release from aldolase but prior to reduction by αPG dehydrogenase.

In conclusion, the results from this work demonstrate a mechanistic difference between yeast and muscle aldolase. Presumably, this difference also applies to the two classes these enzymes represent. The techniques used for this work present an unambiguous test for distinguishing between class I and class II aldolases. Investigations are currently in progress to determine if other aldolases now considered to be class I or class II behave in the same manner as their prototypes. Those aldolases which cannot clearly be placed in either class according to their chemical and physical properties are of particular interest.

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The authors thank Dr. R. L. VanEtten for supplying the wheat germ acid phosphatase, and Mr. J. Carter Cook (University of Illinois) for analyzing the combusted water samples.

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Physical–Chemical Studies of Phospholipids and Poly(amino acids) Interactions†

Kam-Yee Yu, Joseph J. Baldassare, and Chien Ho*

ABSTRACT: The interactions of phospholipid vesicles with poly(L-glutamic acid) and poly(L-tyrosine) were investigated as a model for the molecular interactions between proteins and phospholipids in biological membranes. We have used the spin-label and glucose permeability techniques to study the interactions between poly(amino acids) and phosphatidylcholine. The spin-labels that we used are the spin-labeled stearic acids and the spin-labeled phosphatidylcholines. The spin-label results suggest that these two poly(amino acids) interact on the surface of the phosphatidylcholine vesicles and that this interaction might cause a lateral tightening up of the polar region of the phospholipid molecule, but the flexibility gradient in the methylene chain is still preserved in the model membranes. In addition, the slower rate of glucose permeability in the complexes provides another piece of evidence that there is a tightening up of the bilayer structure of the phosphatidylcholine vesicles upon complex formation with negatively charged poly(amino acids) in aqueous solution.

For many years the structure and function of biological membranes have been the object of intensive research. A typical membrane is known to contain both proteins and lipids. From the time Danielli and Davson (1935) first proposed the classical lipoid model of a plasma membrane, a number of membrane models have been proposed. These range from the unit membrane structure which consists of a continuous biomolecular lipid leaflet surrounded by a layer of protein on either side of the membrane as proposed by Robertson (1964) to a structure which consists of associated, repeating proteolipid structural units as proposed by Green and Purdue (1967). Due to the complexity in the molecular structure as well as the diversity of the functional properties of membranes, it is unlikely that any one of the

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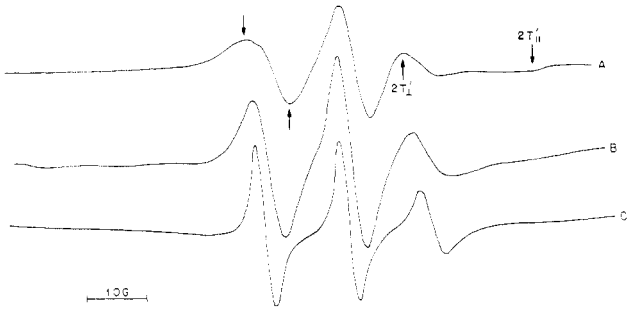


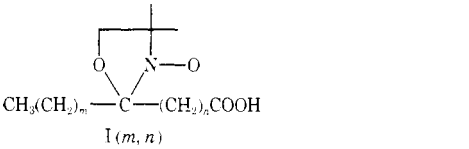
FIGURE 1: The epr spectra of *N*-oxyl-4,4-dimethyloxazolidine derivatives of ketostearic acid (spin-label I) in the complex of poly(L-glutamic acid) and phosphatidylcholine at pH 5.6 and 0.1 M NaCl: (A) I(12,3); (B) I(5,10); and (C) I(1,14).

proposed models will be the correct representation for all biological membranes. Hence, many different models for membranes are likely to be needed.

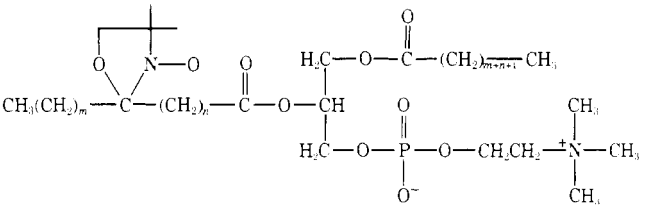
Aqueous phospholipid dispersions (liposomes) consisting of closed, concentric bilayers separated by aqueous channels have been used as an attractive model for biological membranes. Liposomes prepared from a variety of phospholipids have been found to exhibit many properties similar to those in the natural membranes, such as permeability and sensitivity to antibiotics, detergents, hormones, and drugs (Bangham, 1970). In order to account for all or most of the functional properties of cell membranes, we need to consider the interactions between the two essential components of a membrane, namely proteins and lipids. Benson (1968) reported that in a number of membrane lipoproteins studied, there is a higher ratio of the acidic amino acids than that of the basic amino acids. Hence, as a first step in our understanding of the nature of protein–lipid interactions, we have chosen to study the interactions between poly(L-glutamic acid) (poly(Glu))[1] and phosphatidylcholine (PC) as well as between poly(L-tyrosine) (poly(Tyr)) and phosphatidylcholine.

