Interactions Between Black Lipid Membranes and the Loosely Bound Proteins from Erythrocyte Membranes

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The loosely bound proteins from human erythrocyte membranes induce an up to more than 10^3-fold increase in the electrical conductivity of black lipid membranes made from oxidized cholesterol. The conductivity increase continues until the membrane breaks. Its magnitude insreases with increasing protein concentration and salt concentration. The results are compared with earlier results obtained with the strongly bound proteins from erythrocyte membranes.

The electrical conductivity of black lipid membranes (BLM) which are one of the most frequently used model systems for the study of biological membranes is several orders of magnitude lower than that of biomembranes (for a review see [1]). There is, however, now an increasing number of reports which demonstrate that 1. it is possible to incorporate solubilized membrane proteins into the bilayers, and 2. by this incorporation of the protein, the conductivity of the black membranes is increased by up to 10^4-fold and thus approaches that of biological membranes (*e. g.* [2–5]). In one of these reports [4], our group has described the interactions between black membranes made from oxidized cholesterol and the strongly bound protein fraction from human erythrocyte membranes [6, 7]. We found that, by addition of the protein to both solutions bathing the membranes, membrane conductivity increased from values of $10^{-7} - 10^{-8}$ ohm^{-1} cm^{-2} to values up to about $1 \cdot 10^{-4}$ ohm^{-1} cm^{-2}. The conductivity increment 1. in most cases did not reach a stable value but increased until the membrane broke; 2. increased with increasing ionic strength, whereas, as a function of protein concentration, it showed a maximum at protein concentrations around 50 μg/ml; 3. was not observed if the protein was present only on one side of the BLM. We now thought it worthwhile to study whether the effects described are specific for the protein fraction we have used, or whether they are shared by other proteins. Therefore, we have repeated our measurements using the other of our protein fractions from red cell membranes, namely the "loosely bound" proteins [7]. This protein fraction is different from the strongly bound proteins not only in its binding properties to the red cell membrane but also in its composition: its polypeptides are only found in trace amounts in the strongly bound fraction [7].

For the preparation of the loosely bound proteins and the lipid and for the BLM measurements, we have applied the procedures described earlier [4, 7]. The protein solutions used for the measurements were prepared by diluting a stock solution (pH 9, ionic strength approx. 10^{-3} M, protein concentration approx. 1 mg/ml) with phosphate buffers. Protein concentration was determined by UV absorbance measurements using a "differential extinction coefficient" [4] of 11.2. All BLM measurements were done at pH 7.0, $T = 22 - 24$ °C.

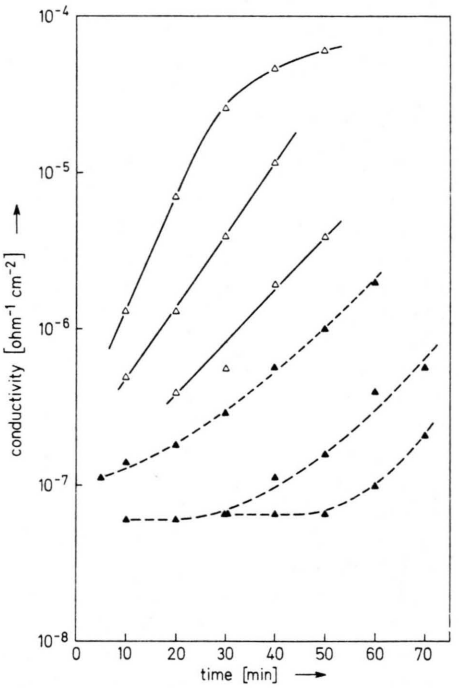

Fig. 1. Time-course of BLM conductivity in the presence of the loosely bound proteins. The membranes were formed at $t=0$ in buffers with $c_p=6.4$ μg/ml. Buffer composition: 10 mM sodium phosphate (pH 7.0) plus 20 mM NaCl (- - -) or 100 mM NaCl (——). For each salt concentration, an "average" curve and two curves showing typical deviation from the average are drawn (at $t=40$ min, only 3 out of 8 curves at the lower and 6 out of 18 curves at the higher salt concentration were outside the range given by the curves shown).

