control experiments showed an extensive binding to human serum albumin.

Haemopexin is a glycoprotein, its sugar component representing ~20% of its molecular weight[16]. The generally low reactivity of its functional groups[9,12] and high solubility suggest that the molecular surface is shielded by sugar chains and that the compact hydrophobic peptide core is not in contact with the solvent. Nevertheless, some hydrophobic groups of native haemopexin can be affected by chemical agents, e.g. three tryptophan residues[9] and especially a large area of the haem-binding site. From this point of view it seems worthwhile to discuss the inability of haemopexin to interact with small molecules of hydrophobic fluorescent probes.

Morgan and coworkers[1,2] have studied the interaction of various porphyrins with rabbit haemopexin. It was found that all the porphyrins studied were bound to the same binding site; their binding constants depended on the nature of the central metal atom as well as on the character of the side chains on the periphery of the porphyrin ring. A non-planar acyclic structure of bilirubin[17] is bound to the same site by a relatively weak bond ($K_D = 7.5 \times 10^{-7}$ M) similar to that of uroporphyrins[2]. Morgan *et al.* conclude[17] that the haem-binding site is sterically non-restrictive.

According to this concept haemopexin might be expected to bind any hydrophobic aromatic structure smaller than a porphyrin. However, the fluorescent probes we have used are not bound at all. This observation can be understood in the light of Koshland's induced-fit theory[18]. The extremely strong binding of haem, deuterohaem and mesohaem[1] by haemopexin clearly has a bearing upon their ability to adopt the protein conformation according to their structural requirements; this adaptation is shown by the rise of the 231 nm band in the circular dichroic spectra[1,6]. The more weakly bound structures (deuteroporphyrin, bilirubin)[1,17] are not able to induce this conformational change to a sufficient extent, so that their binding constants decrease by 2–3 orders. Finally, the ability of the small hydrophobic molecules to affect the protein conformation seems to be so poor that even the sensitive fluorescent technique fails to detect any interaction.

Conclusions

(a) The dependence of haemopexin tryptophyl fluorescence on iodide concentration was measured. It exhibited a two-step course with a distinct plateau. Fluorescence intensity of the haem–haemopexin complex is decreased due to the presence of the haem group but the shape of the quenching curve is similar to the apohaemopexin.

(b) The possibility of tryptophan participation in the haem-binding site architecture is discussed. The direct interaction of indole group with haem seems to be unlikely.

(c) An attempt was made to bind three types of fluorescence probe, anionic, cationic and neutral; no interaction was detected either with haemopexin or its haem complex.

(d) An effect of haem binding on the conformation of the peptide part of the haemopexin molecule can be understood in the light of Koshland's induced-fit theory.

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Could egg albumin be egg shaped?

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Ovalbumin is a glycoprotein found in abundance in egg white. Its function is suggested to be that of a protease inhibitor[1] and its polypeptide chain, of molecular weight 42 699, has now been sequenced[1]. Although it has been extensively examined physiochemically (*Table 1*) workers have generally found it difficult to comment on its gross conformation, either because of problems of hydration or the insensitivity of the hydrodynamic functions previously used to axial ratio (or equivalently high sensitivity to experimental error). For example, the recent analysis of Jeffrey *et al.*[2] could only predict the axial ratio (of the equivalent ellipsoid of revolution) to be between 5:1 and 1:1 for either a prolate model or an oblate model.

The ratio $k_s/[\eta]$ ($\equiv R$) has now been shown by Rowe[3] to be a sensitive function of the axial ratio, independent of any assumptions about the hydrodynamic volume other than it being the same in both the viscosity and sedimentation experiments. From *Table 1*, $R = 1.56$, corresponding to an axial ratio of 1.5, for both a prolate

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Table 1 Physicochemical data

	T(°C)	pH	Ref
s^a $(3.87 \pm 0.02) \times 10^{-13}$ s	25	6→7[b]	8,9
k_s^a 5.45 ml g^{-1}	25	6→7[b]	From 8 and 9
$[\eta]$ 3.49 ml g^{-1}	25	6.8	9
k_η 6.6 ml g^{-1}	25	6.8	From 9
U $(5.0 \pm 0.2) \times 10^5$ ml mol^{-1}	20	4.59 and 7.50	2
$\bar{v}$ 0.748 ml g^{-1}			2,10
M 45 000			2,11

s, Sedimentation coefficient (extrapolated to infinite dilution); k_s, sedimentation regression coefficient; $[\eta]$, intrinsic viscosity; k_η, viscosity regression coefficient; U, molecular covolume; M, molecular weight.

