INTERACTION OF ERYTHROCYTE APOPROTEIN WITH BIMOLECULAR LIPID MEMBRANES

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SUMMARY

Conductance and optical reflectance techniques have been used to study the interaction of erythrocyte apoprotein with erythrocyte lipid bilayers. It is found that addition of apoprotein increases the conductance and reduces the stability of the bilayer. Reflectance measurements demonstrate that the apoprotein forms a surface layer on the lipid membrane; the thickness of this layer is estimated to be about 13Å. This finding, together with infra red data, indicates that the protein is extended over the membrane surface rather than in a compact globular form.

The butanol - water partition procedure of Maddy[1] enables the major apoproteins† of erythrocyte membranes to be obtained in water-soluble form.[2,3] Zwaal and van Deenen[4] have developed a method of recombining the apoprotein with membrane lipid in an all aqueous medium. The recombinant has previously been studied using proton magnetic resonance[5] and electron spin resonance[6,7] methods.

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†i.e. lipid-free proteins.

The techniques for forming isolated bimolecular lipid membranes separating two aqueous phases are now well established.[8,9] Many functional properties of membranes have been induced in these artificial membranes by the addition of polypeptides and other small molecules.[9] However, relatively little progress has so far been made towards reconstituting lipid-protein membranes. Maddy, Huang and Thompson have observed an interaction between sialic acid-containing erythrocyte apoprotein and egg-lecithin bilayers.[10] Here we make a preliminary report of the interaction of sialic acid-free (s.a.f.) erythrocyte apoprotein with single bilayer membranes formed from erythrocyte lipids.

EXPERIMENTAL

Methods of preparing erythrocyte membranes, erythrocyte lipids and s.a.f. apoprotein were as previously described.[1,3,11,12] Phosphatidyl serine was supplied by Lipid Products and further purified on a silicic acid column by the method of Long, Shapiro and Staples.[13] Membranes were formed from solutions of lipid (20mg/ml) in n-decane (Koch Light Puriss). Electrical measurements were made in a cell similar to that of Mueller and Rudin.[8] Optical reflectance measurements were made as previously described.[14] The aqueous phase was either 0.05M acetate buffer p_H 4 or 0.05M phosphate buffer p_H 7.

RESULTS AND DISCUSSION

Bilayers formed from total membrane lipids in n-decane had specific resistances of greater than $10^8 \Omega$ cm.^{2} and were stable for at least several hours. When the aqueous phase was at p_H 4 addition of s.a.f. apoprotein after bilayer formation resulted in a marked increase in bilayer conductance. Changes in

conductance of up to three orders of magnitude were observed. However, the bilayer became both electrically and physically unstable in the presence of the apoprotein. While the conductance was increasing, large random fluctuations in current were observed and the bilayer eventually ruptured (Figure 1). As far as could be ascertained under these conditions, the current-voltage curve was linear at least up to 60mv in the presence of protein.

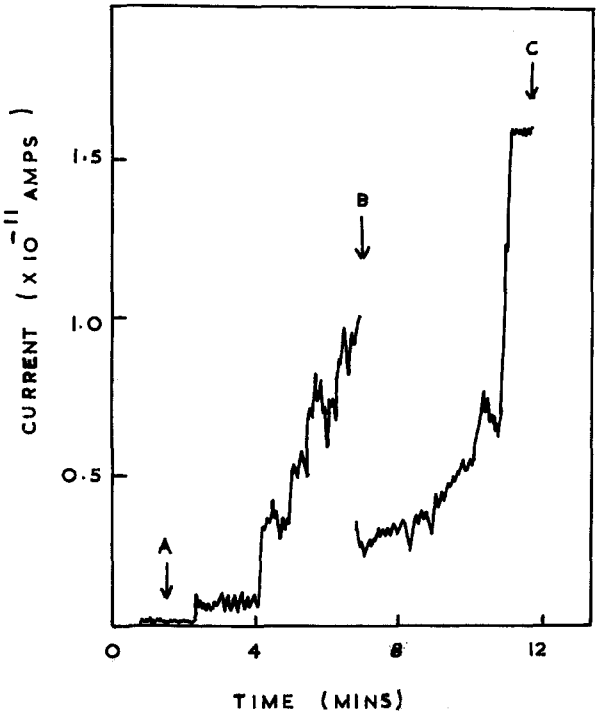

Figure 1. Conductance of bilayer after addition of s.a.f. apoprotein to aqueous phase. Bilayer formed from total erythrocyte lipids 20 mg/ml in n-decane Aqueous phase 0.05 M sodium acetate buffer pH 4. Applied potential 30 mV.

