PROTEIN-INDUCED CONDUCTIVITY CHANGES IN BLACK LIPID MEMBRANES AND PROTEIN AGGREGATION

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1. Introduction

Solubilized membrane proteins, especially the 'integral' ones, show a strong tendency towards aggregation. Little is known of the consequences this behaviour might have in experiments on protein–lipid interactions. Recently, however, it was briefly reported that protein association has an influence on lipid binding of apoprotein A-1 from serum high-density lipoprotein [1]. It may be anticipated that similar effects might also occur with membrane apoproteins.

During the last several years, we have investigated the interactions of the 'strongly bound' protein fraction from human erythrocyte membranes with spherical and planar lipid bilayers. This protein fraction essentially consists of the two main integral proteins of the erythrocyte membrane: a protein of molecular weight 100 000 ('band 3-protein' [2] and 'glycophorin', the membrane sialoglycoprotein; the band 3-protein being the dominating component [3,4]. During our studies on the planar bilayers (black lipid membranes, BLM) we have found that the main peculiarity of the protein–BLM-system, the occurrence of large protein-induced changes in membrane conductivity [5,6], varies considerably with protein subfractions of different states of aggregation.

2. Materials and methods

2.1. *Materials*

The preparation of the strongly bound protein fraction from human erythrocyte membranes was performed as described earlier [3,4]. However, dialysis of the protein after gel-filtration in 90% acetic acid was done against 0.1 mM HCl instead of water. After dialysis, the protein was brought to pH 10.0 by addition of 0.1 M NaOH.

The lipid, phosphatidylserine (PS) from bovine spinal cord, was purchased from Lipid Products, South Nutfield (Surrey) England.

2.2. *Fractionation of the protein according to the state of aggregation*

Tubes of the Spinco rotor SW were filled with the following solutions (adjusted to pH 10.0), 0.6 ml sucrose (0.7 g/ml), 6.0 ml of a linear sucrose gradient (0.20–0.05 g/ml), 7.4 ml protein sample. The tubes were then centrifuged at 4°C for 20 h at 280 000 × g. Afterwards, the upper 9 ml (fraction A) and 1.2 ml from the bottom of the tube (fraction B) were collected, freed from sucrose by dialysis against 0.1 mM NaOH and used for the studies described below. Protein concentration was approx. 30 μg/ml in fraction A and 500 μg/ml in fraction B. For one series of measurements, fraction A was concentrated about 10-fold by ultrafiltration (Amicon cell model 52, filters PM 10).

2.3. *BLM measurements*

Black membranes were formed from a solution of 9 mg PS/ml in chloroform: n-decane (1:10) by the brush technique [7], across a hole (diameter 1.3 mm) in a teflon partition separating two compartments (V = 6 ml). The compartments were filled with 100 mM NaCl, 10 mM sodium phosphate (pH 7.1) to which up to 0.05 vol. of protein fraction A or B had been added. Buffer temperature was 28–29°C. Membrane conductivity was measured in a current clamp circuit using Ag–AgCl electrodes.

2.4. *Other methods*

Protein–liposome association, analytical ultracentrifugation and the determination of protein, phospholipid and neuraminic acid concentration were done as described earlier [3]. Sodium dodecyl sulphate (SDS)–gel electrophoresis was performed similar to Fairbanks et al. [2].

3. Results

3.1. *Chemical and physical properties of the two protein fractions*

After centrifugation of the protein at pH 10.0, approx. 20% of the protein applied to the centrifuge tubes was recovered in fraction A (rather homogeneously distributed over the volume), whereas fraction B contained about 60%. The chemical compositions of the two protein fractions were very similar to each other. With both fractions, the band patterns obtained by SDS–gel electrophoresis were practically indistinguishable, by staining for protein and for carbohydrates, from that of the unfractionated strongly bound proteins [4]. However, the ratio of neuraminic acid/protein and thus of sialoprotein/total protein was about 1.5-times higher in fraction A as compared to B. Similarity between A and B was also observed with respect to the phosphorus content (corresponding to 0.07–0.10 mg phospholipid/mg protein). The main difference between the two fractions was found in the *s*-values. Fraction A, in 0.1 mM NaOH or 1 mM glycine–NaOH (pH 10.0) plus 1 mM NaCl, showed a well-defined boundary with $s_{20} = 4.3$ (as would be expected for the monomer of the band 3-protein [8]. For fraction B, s_{20} was approx. 20, the boundary being sharp for some samples but broad, with a partly resolved fine structure, for others.