Hammes and Schullery (1970) had used circular dichroism (CD), optical rotatory dispersion (ORD), electron microscopy, nuclear magnetic resonance (nmr), and stopped-flow techniques to investigate the interaction of aqueous phospholipid dispersions with a variety of water-soluble polypeptides. From their CD results, they found that there was an increase in the α-helical content of poly(L-lysine) in the presence of phosphatidyl-L-serine at pH 7.0 and that there was no interaction between polypeptides with net negative charges (such as poly(L-glutamic acid)) and phosphatidylcholine or phosphatidyl-L-serine. Their approach emphasized the effects of phospholipids on the conformations of the polypeptides.

It has been found that spin-labels, particularly amphiphilic labels (such as I and II), may be used to obtain information about structural and kinetic properties of pure phospholipid bilayers and biological membrane (Hubbell and McConnell, 1968,1969a,b,1971; Waggoner *et al.*, 1969; Rottem *et al.*, 1970; Hubbell *et al.*, 1970; Seelig, 1970; McConnell and McFarland, 1970,1972; McFarland and McConnell, 1971; Jost *et al.*, 1971a). These labels exhibit electron paramagnetic resonance (epr) spectra in a number of biological membranes that are strikingly similar to those present in pure phospholipid bilayers. In addition, the epr spectra of these labels reveal the different degree of motional freedom in the methylene chains. That is, methylene groups near the terminal methyl group have higher rotational freedom whereas the motional freedom near the polar head group can approach that of a crystalline hydrocarbon. By choosing appropriate spin-labeled fatty acids (I) and spin-labeled phospholipids (II), we hope to gain some information on the nature of the interactions between phospholipids and poly(amino acids), especially the effects of poly(amino acids) on the conformations of the phospholipids.



I (*m*, *n*)



II (*m*, *n*)

Experimental Section

Materials. Chromatographically pure egg-yolk phosphatidylcholine (lecithin) was purchased from General Biochemicals and stored in absolute ethanol at −20°. Phosphatidylcholine dispersions were prepared by three different methods, namely Huang's (1969), Fleischer and Klouwen's (1961), and Hubbell and McConnell's (1971).

In Huang's method, phosphatidylcholine in absolute ethanol solution was dried in a rotatory evaporator under reduced pressure at 40°; an appropriate amount of 0.01 M Tris buffer in 0.1 M NaCl at pH 8.5 was added to the phospholipid dry film. The solution was sonicated for 90 min under nitrogen at 4°, and then centrifuged for 10 min at 12,000 rpm. The solution was then put through a Sepharose 4B column to obtain homogeneous phospholipid vesicles. The size of these vesicles is about 250 Å in diameter with a molecular weight of 2.1×10^6 as estimated by Huang (1969). In Fleischer and Klouwen's method, phosphatidylcholine was dissolved in a mixture containing butanol and 20% potassium cholate at pH 7.5 in a ratio of 87:13 (v/v) and dialyzed at 4° in a rocker dialysis apparatus for 7 days against 0.02 M Tris buffer in 0.001 M EDTA at pH 8. Phosphatidylcholine then formed homogeneous vesicles with particle weight of approximately 2×10^6 as estimated by the method of sedimentation equilibrium (Yu, 1972). Hubbell and McConnell's method resembles Huang's procedure except that the sonication and Sepharose 4B column procedures were omitted. The phosphatidylcholine vesicles obtained by the last method are larger than those by the other two methods. There is no observable difference in the epr spectra among the spin-labeled liposomes prepared by these three methods. This suggests that the bilayer structure is the only determining factor for the epr spectra.

Spin-labels I were obtained from the Synvar Association and spin-labels II were synthesized according to Hubbell and McConnell (1971). Spin-labeled stearic acid (spin-label I) was mixed with phospholipid dispersions at a molar ratio of 1:100. Spin-label II was mixed with unlabeled lecithin prior to the formation of aqueous dispersions at a molar

[1]Abbreviations used are: poly(Glu), poly(L-glutamic acid); poly(Tyr), poly(L-tyrosine); PC, phosphatidylcholine.

