THE EFFECT OF ADDED PROTEIN ON THE INTERCHAIN X-RAY PEAK PROFILE IN EGG LECITHIN

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ABSTRACT The effect of added protein on the phospholipid interchain peak profile has been measured. The results indicate that the basic organization of the bilayer is preserved, and that the added protein affects only the arrangement of the lipid hydrocarbon chains in the first few adjacent layers.

INTRODUCTION

In a preceding article we presented results of a study of the egg lecithin and myelin protein (N-2) membrane system (Brady et al., 1979). Varying amounts of protein were combined with the phospholipid, and from the changes produced in the scattering, an attempt was made to isolate the individual components of the scattering. Some knowledge of how the interchain structure was affected by the protein proved to be a useful guide in the analysis. In its own right, this problem is a fundamental one in membrane research; there exists a large body of work on it, using various chemical and physical techniques. An up-to-date bibliography is found in a review article by Papahadjopoulos (1974). X-ray diffraction is one of the techniques used, and in this field the work of Luzzati and collaborators (1962, 1973) occupies a prominent place. An extension of these studies, using transform techniques, is being pursued; these will take some time to complete. In the interim we did a study of the effect of the added protein on the interchain peak profiles, and we present the results below, since they have a bearing on the previous study. The isolation of the profiles from the rest of the scattering is not at all a routine operation (Brady and Fein, 1977); it can in fact be a delicate one, and we allude briefly to some of the details involved in the procedure. A useful attribute of applying careful intensity comparison techniques to the study of an isolated feature, such as a peak profile, is that Fourier transform techniques are more properly applied to the whole intensity pattern, of which these features comprise only a small part; their details can thus be obscured. A separate study highlights them.

METHODS

The X-ray measurements were made in a transmission goniometer fitted with a LiF singly bent crystal monochromator. An enclosed helium path between source and detector eliminated air scattering.

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The sample was held in a 1-mm thick cell fitted with 5-mm beryllium windows. The whole apparatus has been described elsewhere (Brady and Greenfield, 1967), as well as the absorption correction technique (Brady et al., 1969) used in correcting the data. This latter is an important part of the procedure.

The intensity patterns were measured with CuK radiation, by step-scanning in steps of 0.2° 2θ between 8 and 30° 2θ. Multiple scans were taken and the data collected and averaged on a PDP8/E computer (Digital Equipment Corp., Maynard, Mass.). 16,000 counts were collected for each step and a least-squares program averaged over 7 adjacent steps to give a probability error of 0.3% for each point. The scans were programmed to measure different parts of the pattern at different times, allowing sufficient overlap to prevent long-term drift and settling of the suspension. Polarization corrections were applied. The intensities (corrected for solvent background) shown in Fig. 1 are plotted against $s = 4\pi/\lambda \sin\theta$, where λ is the wavelength and θ is one-half the sccattering angle.

In the evaluation of the solvent background to be subtracted from the measured curves, a correction was made for the amount of H_2O bound to, or perturbed by, the membrane surface. For this purpose the scattering curve for pure H_2O scaled to the appropriate mole fraction was used, since there was no detectable difference between it and that of the buffered solvent. The scattering curve for pure H_2O is given by $I = F_{H_2O} + f^2_{H_2O}[1 + 4\pi\rho \int_0^\infty r^2 \{g_{H_2O}(r) - 1\} (\sin sr/sr)\, dr]$, where F_{H_2O} and f_{H_2O} are the incoherent and coherent scattering factors of H_2O, ρ is the density, and $g_{H_2O}(r)$ the radial distribution function, defined such that $4\pi r^2 \rho g(r)\, dr$ gives the number of H_2O's in a spherical shell of radius r and thickness dr about each H_2O. In the scattering range under study, f^2 is large and positive, and the product of f^2 and the integral term is large and negative; I_{H_2O} is consequently quite small. Any effect of the membrane surface will be reflected in a change in $g_{H_2O}(r)$. The number of H_2O molecules affected by the membrane surface at these concentrations is of the order of 2%, assuming that each polar group interacts with ~10 H_2O molecules (Brady and Fein, 1977). A correction of this order when applied to the $g(r)$ term gives rise to a ~10% correction in I. This is significant. Applying a correction of 2% directly to I is not a correct procedure. The scattering factors are tabulated (James and Brindley, 1931) and can be scaled to the experimental units at $s = 1.76$, at which value the integral term in Eq. 1 is zero (Brady and Romanow, 1960). The measured intensity is equated to $f^2_{H_2O} + F_{H_2O}$ at $s = 1.76$, thus permitting the scattering factors to be expressed in the experimental units at all values of s. The integral term can then be obtained from Eq. 1 by difference, and the correction for bound H_2O applied directly to it. The scaling can next be extended to the membrane scattering and its scattering factors can be evaluated. This last step permits a correction for the membrane incoherent scattering; it was found to be minor under the conditions of these experiments. The whole procedure is set out in detail in an earlier publication (Brady and Kaplan, 1973).