A. Addition of 0.02 mg/ml apoprotein to aqueous phase, stirring for 30 sec.

B. Current sensitivity decreased by 3X.

C. Bilayer broke.

The sensitivity of the bilayer to the protein was high, the effects being detectable with as little as 3μg/ml of protein in the aqueous phase. Varying the protein concentration mainly affected the rate at which the bilayer responded to the presence of protein. If sufficient protein was added to produce an observable conductance change, the bilayer always eventually ruptured. Typically the lifetime of the membrane was 5 - 15 min. in the presence of 10μg/ml of protein.

If the protein was added prior to bilayer formation different behaviour was observed. In this case the thick film thinned to a silvery state (probably ~ 1000Å thick) and then appeared to be indefinitely stable. In one experiment this silvery film still showed no sign of further thinning after a period of 18 hours had elapsed.

None of the above effects were observed when the aqueous phase was at p_H 7. At this p_H the membrane conductance, stability and rate of formation were unchanged in the presence of protein. When bilayers were formed from the single lipid, phosphatidyl serine, the effects of the protein were essentially the same as those observed with the total membrane lipids.

The interaction of the s.a.f. apoprotein with the lipid membrane was also studied optically. Figure 2 shows how the reflectance of the bilayer increases following the addition of protein to the aqueous phase. The increasing reflectance results from adsorption of the protein to the surface of the bilayer. As with the conductance effects the reflectance changes are observed at p_H 4 but not at p_H 7.

The rate at which protein adsorbs as measured optically is comparable with the time course of the conductance changes. However, unlike the conductance effects, the optical change

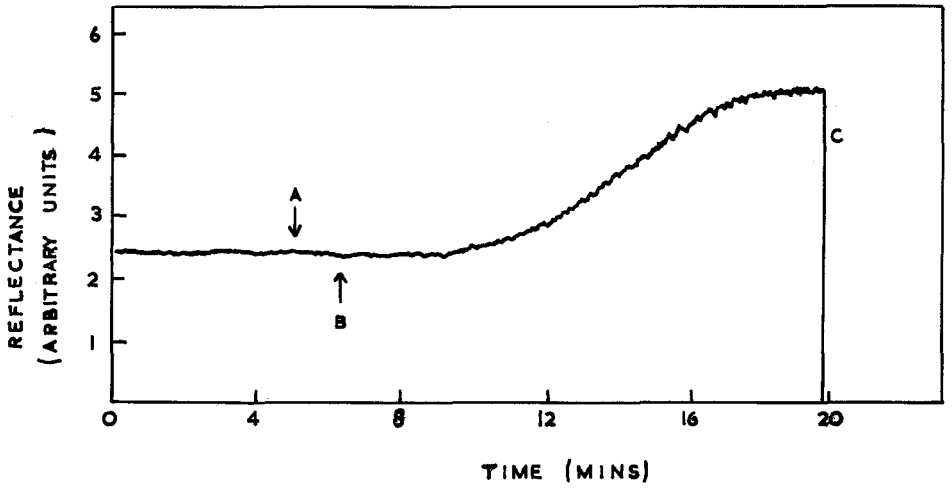

Figure 2. Reflectance of bilayer after addition of s.a.f. apoprotein to aqueous phase. Bilayer formed from total erythrocyte lipids 20 mg/ml in n-decane. Aqueous phase 0.05 M sodium acetate buffer p_H 4.

A. Addition of 0.1mg/ml apoprotein to aqueous phase.
B. Stirring for 30 sec.
C. Bilayer broke.

follows a smooth curve. Thus we may distinguish two processes, a steadily progressing surface adsorption followed by a secondary interaction which produces the observed conductance changes. From the p_H dependence, the initial interaction requires an electrostatic attraction between lipid and protein, this conclusion being in accord with previous bulk studies,[4-7] (previous ESR studies[7] were, however, somewhat ambiguous concerning the possibility of interaction at p_H 7, the present optical measurements clearly show that the protein does not adsorb to the membrane at this p_H.) Because the conductance changes are fairly large, it is most probable that the secondary interaction involves some disruption of the bilayer structure. This leads to instability and eventual rupture of the membrane.

