



by chromatography on carboxymethylcellulose in 2 M urea/0.08 M glycine, pH 10.4 [6]. For circular dichroism (CD) and sedimentation measurements the protein was dialysed at 4°C against 0.1 M sodium phosphate buffer at pH 7.4, containing 0.2 M sodium chloride and 2 mM sodium azide.

Sedimentation equilibrium experiments and calculations were performed as outlined in [7]. CD spectra were recorded on a Jobin Yvon Mark III instrument using 0.10 or 0.50 mm path length quartz cells, a band pass of 2 nm, scanning at 0.05 nm/s with a time constant of 10 or 20 s. A mean residue  $M_r$  of 108.5 was assumed. Protein concentrations were measured spectrophotometrically using  $E_{1\text{ cm}}^{1\%} = 5.44$  at 280 nm.

Myristoyllysophosphatidylcholine (lyso PC, Sigma, St Louis, MO) was added as small aliquots of a 20 or 40 g/l solution in buffer.

### 3. RESULTS AND DISCUSSION

Sedimentation equilibrium studies of MBP have revealed a self-association equilibrium which was earlier analysed as a linear isodesmic association [6,7]. However, re-examination of the  $\Omega(r)$  analysis [8] of those data showed this function to be almost linearly dependent on the total protein concentration ( $\bar{c}$ ) at high  $\bar{c}$  and to be independent of concentration at low values of  $\bar{c}$ , a situation more typical of a two state monomer-polymer equilibrium [9] than of linear isodesmic self-association. To this end fig.1 presents analyses of the sedimentation equilibrium data for MBP in terms of the logarithmic form of the law of mass action for a two-state self-associating system ( $nP \rightleftharpoons P_n$ ): clearly the linearity of these plots signifies reasonable conformity with such a description, the value of  $n$  derived from the slope being  $6.1 \pm 0.2$  in the presence of lyso PC (fig.1, line b). Although the slope of line a in fig.1 provides a poorer definition of  $n$  ( $5 \pm 1$ ) in the absence of lyso PC, it seems highly likely that the same polymeric species is formed in the presence and absence of the detergent. On the basis that  $n = 6$  a plot of the hexamer concentration against the sixth power of the monomer concentration gave an association equilibrium constant of  $0.19 \pm 0.2 \text{ l}^5 \cdot \text{g}^{-5}$  for MBP in the presence of lyso PC, and a value several orders of magnitude lower in the absence of detergent.

Measurements of secondary structure using CD

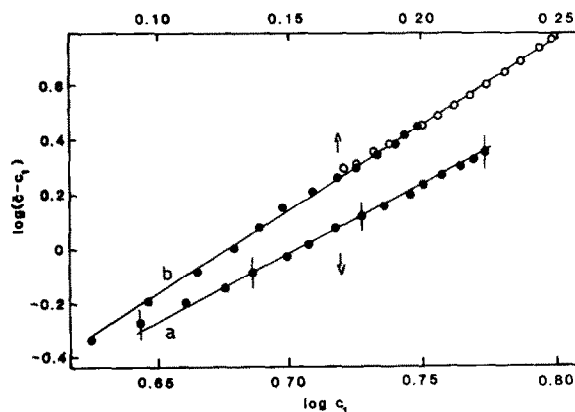


Fig.1. Total ( $\bar{c}$ ) and monomer ( $c_1$ ) MBP concentrations (in g/l) derived from sedimentation equilibrium data obtained in the absence (a) or presence (b) of lyso PC and plotted as the logarithmic form of the equilibrium equation for a two state monomer-polymer association. Lyso PC was added to give a 6:1 lipid:protein molar ratio. (●) Data from earlier studies, (○) data obtained here. In (a) the points represent average values and the range of results is indicated by the vertical bars: the uncertainty in the values on line (b) is comparable with the circle diameter. The arrows show the correct axes for both lines.

measurements generally do not require protein concentrations in excess of 1 g/l and typically concentrations of this order or lower have been used for MBP. At 1 g/l greater than 99% of the MBP is monomeric in the absence of lyso PC. Fig.2 (curve a) shows the mean residue ellipticity of the protein in the far ultraviolet region derived from several spectra recorded from solutions containing 0.3–0.9 g/l protein. It follows closely those recorded earlier in other solvents, having qualitatively the form expected for a protein devoid of regular secondary structures such as the  $\alpha$ -helix or  $\beta$ -pleated sheet though, as noted earlier [10], the intensity of the trough near 200 nm is lower than that deduced for other unordered proteins.

To observe the spectrum of the hexamer two approaches were followed: solutions containing considerably higher protein concentrations were used, and in other measurements lyso PC was added. At concentrations near 6 g/l in the absence of lipid, about 15% of the protein is present as hexamer. The resultant spectrum (fig.2, curve b) differs markedly from that of the pure monomer: the large negative peak near 200 nm is replaced by a