3.2. *BLM measurements*

Black PS membranes, in the absence of protein, were found to have specific conductivities λ of $1 \cdot 10^{-10}$–$5 \cdot 10^{-10}$ Ω^{-1} cm^{-2} [6]. However, when formed in the presence of protein fraction A (c = 1.0 μg/ml) λ increased to approx. $2 \cdot 10^{-7}$ Ω^{-1} cm^{-2}. On the other hand, the same concentration c of protein fraction B led to a membrane conductivity of only $2 \cdot 10^{-9}$ Ω^{-1} cm^{-2} (fig.1). This difference in the behaviour of the two protein fractions was confirmed at the other values of

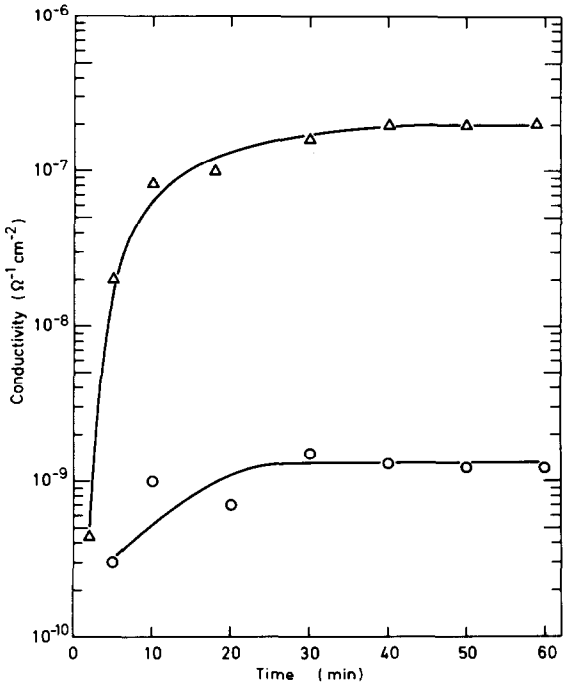

Fig.1. Typical time course of BLM conductivity in the presence of protein fraction A (△) and B (○). Protein concentration c = 1.0 μg/ml.

c tested: λ was always higher in the presence of fraction A and was proportional to the fourth power of c (as with the unfractionated protein. [6]) whereas λ was barely dependent on c with fraction B (fig.2).

When aggregation was induced in protein fraction A by concentration and subsequent incubation in salt solutions, yielding proteins with an average $s_{20,w}$-value of 16, a sharp decrease in the ability of the protein to increase BLM conductivity was observed (fig.2). Thus, the ability of protein fraction A to induce large changes in BLM conductivity is related to its low state of aggregation.

3.3. *Protein–liposome interactions*

In recombination experiments on protein fractions A and B and liposomes, performed at a protein/PS weight ratio in the starting mixture of 0.4, both protein fractions yielded lipoprotein aggregates of a protein/PS-ratio of approx. 0.5. In both types of recombinates, washing in 1.0 M NaCl did not lead to a decomposition of the aggregates. Thus, there is not marked differ-

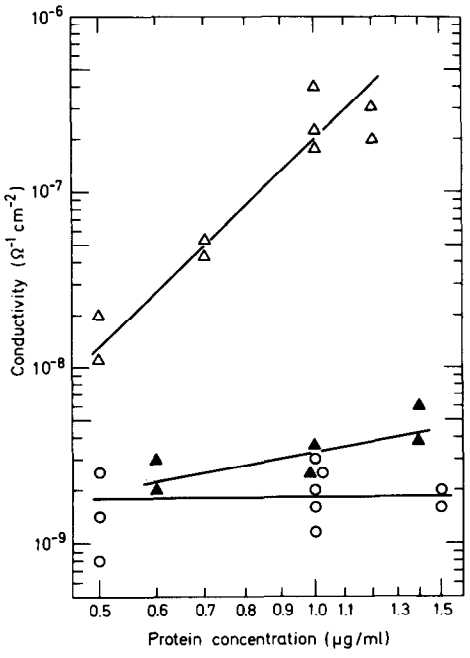

Fig.2. Double-logarithmic plot of final membrane conductivity (taken from the plateau region of the $\lambda = f(t)$-curves) as a function of protein concentration c. (△) Protein fraction A, (○) fraction B. (▲) λ-values obtained with fraction A after 10-fold concentration and subsequent dialysis against 100 mM NaCl, 10 mM sodium phosphate (pH 7.1).

ence in the ability of the two fractions to bind hydrophobically to lipid bilayers.

4. Discussion

In aqueous solutions, the strongly bound proteins from erythrocyte membranes show a broad distribution of aggregational states [3]. We have now shown that two subfractions of different states of aggregation display large differences in their interactions with black lipid membranes. The results of the chemical analyses of the two protein fractions, together with the dependency of the conductivity values on protein concentration (fig.2), suggest that these differences are not due to a different composition but are related to the differences in the aggregational state. This is strongly supported by experiments on protein fraction A where the aggregational state had been changed. We conclude that the state of protein aggregation can be a major parameter controlling protein–lipid interactions.

In studies on protein–lipid recombination, details of the experimental conditions often had a profound influence on the properties of the recombined lipoproteins, especially with respect to the reconstitution of structural or functional properties of the native membranes (e.g. [9,10]). We suppose that changes in the state of protein aggregation were a main factor of influence at least in some of these experiments.

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