TABLE I: EPR Data of Spin-Labels I and II in Phospholipid Dispersions and Mixtures of Phospholipids and Poly(amino acids) in 0.1 M NaCl.

Spin-Label	System	Hyperfine Splitting	
		Perpendicular, $T_{\perp}'$ (G)	Parallel, $T_{\parallel}'$ (G)
I(12,3)	PC dispersions at pH 11.2	9.2 ± 0.1	25.7 ± 0.1
I(12,3)	Mixture of PC and poly(Tyr) at pH 11.2	9.3 ± 0.1	27.4 ± 0.1
I(12,3)	PC dispersions at pH 7.0	9.0 ± 0.1	25.6 ± 0.1
I(12,3)	Mixture of PC and poly(Glu) at pH 7.0	9.1 ± 0.1	27.2 ± 0.1
II(10,3)	PC dispersions at pH 8.5	9.0 ± 0.1	25.6 ± 0.1
II(10,3)	Mixture of PC and poly(Glu) at pH 5.6	9.2 ± 0.1	25.9 ± 0.1
II(10,3)	Mixture of PC and poly(Tyr) at pH 11.2	9.2 ± 0.1	26.1 ± 0.1

ratio of 1:100. The concentration of the spin-label is between 10^{-4} and 10^{-5} M in all preparations.

For the preparation of a mixture of poly(amino acids) and phospholipids, the appropriate amount of lyophilized poly(L-tyrosine) (molecular weight 100,000, Pilot Chemicals, Inc.) or poly(L-glutamic acid) (molecular weight 80,000, Pilot Chemicals, Inc.) was added to the spin-labeled phospholipid dispersions, the mixture was adjusted to the desired pH by adding 1 N HCl or 1 N NaOH, and the epr spectra were recorded. An alternate way of preparation was that the aqueous solution of poly(amino acids) was added directly to the phospholipid dry film containing the appropriate spin-labels. There is no observable difference in the epr spectra of the samples prepared by these two methods.[2] The concentration of phospholipid was determined by the phosphorus analysis procedure of Bartlett (1958) and the concentration of poly(amino acid) was determined either by the dry weight method or by the micro-Kjeldahl method (Nobel, 1966).

Method. A Bruker X-band (Model B-ER 418s) or a Varian E-4 electron paramagnetic resonance spectrometer was used to obtain the epr spectra. The ambient temperature inside the microwave cavity was 22°. Samples were transferred to either quartz aqueous flat cell or thin glass capillary tubes for epr measurement.

The glucose permeability of liposomes was measured enzymatically according to Demel *et al.* (1968). The enzymes, hexokinase and glucose-6-phosphate dehydrogenase, needed for the assay were obtained from Boehringer-Mannheim. Liposomes with glucose trapped inside the vesicles were prepared by adding 0.3 M glucose solution to phospholipid dry film in the flask. The "untrapped" glucose was removed by dialysis of the vesicles against a large volume of a solution containing 0.075 M KCl and 0.075 M NaCl for 12 hr. An appropriate amount of either poly(L-tyrosine) or poly(L-glutamic acid) was added to form the complex. The change in glucose concentration in solution is proportional to the absorbance at 340 nm. A Cary 14 spectrophotometer was used to measure the absorbance for both the liposomes and the complexes at 340 nm for a period of 9 hr thermostated at 25° and at *ca.* pH 7.5. For details, refer to Yu (1972).

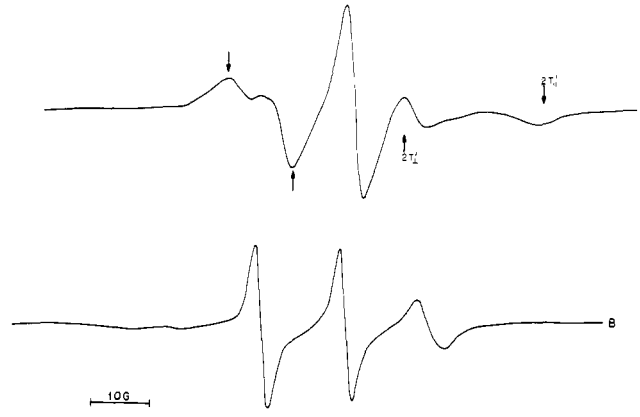


FIGURE 2: The epr spectra of spin-labeled phosphatidylcholine (spin-label II) in the complex of poly(L-tyrosine) and phosphatidylcholine at pH 11.2 and 0.1 M NaCl: (A) II(10,3), and (B) II(1,14).

