles. The possibility cannot be excluded, however, of interaction between the block copolymer unimers and these proteins, which would arguably be of much less physiological significance. This together with an investigation of possible interactions of copolymers of this type with other proteins, particularly immunoglobulins, will be reported at a later date.

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Stimulation and inhibition of matrix metalloproteinase activities in articular cartilage

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The ability of interleukin-1 (IL-1) to stimulate cartilage matrix degradation is well established [1, 2], and one mechanism likely to be involved is an increase in the synthesis and release of the matrix-degrading metalloproteinases (MMPs) by chondrocytes [3, 4]. Non-steroidal anti-inflammatory drugs (NSAIDs) are clinically beneficial in inflammatory and degenerative joint diseases [5], although their influence on cartilage is poorly understood. The aim of this study was to determine the IL-1 and a NSAID (naproxen) dose and kinetic response of articular cartilage proteoglycan turnover and total MMP activities.

Adult bovine articular cartilage explants were maintained in serum-free conditions in the presence of human recombinant IL-1α at 0, 5 or 25 units/ml for 4 days, and in the presence of naproxen sodium at 0, 1, 10, 50 or 200 μg/ml. The media was changed daily and analysed to determine MMP activities and proteoglycan content. The explants were analysed to determine hydroxyproline and proteoglycan content. Collagenase was measured using [^{3}H]collagen (type II) substrate [6]. The acid and neutral MMP activities were determined using the [^{3}H]proteoglycan bead assay [7]. Gelatinase was measured using the Azocoll substrate (Calbiochem, La Jolla, U.S.A.). Tissue inhibitor of metalloproteinases (TIMP) was measured using an inhibition assay of rat uterine MMP [8]. All assays for MMPs measure latent and active forms and they do not detect the MMP–TIMP complexes.

IL-1 caused a dose- and time-dependent increase of proteoglycan release from the articular cartilage explants into the media, and a corresponding dose-dependent loss of proteoglycan from the cartilage matrix. The proportion of MMPs activities released into the media represented the majority of the total activities found in the whole explant culture (cartilage and media).

Stromelysin activity in the media was IL-1 dose and time dependent, and increased to 550% of control values with 25 units of IL-1/ml at day 4. Acid metalloproteinase activity was elevated at day 1 in a dose-dependent manner, up to 220% of controls, with 25 units of IL-1/ml. However, at subsequent times this was reduced, and by day 4 there was no significant elevation. Collagenase activity was significantly elevated at day 1 only with 25 units of IL-1/ml, to 160% of the control

Abbreviations used: IL-1, interleukin-1; MMP, matrix metalloproteinase; NSAID, non-steroidal anti-inflammatory drug; TIMP, tissue inhibitor of metalloproteinases.