The interaction between bovine plasma albumin and dodecyltrimethylammonium bromide

Relatively few investigations have been concerned with the interaction of cationic detergents with bovine plasma albumin (BPA), although the fact that complexes are formed between this protein and cationic detergents has been reported in the literature[1,2,3]. In this communication, the interaction between BPA and a pure sample of dodecyltrimethylammonium bromide (DTAB) has been studied by the equilibrium dialysis technique and by viscosity measurements.

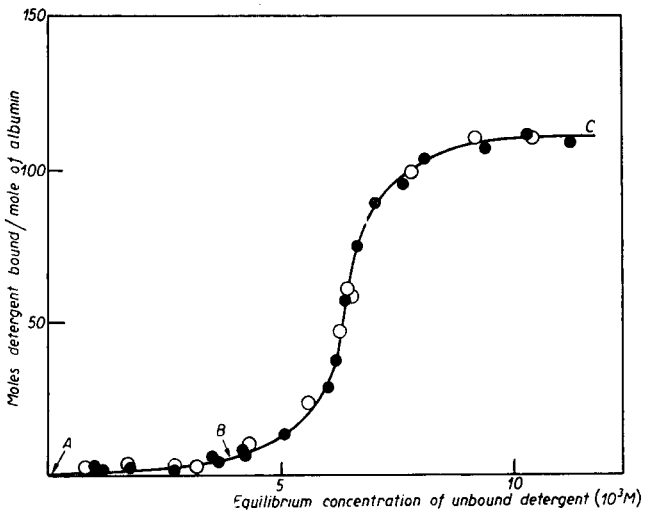

Fig. 1. Binding curve for the interaction of dodecyltrimethylammonium bromide with bovine plasma albumin (1 % w/v) in 0.1 *M* sodium chloride at 20°. Detergent estimated interferometrically (—●—) and spectrophotometrically (—○—).

Fig. 1 shows the interaction isotherm obtained for the binding of the detergent by the protein in the presence of 0.1 *M* sodium chloride. 5 ml of protein solution (1 % w/v) was contained in a dialysis sac, and equilibrated against 10 ml of detergent solution at 20° for three days. Control experiments on detergent solutions alone were also carried out to enable a correction to be applied, where necessary, for the unequal distribution of detergent ions[4] above the micelle point. The *unbound* detergent was estimated interferometrically (using $dn/dc = 0.155$ ml/g for the detergent at $\lambda = 5895$ Å) or by using a spectrophotometric dye method[5]. The curve shows that initially only a small fraction of the detergent is bound by the protein, in contrast to the case with the anionic detergent sodium dodecyl sulphate (SDS), where in the very early stages of this interaction 8–10 detergent ions are bound[6]. When the number of bound detergent ions reaches *ca.* 30 the curve becomes very nearly vertical, the protein thus exhibiting a much greater binding capacity for the detergent in this region. Eventually, when *ca.* 109 detergent ions are bound, saturation of the binding sites is obtained. This value corresponds closely to the number of free carboxyl groups (102) in the protein[7], and is consistent with the view that the detergent associates with the protein by electrostatic interaction of the cationic groups with the carboxyl groups of the protein.

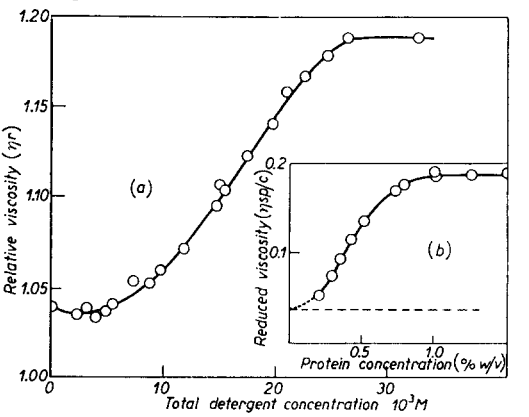

Fig. 2(a). Relative viscosity at 20° of bovine plasma albumin/detergent mixtures (constant protein concentration of 1 % w/v in 0.1 *M* sodium chloride) *vs.* total detergent concentration. Fig. 2(b). —○—, reduced viscosity of detergent/protein mixtures in 0.1 *M* sodium chloride at 20° (molar ratio 200/1) *vs.* protein concentration. – – – – reduced viscosity *vs.* protein concentration for native bovine plasma albumin, YANG AND FOSTER[8].