Results

1. Spin-Label Study. The epr spectra of the spin-labels I and II in the mixture of poly(L-tyrosine) and phospholipid are very similar to those in the mixture of poly(L-glutamic acid) and phospholipid. Both mixtures have the molar ratio of four amino acid residues to one phospholipid molecule. Typical sets of epr spectra are shown in Figures 1 and 2. In the case when n is equal to 10 or 14, the epr spectra show the intermediate isotropic motion of the labels similar to those reported by other investigators (Hubbell and McCon-

[2] In principle, there should be differences in the epr spectra of the mixtures of poly(amino acids) and phospholipids prepared by the two methods. The first method should give vesicles with the outermost layer exposed to the poly(amino acids) whereas the alternate method should yield vesicles with both layers exposed to the poly(amino acids). Hence, one would expect that the mixtures prepared by the first method should give a composite epr spectrum and that those prepared by the second method should not. Due to the broadness of the low-field hyperfine resonances and the low intensity of the high-field lines, it is difficult to decide if the observed spectrum of the mixtures prepared by the first method is indeed composite in nature (refer to Figure 1A). The similarity of the epr spectra of the mixtures prepared by both methods could be due to a lack of interactions between the inner layers of the vesicles and the poly(amino acids). In other words, the poly(amino acids) used in our studies (with molecular weights greater than 80,000) are too big to be accommodated into the inner layers of the vesicles. Further experiments (such as obtaining the epr spectra with a spectrometer operated at higher frequencies and varying the size of the poly(amino acids)) are needed to clarify this problem.

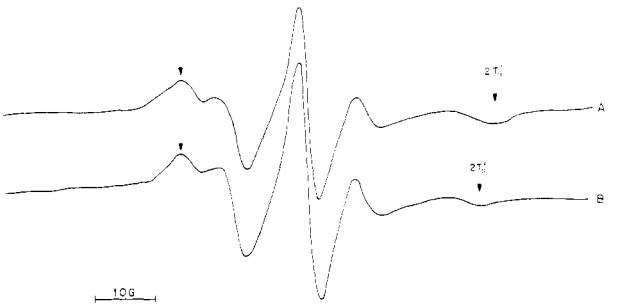


FIGURE 3: The epr spectra of spin-labeled phosphatidylcholine [(spin-label II(10,3)]: (A) complex of poly(L-tyrosine) and phosphatidylcholine at pH 11.2 and 0.1 M NaCl; and (B) aqueous dispersions of phosphatidylcholine at pH 11.2 and 0.1 M NaCl.

nell, 1969a,b; Hubbell *et al.*, 1970; Jost *et al.*, 1971a,b). The tumbling time is about 4 nsec for I(5,10), and is about 1 nsec for labels I(1,14) and II(1,14). The epr spectra of labels I(12,3) and II(10,3) are distinctly different from those of I(5,10), I(1,14), and II(5,10). Both $T_{\perp}'$ and $T_{\parallel}'$ are well resolved in the spectra of I(12,3) and II(10,3). Although there is great similarity between the spectra of the mixtures and the pure phospholipid dispersions, the hyperfine splittings of I(12,3) and II(10,3) are somewhat greater in the mixture than those in the pure phospholipid dispersions (Table I, Figure 3).

Ascorbic acid is known to reduce the oxazolidine ring and thus to abolish the epr signal of the free radical (Hubbell and McConnell, 1969a; McConnell and McFarland, 1970; Kornberg and McConnell, 1971). Ascorbic acid is very soluble in aqueous solution (30 g/100 ml at 20°), but insoluble in organic solvents. Figure 4 shows the effect of ascorbic acid on label I which has the nitroxide ring attached to different positions along the polymethylene chain. In the presence of 200-fold excess of ascorbic acid, the reduction occurred faster when the nitroxide ring was closer to the polar head group. At 22° there was a 67% reduction for $n = 2$ after 10 min whereas only an 8% reduction for $n = 14$. Figures 5 and 6 show the effect of 100-fold excess of ascorbic acid on the spin-labeled phospholipid dispersions and the mixtures of phospholipids and poly(amino acids). Comparing the reduction rates of the mixtures and phospholipid dispersions, it shows that the presence of poly(amino acids) (poly(L-tyrosine) and poly(L-glutamic acid)) can slow down the rate of reduction of the nitroxide groups in both II(1,14) and II(10,3).