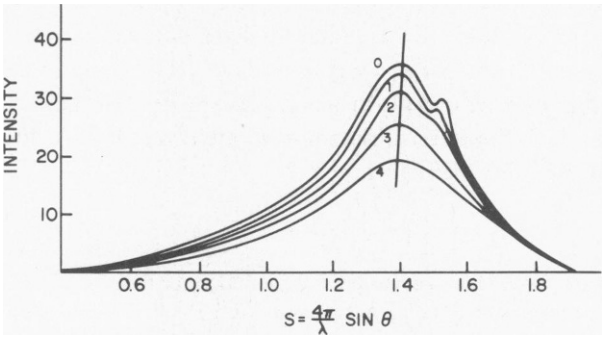

FIGURE 1 Isolated interchain peaks. The numbers refer to the concentrations in the text. Estimated accuracy is 5.0%. The overall error in the X-ray measurements and corrections was assessed at ±1.0%; the remainder is in the concentration determination.

The stock samples were the same as those used in the previous series of experiments (Brady et al., 1979). They were concentrated by centrifuging, and allowed to stand for 2 h before the supernatant was pipetted off. The membrane concentrations, determined by phosphorous assay, were in the range of 60–70 mg/ml. The curves (Fig. 1) were all scaled to the same total concentration. The protein concentrations and scattering units for the systems studied were: 0. 0% ($lip_{1.00}$); 1. 6.0% ($prot_{0.060} \cdot lip_{0.940}$); 2. 10.2% ($prot_{0.102} \cdot lip_{0.898}$); 3.21% ($prot_{0.210} \cdot lip_{0.790}$); 4.35% ($prot_{0.350} \cdot lip_{0.650}$).

RESULTS AND DISCUSSION

The resolved peak profiles are shown in Fig. 1. A notable feature is the presence of a spike at $s = 1.53$, corresponding to a Bragg spacing of 4.15 Å. This is the interchain distance in a "crystalline" state of chain organization and indicates that there are domains of such ordered material, extended enough to give rise to a diffraction effect. The area of the spike is ~ 1–2% of the total. It broadens with increasing protein concentration and finally melds into the main peak. This is an important observation and will have to be taken into account in more detailed studies of chain organization. Another observation is that there is a small shift toward smaller angle at the higher concentration, as indicated by the sloped line drawn through the apparent peak maxima. This is to be interpreted as a shift toward larger values of the moments of the interchain distance distribution and the effect is of course partly related to the disappearance of the crystalline spike. Or, qualitatively, there is a slight decrease in the average density of the hydrocarbon region of the membrane.

The second column of Table I lists the measured peak areas, A_M, with the 0% protein value normalized to unity. The third column gives the 0% value scaled to the concentration of phospholipid in each sample (A_S). Column 4 lists the ratios $(A_M - A_S)/A_S$. These are plotted against the weight fraction of protein in Fig. 2.