[a] Miller's original data[8] was corrected to 25°C and for radial dilution by Holt[9]. k_s has been further corrected to solution density according to a relation given by Rowe[3].

[b] The exact pH was apparently not specified.

and oblate ellipsoid model. Allowing for errors of ± 1% in the measured values of k_s and $[\eta]$, $R = 1.56 \pm 0.03$, corresponding to axial ratios of 1.5 ± 0.3 for both prolate and oblate models. This is much more precise than previous estimates[2]. Unfortunately, it is impossible to decide from the R function directly whether the prolate or the oblate ellipsoid is the better model, because of the inherent property in R that at least for axial ratios < 50, a given value of R does not uniquely define the geometry of the ellipsoid (viz. is it oblate or is is prolate?). The ratios $U\eta_0^3 N_A^2 s^3 / M^3 (1 - \bar{v}\rho_0)^3 \equiv \psi$ (Ref 2) and $U/[\eta]M \equiv \Pi$ (Refs 4 and 5), although far less sensitive to axial ratio and more sensitive to experimental error, do not suffer from this problem: values of $\psi < 5 \times 10^3$ and $\Pi < 3.2$ define a prolate ellipsoid and values of $\psi > 5 \times 10^3$ and $\Pi > 3.2$ define an oblate ellipsoid. For ovalbumin $\psi = (4.97 \pm 0.24) \times 10^3$ (Ref 2) and $\Pi = 3.18 \pm 0.20$. Although the mean values correspond to a prolate ellipsoid, because of the low asymmetry the error envelopes go well into the oblate range. This uncertainty still remains, even after comparison of model independent with model-dependent estimates for the swelling due to solvation $S_w = (\bar{v}_s/\bar{v})$, of the macromolecule in solution, were $\bar{v}_s$ is the swollen volume in ml g^{-1}. The model-dependent estimate is found by finding the Simha–Saito[6,7] function ν corresponding to the axial ratio found from R for each particular model and then substituting in the relation[4]:

$$\nu = \frac{[\eta]}{\bar{v}} \times \frac{1}{S_w}$$

A value for S_w independent of any assumed model can be found from the relation $S_w = k_\eta / k_s$ (Rowe, 1977)[3]. For ovalbumin, the model independent S_w is 1.2; however, for both prolate and oblate ellipsoids, the model dependent S_w is 1.5. We cannot conclude further than that ovalbumin is spheroidal (possibly prolate) of axial ratio 1.5 ± 0.3. If it is indeed prolate then it is interesting to note that the axial ratio of an average standard egg is about 1.5.

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Factors affecting the ethanol stability of bovine casein micelles: 3. Substitution of ethanol by other organic solvents

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Introduction

The interactions of organic solvents, including alcohols, with protein systems are many and complex. Of the forces that stabilize the unique folded structure of proteins in solution — hydrophobic interactions, hydrogen bonds and electrostatic forces — each is affected in some way by the addition of organic solvent to the solution. Such additions can lead to denaturation, inhibition of enzyme reactions or, in the case of skim-milk, breakdown of the stability of the micellar system and subsequent coagulation. Intensive research on casein micelle structure has failed to answer the question of what determines the stability of the micelle in milk. Centrifugation, heating, cooling or drying of milk apparently do not destroy micellar integrity[1]. Nevertheless, this stability does have limits with respect to environmental conditions. Among the reactions which can be used as sensitive indicators of micellar properties and probes of micellar stability are the coagulation of milk by chymosin, by heating at high temperature or by addition of alcohol, the subject of our present investigation.

In the last forty years, the ethanol stability of milk has been the subject of two comprehensive investigations[2,3]. Both of these concentrated on the properties and compositions of the milks giving rise to changes in the ethanol stability rather than on the mechanism of the coagulation process *per se*. More recently, Horne and Parker[4] have noted the effect of artificial adjustment of the pH on the ethanol stability of the milk enabling an ethanol stability/pH profile characteristic of that skim-milk to be obtained. Further investigations[5,6] into the factors affecting the shape and position of this pH profile

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