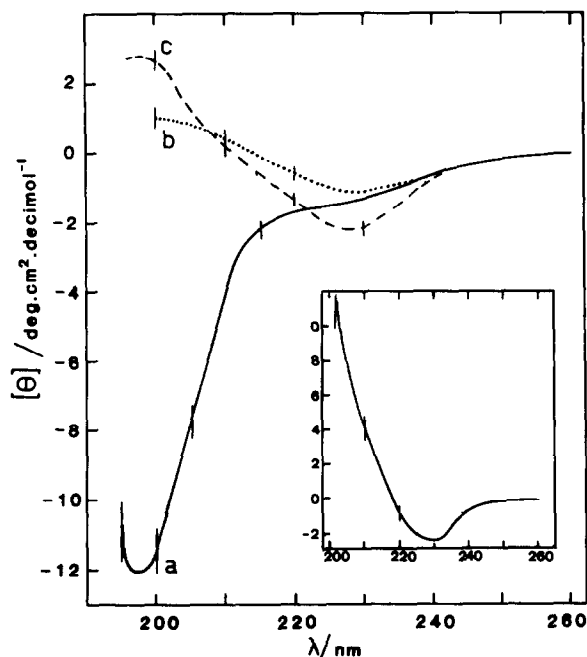


Fig.2. Circular dichroism spectra of MBP at 20°C. (a) Spectrum for MBP at concentrations below 1 g/l. (b) MBP at 5.8 g/l. (c) 3.7 g/l MBP to which lyso PC had been added to a lipid:protein molar ratio of 6:1. The inset shows the CD spectrum of hexameric MBP deduced from curves (a) and (c) (see text). The vertical bars show the range of results from several experiments.

small positive peak, and a small trough appears at 230 nm. Identical spectra were obtained with the post-translationally modified forms of the protein obtained by taking the penultimate and antepenultimate peaks eluted from the carboxymethyl-cellulose column [11] rather than the final peak containing the unmodified form of MBP used in most of the experiments. The magnitude of the changes, particularly around 200–210 nm, indicates that the hexamer spectrum differs substantially from the monomer spectrum, but the former could not be reliably deduced from these results, as the hexamer is still a minor constituent.

Addition of lyso PC at a 6:1 lipid:protein molar ratio promotes self-association [7] so that at 1 g/l about 10% is present as hexamer compared with less than 1% in the absence of this zwitterionic amphiphile. This shift in the equilibrium leads also to the adoption of an ordered structure at lower concentrations. The spectrum (curve c) in fig.2 was derived from solutions containing 3.7 g/l protein;

at this concentration only 40% of the protein remains monomeric. Compared with the lipid-free spectrum it shows increased intensity of both the trough at 230 nm and the peak at 195 nm.

The folding of the protein appears to be rapid and reversible. Following addition of a 6-fold molar excess of lyso PC to a 5.7 g/l protein solution the ellipticity at 210 nm reached its equilibrium value within 30 s, the time taken to mix the solutions and place them in the spectrometer. Dilution of a 14 g/l solution not containing lipid to 1.0 g/l caused a similarly rapid decrease in the ellipticity at 205 nm to the value expected for the monomer.

The spectrum of the hexamer was deduced by assuming that the spectrum obtained in the presence of lyso PC (fig.2, curve c) was the sum of the spectra of the monomer (fig.2, curve a) and of the hexamer added in the appropriate ratio (40:60, see above). The result of this spectral deconvolution is shown in the inset to fig.2 which has the same general form as fig.2, curves b and c, but with a substantially larger peak near 200 nm. The form of the MBP spectrum is close to that exhibited by proteins in a  $\beta$ -pleated sheet conformation; it does not have the double minima near 209 and 222 nm that characterize  $\alpha$ -helical proteins. But the trough is red-shifted 10–13 nm, and is of lower intensity relative to the 200 nm peak in comparison with the spectra of  $\beta$ -structured synthetic polypeptides [12] and the  $\beta$ -spectrum deduced from CD studies of globular proteins [13]. However, the spectra deduced for  $\beta$ -sheets from observations on proteins of known three-dimensional structure have been quite variable, indicating a sensitivity to minor changes in conformation. Immunoglobulin molecules, which have a predominantly anti-parallel pleated-sheet conformation within each domain, also have a very low intensity trough which is centered around 217 nm, but appears at 225–230 nm in some immunoglobulin fragments [14].

Thus, MBP appears to exist in solution as an equilibrium mixture of relatively unstructured monomers and hexamers in which the protein molecules have an ordered conformation. In this respect it parallels the smaller polypeptides melittin [15], a venom polypeptide, and glucagon [16], which adopt a largely  $\alpha$ -helical conformation on aggregation. These equilibria serve to emphasize the relatively small free energy changes that ac-

company the unfolding of complex polypeptides [17]. For myelin basic protein the equilibrium is perhaps shifted in favour of the unfolded monomer by the reduction in intramolecular electrostatic repulsions that would accompany such a conformational transition and the absence of intramolecular disulphide bonds or a prosthetic group. Although the conformation of the hexamer formed from the acid-extracted protein may differ from that of MBP within myelin there is evidence that protein not subjected to acid extraction undergoes a similar structural transition. We have found that prolonged washing of myelin with 0.5–1.0 M sodium chloride releases some MBP, which at low concentrations has a CD spectrum equivalent to that in fig.2 (curve a). Also, after completion of this work Riccio et al. [18] reported isolation of MBP from myelin using a non-ionic detergent and commented that its CD spectra indicated a  $\beta$ -pleated sheet secondary structure. Formation of  $\beta$ -structure by MBP has also recently been theoretically predicted [19].

Although MBP may be synthesized primarily on free ribosomes [20] as an unstructured molecule, it is likely that on binding to myelin it adopts a highly ordered structure. Possession of an ordered structure strengthens the possibility of highly specific interactions with a range of other molecules: the folded structure would provide the three-dimensionally structured binding sites that characteristically participate in important biochemical interactions.

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