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The typical behaviour of membrane conductivity in our protein-BLM system is illustrated, for two values of salt concentration, in Fig. 1. In the absence of protein, membrane conductivity was found to be approx. 8×10^{-8} ohm^{-1} cm^{-2} and was nearly time-independent. However, if the membranes were formed in buffer solutions containing some μg/ml of the loosely bound proteins, a steady increase in BLM conductivity was observed which continued until the membrane broke. The values of membrane conductivity near the end of the membrane life-time were up to about 3×10^3-fold higher than in the absence of protein. Essentially the same results were obtained if the protein, instead of being present during membrane formation, was added to both buffer compartments after the membranes had become black. In both cases, quantitative relationships could, however, not be established because of the very high scatter of the data obtained with different membranes.

Despite of the scatter of the experimental data which was much larger than that observed with the strongly bound proteins, some qualitative relationships on the influence of salt concentration and protein concentration could be found. Thus, higher salt concentrations in the buffer compartments led to a higher increase in BLM conductivity (Fig. 1). Also, in the range of protein concentrations studied ($c_p \leqq 13$ μg/ml), increasing protein concentration led to distinct conductivity increases. *E. g.*, for $c_p =$ 6.4 μg/ml average BLM conductivity was about 50-fold higher than for $c_p = 3.2$ μg/ml. For $c_p > 13$ μg/ml, sufficient numbers of measurements could not be performed since most of the membranes broke within a few minutes. – It is interesting that significant conductivity increases as well as the upper limit of BLM stability were found at much lower values of c_p than with the strongly bound protein fraction. However, this difference is not large enough to be explained by the assumption that the results obtained with the strongly bound protein fraction are due to the traces found in this fraction of the loosely bound proteins.

If, different from the experiments described above, protein was added to one side of the BLM only, membrane conductivity first remained constant. However, after 10 – 20 min, a rapid conductivity increase was observed (Fig. 2). This contrasts the results found with the strongly bound proteins which induced an increase in BLM conductivity only if present on both sides of the membrane [4]. It should, however, be noted that with membranes from another batch of oxidized cholesterol, the effect of the loosely bound proteins just described was much less pronounced than shown in Fig. 2.

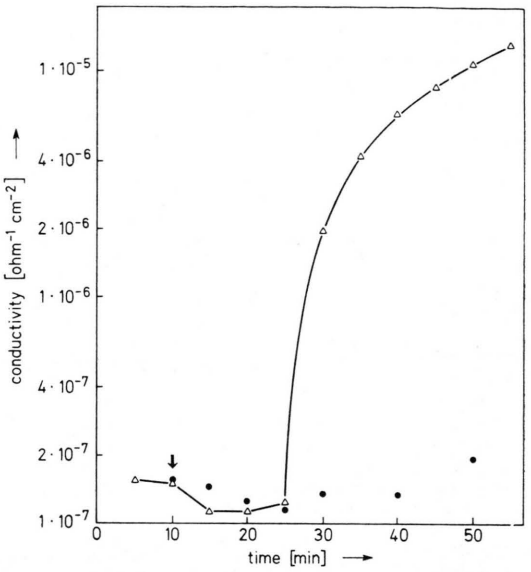

Fig. 2. Time-course of BLM conductivity in the absence of protein (●) and with protein present only on one side of the BLM (△). (↓): Time of protein addition. Buffer: 10 mM sodium phosphate (pH 7.0), 100 mM NaCl. $c_p =$ 6.4 μg/ml.

Comparing the results obtained for the two different BLM-protein systems, it is evident that the general properties of the two systems are quite similar. This concerns the effect and the order of magnitude of the increase in BLM conductivity induced by the protein as well as the qualitative effect of ionic strength and, at least for low values of c_p, of protein concentration. On the other hand, details of the behaviour of the two systems are different, and these differences probably would be much larger if pure protein components instead of heterogeneous protein fractions would have been used. Nevertheless, the suggestion that many properties of different membrane protein-BLM systems agree with each other, regardless of the protein source, can be supported not only by our results but also by the data of other investigators [3, 5].

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