THE INTERACTION BETWEEN A SYNTHETIC AMPHIPHILIC POLYPEPTIDE AND LIPIDS

JAMES H. DAVIS
Department of Physics, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

ROBERT S. HODGES
Department of Biochemistry and Medical Research Council Group in Protein Structure and Function, University of Alberta, Edmonton, Alberta, T6G 2H7, Canada

MYER BLOOM
Department of Physics, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada

Most spectroscopic studies designed to examine the interactions between phospholipid and protein molecules in biological membranes have involved measurements of the perturbation of the orientational order of lipids due to the presence of proteins (1). Such measurements are difficult to interpret on the molecular level because of the complexity of proteins and the paucity of experimental information on the three-dimensional structure of integral membrane proteins. In addition, the proteins are often relatively short lived under the variety of experimental conditions required for physical measurements and a large biochemical effort is required to produce an adequate renewable supply of well-characterized integral membrane proteins.

For these reasons, we have been led to develop a novel class of model membranes which have properties similar to those of protein-lipid reconstituents and which are stable and well-defined physical systems. Our model membrane consists of amphiphilic polypeptide molecules incorporated in phospholipid bilayers. The polypeptide is synthesized with hydrophilic amino acids at each end and a number (N) of hydrophobic amino acids in the central part to span the hydrophobic region of the phospholipid bilayer. From the structure of bacteriorhodopsin and general energy considerations of polypeptides in hydrophobic solvents (2), it is anticipated that the most stable type of protein secondary structural element in membranes should be the α-helix, which has a length of 1.5 Å/residue.

We report here a study of a mixture of the amphilic polypeptide (Lys_2-Leu_{24}-Lys_2-Ala amide) with dipalmitoylphosphatidylcholine (DPPC) in excess water (50% by weight). Since the hydrophobic region of DPPC has a width of ~ 33 Å in the liquid crystalline (L_α) phase and 47 Å in the gel (L_β phase (reference 1, page 38) we have decided to study these lipid/polypeptide mixtures as a function of values of N in the range 20–30 and of molar ratio (L/P). This paper should be considered as a first report of a systematic study.

MATERIALS AND METHODS

The 29-residue polypeptide was synthesized on benzhydrylamine resin using the general procedures for solid-phase peptide synthesis (3) on a Beckman peptide synthesizer model 990 (Beckman Instruments, Palo Alto, CA).

The sample with L/P = 50 was prepared by dissolving the polypeptide and lipid in methanol which was then evaporated. The lipid/polypeptide mixture was combined with water and mixed thoroughly. The ^{2}H-NMR spectra of the perdeuterated chains of DPPC were studied using methods described elsewhere (4). The oriented spectra were extracted from the "powder spectra" using a recently developed "de-Pakeing" procedure (5).

RESULTS AND DISCUSSION

The DPPC/H_2O system is known to have a phase transition of width < 1°C between the gel and liquid crystalline phases (4). The transition occurs near 41°C for protiated DPPC/H_2O and near 36°C for DPPC-d_{62}/H_2O. It is marked by a distinctive change in the ^{2}H-NMR lineshape as shown in Fig. 1 *a* and *b*. As may be seen from Fig. 1 *c*, the presence of the polypeptide hardly changes the distribution of quadrupole splittings in the liquid crystalline