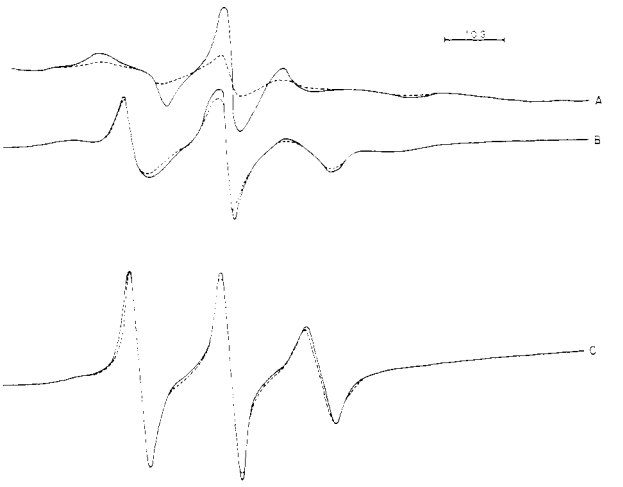


FIGURE 4: Effects of ascorbic acid on *N*-oxyl-4,4-dimethyloxazolidine derivatives of ketostearic acid (spin-label I) in aqueous dispersions of phosphatidylcholine at pH 8.5 and 0.1 M NaCl:(—)without ascorbic acid; and (- - - -) with 200-fold excess of ascorbic acid. The epr spectra were taken 10 min after mixing ascorbic acid with the spin-label I: (A) I(12,3); (B) I(5,10); and (C) I(1,14).

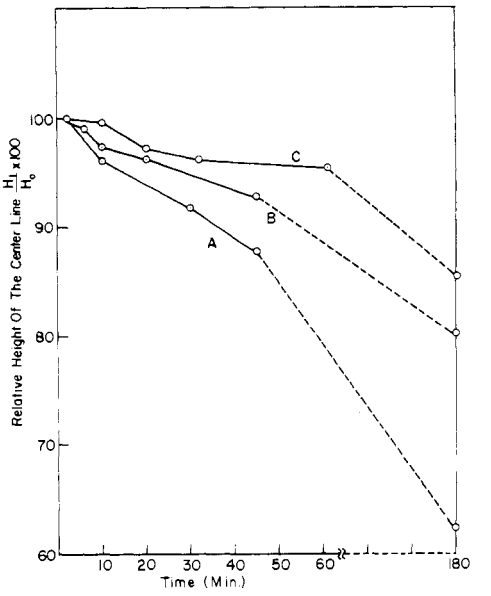


FIGURE 5: Effects of ascorbic acid on spin-labeled phosphatidylcholine [(spin-label II(1,14)]: (A) aqueous dispersions of phosphatidylcholine at pH 8; (B) complex of phosphatidylcholine and poly(L-glutamic acid) at pH 5.6; and (C) complex of phosphatidylcholine and poly(L-tyrosine) at pH 11.2.

2. Glucose Permeability Study. Glucose permeability was also found to be slower in both poly(L-tyrosine)-phosphatidycholine complex and poly(L-glutamic acid)-phosphatidylcholine complex than in the phosphatidylcholine liposomes alone (Figure 7). Each experiment was repeated at least three times and the "release rate constant" of glucose in the liposomes is about twice as fast as that in these two complexes.

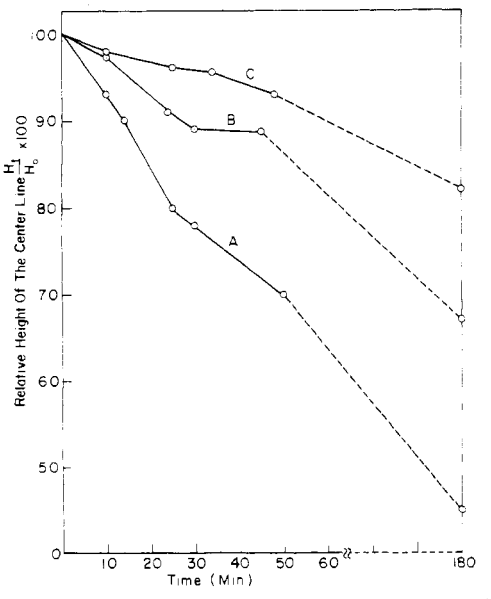


FIGURE 6: Effects of ascorbic acid on spin-labeled phosphatidylcholine [(spin-label II(10,3)]: (A) aqueous dispersions of phosphatidylcholine at pH 8; (B) complex of phosphatidylcholine and poly(L-glutamic acid) at pH 5.6; and (C) complex of phosphatidylcholine and poly(L-tyrosine) at pH 11.2.