Measurements of the relative viscosity of BPA — DTAB mixtures in 0.1 *M* sodium chloride are recorded in Fig. 2(a), in which the protein concentration was kept constant at 1 % w/v and the DTAB concentration was varied. In the presence of low concentrations of detergent little change was observed in the relative viscosity. At higher detergent concentrations the relative viscosity increased rapidly to a value of 1.189 and then remained constant. It is clear from this curve that in the presence of the detergent the protein undergoes an expansion or partial unfolding, this process commencing at a detergent concentration of $5.2 \cdot 10^{-3}$ *M*. From the binding

curve (Fig. 1) it is evident that this change occurs when the number of bound ions exceeds *ca.* 6 per molecule of protein (point B).

Fig. 2(b) shows the curve of reduced viscosity $\eta sp./c$ (where c = protein concentration % w/v) against c, obtained by dilution of a stock solution having a concentration of 1.5 % protein and a molar detergent/protein ratio of 200/1. The reduced viscosity remains constant at 0.189 with decreasing protein concentration but below *ca.* 0.9 %, the reduced viscosity decreases and the curve approaches that obtained for native BPA (YANG AND FOSTER[8]). This indicates dissociation of the protein-detergent complex with decreasing protein concentration, a fact which has been confirmed by light scattering and sedimentation velocity experiments.

It appears from these experiments that DTAB combines less strongly with the protein than does the anionic detergent SDS. This is supported by the facts that less of the DTAB is bound at the same equilibrium molar concentration of detergent, and that the binding of DTAB is dependent upon the protein concentration, a situation which is not found in the BPA-SDS system[9] within a protein concentration range of 0.05 to 0.5 % w/v. However, a similarity between the two detergents is that both are able to act as a "molecular wedge", causing the protein to unfold partially and expose new sites for binding. Thus the interaction isotherm (Fig. 1) may be considered as two isotherms, AB the isotherm for the binding of DTAB onto the native protein (P_n), and BC the isotherm for the binding of DTAB onto partially unfolded protein molecules (P_o). The change from P_n to P_o is substantiated by the changes in relative viscosity which occur with increasing concentrations of DTAB (Fig. 2(a)). Further considerations of the reversibility of the process $P_n \rightleftharpoons P_o$, and other physical measurements on the BPA-DTAB system will be given in a later publication.

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The action of fluorodinitrobenzene on ichthylepidin

Ichthylepidin, a structural protein of fish scales first isolated by MÖRNER[1], is notable for its insolubility in the usual solvents for proteins in spite of a low cystine content[2].

In an attempt to determine the N-terminal amino-acid residues of ichthylepidin from scales of the pilchard (*Sardina ocellata* Jenyns) by SANGER's method[3], we have observed that the action of fluorodinitrobenzene (FDNB) in an aqueous-alcoholic solution of sodium bicarbonate brings about the dissolution of more than half the protein, whereas very little is dissolved in the same mixture without the FDNB, or when this is replaced by chlorodinitrobenzene or dinitrophenol. The experimental conditions were as follows:

A sample of scales (10 g) that had been purified by washing with water and Soxhlet extraction for 24 hours with hexane, was demineralised by soaking in a 0.4 *M* solution of trichloracetic acid (500 ml) for 6 hours at 5°. The protein was 44 % by weight of the original scales on an oven-dry basis. Ichthylepidin was then separated from the soluble gelatin by heating the demineralised scales (2 g) in water (100 ml) at 80° for 2 hours. The ichthylepidin was 23 % by weight of the original scales and heating it for another 2 hours in a fresh volume of water dissolved less than 2 % more protein.

To examine the action of FDNB, samples of ichthylepidin (each of 0.10 g of known moisture content) were added to mixtures containing 0.18 g of 1, 2, 4-fluorodinitrobenzene (from Light and Co. Ltd., Colnbrook, England), 0.10 g of sodium bicarbonate ("Analar" from British Drug Houses Ltd.), 2.0 ml of water, and 4.0 ml of ethyl alcohol. Not all of the bicarbonate dissolved under these con-