The conclusions to be drawn from these data are twofold. First, the phospholipid can retain large amounts of protein without losing its essential character as a discrete oriented phase. When a protein is inserted, the bilayer lipid molecules in the vicinity appear capable of moving apart to accommodate it. This is a unique feature of the role of phospholipids in membranes and is the result of the strong interchain attraction and amphipathic character of these compounds. Secondly, although the membrane continuity is retained, the presence of protein does have a discernible effect, indicating significant interactions between the lipid and protein. In fact, since the peak areas essentially count the number of (C, C) interchain distances, the ratios in Table I are a direct measure of the number of chains or parts of chains transformed into a configuration, such that they are no longer a part of the inter-

TABLE I
PEAK AREAS AND RATIOS

Sample	Peak areas		Ratio
	Measured (A_M)	Scaled (A_S)	$(A_S - A_M)/A_S$
0	1.0	1.000	0
1	0.919 ± 009	0.940 ± 038	0.022 ± 039
2	0.862 ± 009	0.898 ± 036	0.040 ± 036
3	0.740 ± 007	0.790 ± 032	0.063 ± 033
4	0.596 ± 006	0.650 ± 026	0.083 ± 026

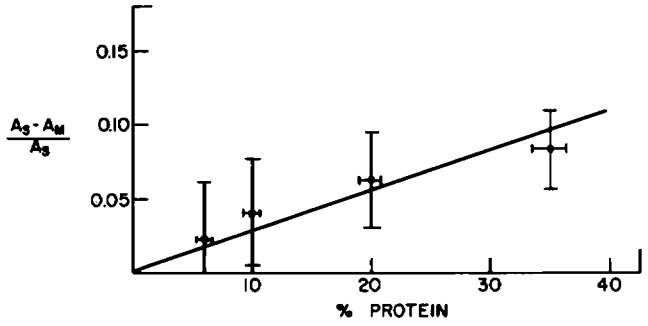

FIGURE 2 Fractional decrease in peak area as a function of protein content. The error is too large to say whether the apparent downward slope is real.

chain distribution. For example, a lipid molecule oriented with its hydrocarbon pointing towards the protein could suffer a partial solubilization of the chain in a hydrophobic region of the protein. Or, a side chain protruding out from the protein surface could, by insertion, disrupt the hydrocarbon packing. The work of Metcalfe and co-workers (Warren et al., 1975; Hesketh et al., 1976) on the lipid annulus around proteins is of significance on these questions. The X-ray results of course say nothing about the nature of the interactions, but they do place a limit on the number of lipids involved and thus furnish a useful constraint on any models proposed for the interactions. The (N-2) protein is a 54 Å radius spherical particle (Brady et al., 1979), giving a "molecular weight" of 9×10^5 (specific volume 0.73). The phospholipid molecular weight is 800; therefore for the 35% protein there are ~2,000 phospholipid molecules per protein particle. From Fig. 2 the fraction of molecules removed is 0.08. Thus a reorganization of lipids resulting from protein insertion would affect ~160 molecules. Our low angle work on this system (Brady et al., 1979) indicated that the protein was centrally placed in the bilayer. For a protein of this size, its surface is about equally divided between the protein embedded in the bilayer and the portion protruding out from the bilayer. The total available surface is 3.7×10^4 Å^2. The phospholipid diameter is ~8 Å (twice the diameter of a single chain). For the embedded surface, assuming that it is surrounded by phospholipids oriented roughly with their long dimension tangent to the surface as in the unperturbed membrane, we then have ~85 molecules of this type in the first neighbor region of the particle. Thus a modification of the chain organization in the first few surrounding layers would account for the number affected by the protein.

It would be speculation to try to draw any further conclusions from the data. We should point out, however, that the curvature of the protein surface is a factor in the disruption and that the surface is not smooth, but has many side chains dangling out, which, as noted above, would dissolve in the lipid chains, forcing them apart and/or reorienting them. There may also be patches of hydrophobic material on that protein surface not immersed in the membrane, and that these would be covered with lipids, oriented with their chains in the protein. In the natural membranes, where the phospholipid could be functional, and the protein-lipid interaction a specific, an analogous conclusion that only the first few layers are involved would still be valid.

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