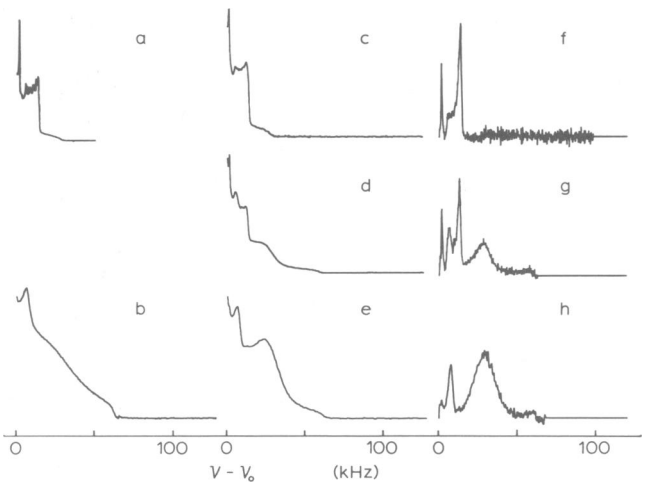

FIGURE 1 ^{2}H-NMR spectra of amphiphilic polypeptide and DPPC. The spectra shown here are one-half of the symmetrical ^{2}H-NMR spectra of perdeuterated hydrocarbon chains. *a* and *b*, DPPC-d_{62} in excess H_2O at 39°C and 20°C; *c*, *d*, and *e*, polypeptide/DPPC-d_{62} in excess H_2O at 45°C, 39°C, and 20°C, respectively. The oriented ("de-Paked" [5]) spectra of *c*, *d*, and *e* are shown in *f*, *g*, and *h*.

BIOPHYS. J. © Biophysical Society · 0006-3495/82/01/170/02 $1.00
Volume 37 January 1982 170–171

phase. This is because the length of the hydrophobic region of the polypeptide approximately matches the hydrophobic region of DPPC. As illustrated by Fig. 1 *c*, *d*, and *e*, however, the phase behavior is considerably modified due to the mismatch of the hydrophobic regions of the polypeptide and lipid in the gel phase (6). A gel phase spectrum appears below 44°C superimposed on the liquid crystalline spectrum. The fractional intensity of the gel spectrum varies continuously from 0 to 1 as the temperature is decreased from 44° to 22°C. The characteristics of the two superimposed spectra do not change markedly over this temperature range. Only their relative intensities vary. This is best seen from the oriented ("de-Paked" [5]) spectra shown in Figs. 1 *f*, *g*, and *h*. The spectrum of Fig. 1 *g* is, to a good approximation, a superposition of the high temperature, liquid crystalline spectrum of Fig. 1 *f* and the low temperature, gel spectrum of Fig. 1 *h*.

If the equilibrium length of the hydrophobic part of the polypeptide were significantly greater than the equilibrium width of the hydrophobic part of the lipid bilayer, the polypeptide would act as a spacer pushing the two halves of the bilayer apart and give rise to an increase in the quarupolar splittings. In potassium palmitate-water mixtures the width of the bilayer is known to be ~ 24 Å (7). We have observed a 50% increase in the quadrupolar splittings of a sample of polypeptide/potassium palmitate with L/P = 100.

Received for publication 30 April 1981.

REFERENCES

1. Seelig, J. 1980. Lipid conformation in model membranes and biological membranes. *Quart. Rev. Biophys.* 13:19–61.
2. McLachlan, A. D., and R. Henderson. 1980. Structure of the bacterio-rhodopsin in proton pump of *Halobacterium halobium* and other membrane proteins. *Biochem. Soc. Trans.* 8:677–678.
3. Hodges, R. S., A. K. Saund, P. C. S. Chong, S. A. St.-Pierre, and R. E. Reid. 1981. Synthetic model for two-stranded α-helical coiled-coils. *J. Biol. Chem.* 256:1214–1224.
4. Davis, J. H. 1979. Deuterium magnetic resonance study of the gel and liquid crystalline phases of DPPC. *Biophys. J.* 27:339–358.
5. Bloom, M., J. H. Davis, and A. L. MacKay. 1981. Direct determination of the oriented sample nuclear magnetic resonance spectrum for systems with local axial symmetry. *Chem. Phys. Lett.* In press.
6. Owicki, J. C., M. W. Springate, and H. M. McConnell. 1978. Theoretical study of protein-lipid interactions in bilayer membranes. *Proc. Natl. Acad. Sci. U.S.A.* 75:1616–1619.
7. Davis, J. H., and K. R. Jeffrey. 1977. The temperature dependence of chain disorder in potassium palmitate-water. A deuteron NMR study. *Chem. Phys. Lipids.* 20:87–104.