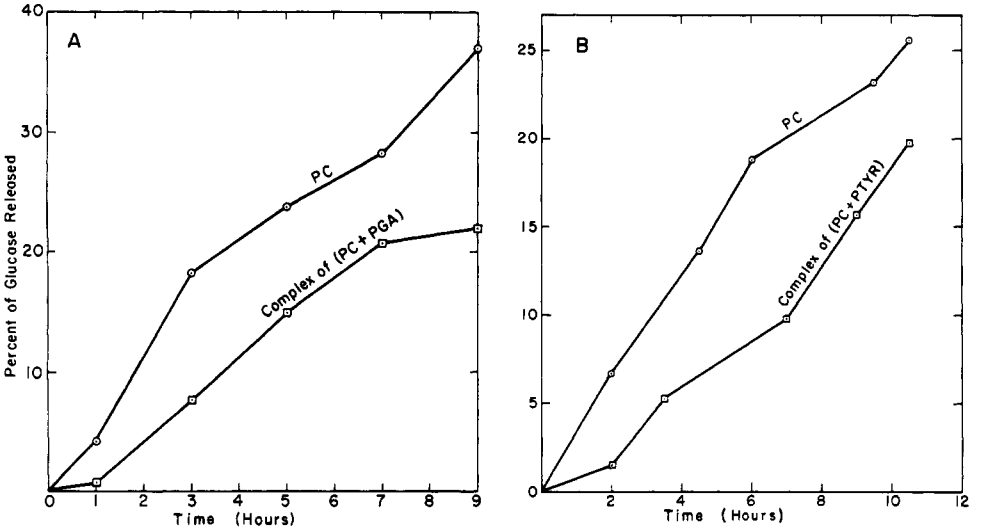


FIGURE 7: Effects of poly(amino acids) on the glucose permeability in phospholipid vesicles at 25°: (A) complex of phosphatidylcholine and poly(L-glutamic acid); and (B) complex of phosphatidylcholine and poly(L-tyrosine). For details, refer to the Experimental Section.

Discussion

The results presented here show that measurable changes in molecular structure in the phospholipid vesicles can occur when mixing poly(amino acids) and phosphatidylcholine aqueous dispersions together. Since the long axis of the spin-labeled fatty acids (I and II) tends to align itself along the axis of the phospholipid molecules, the mobility and orientation parameters measured for the spin-labels directly reflect the different environment in the phospholipid vesicles (Hubbell and McConnell, 1969b,1971; Waggoner *et al.*, 1969; Rottem *et al.*, 1970). The epr spectra of spin-labels I(12,3) and II(10,3) in phospholipid vesicles indicate a preferential orientation and rapid anisotropic motion of the oxazolidine ring about the methylene chain (Hubbell and McConnell, 1969a,b,1971). According to Hubbell and McConnell, the spin-label which is only three carbon atoms away from the polar region of phospholipid molecule sits in a well-organized array and as the position of oxazolidine ring on the fatty acid chain is moved away from the polar end of the phospholipid, the motional freedom is increased. These studies show that the molecular arrangement in the mixture of phosphatidylcholine and poly(amino acids) varies from a well-organized outer layer to a more fluid interior. The same phenomenon is observed in pure phospholipid bilayers and many biological membranes (Hubbell and McConnell, 1969a,b,1971; Rottem *et al.*, 1970; Seelig, 1970; Jost *et al.*, 1971b).

Although the epr spectra of the mixtures are very similar to those in the phosphatidylcholine vesicles, the larger hyperfine splitting in I(12,3) and II(10,3) in the mixtures implies either a lesser degree of freedom or a change in solvent polarity in the region around the oxazolidine ring or a combination of both factors. On the other hand, the hyperfine splittings of labels I(1,14), I(5,10), and II(1,14) are the same in both the phospholipid dispersions and the phospholipid–poly(amino acid) complexes. These results suggest that both poly(Glu) and poly(Tyr) have affected the outer region of the phospholipid bilayers, but have little effect on the inner region of the bilayer. These findings are in agreement with Rottem *et al.* (1970) who found that $T_{\parallel}'$ in native mycoplasma membranes was larger than in dispersed mycoplasma lipids.

The aims of ascorbic acid reduction experiments are twofold: (1) to verify the position of oxazolidine ring in the spin-labeled fatty acids and (2) to investigate the effect of the interaction between phosphatidylcholine and poly(amino acids) on the conformation of phospholipid vesicles. The rate of reduction is faster when the spin-label is closer to the polar region and slower when it is further away. This is due to both the distance that ascorbic acid has to diffuse through before it could reduce the ring and the slight solubility of ascorbic acid in nonaqueous medium. These results suggest that the oxazolidine ring near the polar region is exposed to the aqueous environment while the oxazolidine ring further from the polar region is tucked away into the hydrophobic region of the bilayer.