Reflectance measurements in principle enable the total thickness of the membrane to be determined.[14-16] Because of the

instability of the system it was not possible to accurately determine the thickness of the lipid-protein membrane in the present case. Very often the bilayer broke before a steady value of reflectance was reached. In the few instances where a steady value was reached, the maximum increase in reflectance was about two times (in 0.05M acetate buffer). A rough estimate of the protein thickness may be made by assuming that the mean refractive index of the lipid membrane and lipid-protein membrane are the same. (Measurements on a lipid-toxin membrane suggest that adsorption of protein does not in fact produce any very marked change in mean refractive index.[16]) With this condition the thickness is proportional to the square root of the reflectance[15] so that an increase in reflectance of two times is equivalent to an extra layer of 13Å on either side of the lipid bilayer (taking the lipid thickness as 65Å). Because of the high molecular weight of the membrane apoprotein, such a value suggests that the protein is spread out over the surface rather than in a compact globular form.

An unfolding of the protein should be accompanied by a conformational change. In fact this is suggested by the drastic change of the ESR spectra of spin labeled protein upon recombination with lipid.[6,7] We have tried to gain further, more detailed information on this point by employing infrared absorption spectroscopy. The positions of amide I band (C=O stretching) and amide II band (N-H bending) are functions of protein conformation.[17] Dry films of α-helical and unordered protein structures display an amide I band at about 1652 cm^{-1}, whereas the corresponding band of β-structure occurs at about 1630 cm^{-1}. Exchange of protons of the peptide bond for deuterons can affect either of the amide I and II bands. In contrast to

α-Helix and β-structures the amide I band for randomly coiled proteins is shifted to lower frequencies considerably upon N-deuteration.[17]

We have examined the infrared absorption of dried films of s.a.f. apoprotein, recombinant of protein and lipid, and erythrocyte membranes (Figure 3). The amide I band of the s.a.f. protein shows no shoulder at 1630 cm^{-1} in agreement with Maddy[1]. Likewise the spectra of dried erythrocyte ghosts show no evidence for β-structure in accordance with Chapman[18] and Maddy[19]. Upon

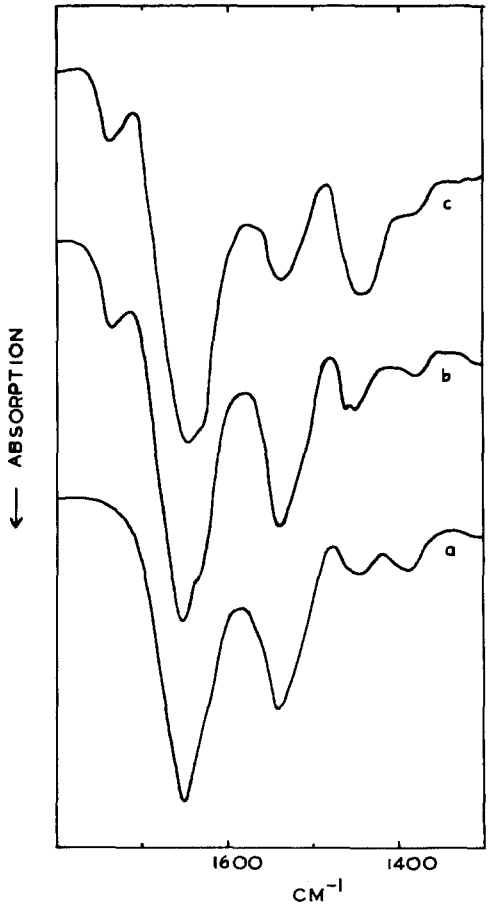

Figure 3. Infra red spectra of dried films of (a) butanol extracted s.a.f. membrane apoprotein; (b) recombinant with membrane lipid, (c) recombinant N-deuterated.

proton exchange we see just a small shift of amide I band and a decrease of amide II band at about 1540 cm^{-1} which is replaced by a band at about 1450 cm^{-1}.

In contrast to these findings recombinant of lipid and protein displays a distinct shoulder at about 1630 cm^{-1} typical of β-structure. Furthermore we observe a significant shift of the amide I band when the film is dried from D_2O-suspension. In consequence of this shift, separation of the C=O stretching bands of the lipid ester bond at 1740 cm^{-1} and of the peptide bond is enhanced. Amide II band at about 1540 cm^{-1} does not vanish completely upon proton exchange. Apparently some of the protons are not exchangeable perhaps because they are buried in the hydrophobic lipid phase. However, the significant shift of amide I band from 1653 cm^{-1} to 1646 cm^{-1} shows that the conformation of a considerable amount of protein has changed to an unordered structure upon recombination with the membrane lipid. This finding correlates well with the bilayer experiments which indicate that the protein is likely to form an extended layer on the surface of the lipid film.

ACKNOWLEDGEMENT

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