The ascorbic acid reduction is much slower in the mixture than in the phospholipid vesicles alone. This finding is consistent with the epr results given in Table I that the interaction between poly(amino acid) and phospholipid has tightened up the outer region of the bilayers, but phosphatidylcholine molecules are still arranged in the bilayer form judging from the epr spectra exhibited by labels I(12,3), I(5,10), I(1,14), II(10,3), and II(1,14).

The results from the glucose permeability study also furnish another piece of evidence to indicate the tightening up of the bilayers. The slower rate of glucose permeability in the complex implies a closer packing of the phospholipid bilayer which suggest that the poly(amino acids) have exerted some kind of barrier to the diffusion of glucose. This barrier could not be in the interior of the hydrocarbon region of the bilayer as indicated by the spin-label experiments, so it is more likely on the surface of the bilayer or in the exterior of the hydrocarbon region. In addition, since the glucose permeability experiments were carried out in the absence of a spin-label, these results suggest that the lateral tightening of the phospholipids is not an artifact due to the presence of an oxazolidine ring in the spin-labeled fatty acids or in the spin-labeled phospholipids.

The effects of phospholipids on the conformation of polypeptides and membrane proteins have been studied by a number of investigators. Ulmer *et al.* (1965) found no change in the ORD spectrum of cytochrome *c* upon the addition of mixed beef heart phospholipids. Using CD and ORD techniques, Hammes and Schullery (1970) observed an increase in the α-helix content of positively charged

polypeptides in the presence of negatively charged phospholipids. They found no evidence of interaction between poly(L-glutamic acid) and phosphatidylcholine. On the other hand, we found that according to our ORD results, poly(L-glutamic acid) underwent a change in conformation from a highly ordered structure (α helix) to a more random coil state upon interaction with phosphatidylcholine vesicles from pH 4.5 to 7.7 and ionic strength <0.01 (K. Y. Yu and C. Ho, unpublished results; Yu, 1972).[3] Our spin-label and glucose permeability results support our ORD data, namely there is a complex formation between poly(L-glutamic acid) and phosphatidylcholine. However, our spin-label and glucose permeability results do not tell us the nature of the conformational changes in the polypeptides. The ORD and/or CD spectra of membrane proteins isolated from different sources as well as model membranes exhibit the following two anomalous features (Wallach and Zahler, 1966; Wallach and Gordon, 1968; Lenard and Singer, 1966; Urry *et al.*, 1967; Glaser *et al.*, 1970): (1) low amplitude as compared with poly(amino acids) of known conformation and (2) displacement of the entire spectrum to longer wavelengths (red shift) than that is observed for α helix. Urry and Krivacic (1970) as well as Glaser and Singer (1971) had suggested that red shift and low amplitude observed in membrane type suspensions could be due to optical artifacts. Using the classical general scattering theory (Mie theory), Gordon and Holzwarth (1971) had recently shown that the characteristic anomalies in the optical activity of membrane suspensions are artifactual. Hence, it is difficult to predict what effects phospholipids will have on the conformations of polypeptides and membrane proteins by optical techniques.

The X-ray diffraction results of Levine and Wilkins (1971) and Wilkins *et al.* (1971) and the spin-label results of McConnell and coworkers (Hubbell and McConnell, 1968,1969a; Rottem *et al.*, 1970) suggest that, at least in some of the membrane systems, phospholipids exist as a bilayer. How this organization is preserved or modified in the presence of membrane proteins is still an open question (Singer and Nicolson, 1972). Our study in these model membranes using the spin-label technique and the glucose permeability experiment suggests that (1) there are interactions between negatively charged poly(amino acids) and phosphatidylcholine and (2) these negatively charged poly(amino acids) and phosphatidylcholine interact on the surface of the phospholipid vesicles. This interaction might cause a lateral tightening up of the polar region of the phospholipid molecules, but the flexibility gradient along the methylene chain is still preserved in these model membranes. We believe that our results may have important implication for biological membranes and that our approach offers some advantage to investigate certain aspects of the interactions between membrane proteins and phospholipids.

Recently, Chang and Chan (1974) have studied the interactions between poly(Glu) and sonicated lecithin vesicles by means of the proton magnetic resonance spectroscopy. Their results suggest that there are strong interactions between poly(Glu) and the bilayer surface. They further proposed that the binding of poly(Glu) to lecithin vesicles leads to a lateral tightening of the bilayer membrane. Hence, their findings strongly support the conclusions reached in this paper.

[3]ORD measurements were made with a Cary 60 spectropolarimeter at 27°. A computer program based on Magar's computation method (Magar, 1968) was written to calculate the percentage of α helix in the sample. ORD measurements of a 1:1 molar ratio of PC–poly(Glu) complex and poly(Glu) were made at pH values 4.5, 5.2, 5.6, 6.0, and 7.7 and ionic strength <0.01. There was a decrease in the helicity of poly(Glu) in the complex as the pH was changed from 4.5 to 7.0. The most drastic difference was found at pH 5.6. The α-helical content in poly(Glu) at pH 5.6 was found to be 91% and that in PC–poly(Glu) was 8%. Since the conformation of the poly(Glu) molecule depends primarily on the degree of ionization of the side-chain carboxyl groups, it may be argued that a change in the helicity of poly(Glu) on mixing with PC vesicles could be due to a shift in the pK_a of the carboxyl groups in the presence of PC. To check this possibility, a titration of acetic acid in the presence of equimolar amount of PC was carried out. The apparent pK_a value of acetic acid in PC was found to be pK_a = 4.78 as compared to that of pK_a = 4.85 for acetic acid alone. It is therefore concluded that the presence of PC has very little effect on the state of ionization of the side-chain carboxyl groups, and any change in the helicity of poly(Glu) in the presence of PC must result from some other effects. For details, refer to Yu (1972).

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Nuclear Magnetic Resonance Studies of the Interactions of Sonicated Lecithin Bilayers with Poly(L-glutamic acid)†

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ABSTRACT: The interactions of sonicated lecithin vesicles with poly(L-glutamic acid) have been studied by high-resolution proton magnetic resonance (pmr) spectroscopy. The choline methyl protons of the lecithin vesicles showed a large decrease in pmr intensities upon mixing with the poly(L-glutamic acid). The observed change in pmr intensities is shown to be mainly due to the interactions between the lecithin vesicles and poly(L-glutamic acid). Electron microscopy study showed isolated vesicles for the pure lecithin solution, but clusters of vesicles when the poly(L-glutamic acid) is present in the solution. The pmr spectra of poly(L-glutamic acid) further indicated that the polypeptide remained in the random coil form upon mixing with the lecithin vesicles.

Membrane proteins play an important role in determining the structures and function of biological membranes (Guidotti, 1972). Studies of the interactions between the phospholipid bilayer and membrane proteins and interactions among the membrane proteins themselves are therefore essential to our understanding of membrane properties. Physical methods can be brought to bear on lipid–protein interactions. However, the heterogeneous composition and the resultant complicated structures in biomembranes render studies of real membranes a difficult if not a formidable task. It has been suggested that useful if not pertinent information might be derived from examining simpler model systems such as membrane vesicles containing synthetic polypeptides and reconstituted membrane vesicles containing well-characterized protein systems (Trudell *et al.,* 1973; Griffith *et al.,* 1973).

In recent years sonicated lecithin vesicles have been used as a model bilayer membrane for studies of membrane properties (Finer *et al.,* 1972; Huang, 1969; Sheetz and Chan, 1972; Kornberg and McConnell, 1971a,b; Lee *et al.,* 1972; Bangham, 1972; Papahadjopoulos and Watkins, 1967), as well as membrane–protein interactions (Finer *et al.,* 1969; Hammes and Schullery, 1970; Yu *et al.,* 1974; Lau and Chan, 1974). Investigations which pertain to lipid–protein interactions include the work of Hammes and Schullery (1970), who studied the interaction of vesicles of phosphatidyl-L-serine (PS)[1] with various polypeptides. The study revealed that a complex was formed between PS and poly(L-lysine). Similar observations were also noted for the mixtures of PS with poly(L-ornithine) and with poly(Glu^{42} $Lys^{28}Ala^{30}$). On the other hand, polypeptides bearing negative charges, for example, poly(L-glutamic acid), were found not to interact with PS or phosphatidylcholine over a wide range of pH. More recently, a study of the interaction of phosphatidylcholine vesicles with poly(L-glutamic acid) was undertaken by Yu *et al.* (1974) using spin-label and glucose permeability measurements. This work indicated that poly(L-glutamic acid) does interact with phosphatidylcholine vesicles and, in fact, these workers concluded that this interaction led to a lateral tightening of the polar region

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[1] Abbreviations used are: PS, phosphatidyl-L-serine; poly(Glu), poly(L-glutamic acid); poly(Lys), poly(L-lysine).