U. S. 56, 1828.
Levine, Y. K., and Wilkins, M. H. F. (1971), *Nature (London), New Biol. 230,* 69.
Magar, M. E. (1968), *Biochemistry 7,* 617.
McConnell, H. M., and McFarland, B. G. (1970), *Quant. Rev. Biophys. 3,* 41.
McConnell, H. M., and McFarland, B. G. (1972), *Ann. N. Y. Acad. Sci. 195,* 207.
McFarland, B. G., and McConnell, H. M. (1971), *Proc. Nat. Acad. Sci. U. S. 68,* 1276.
Nobel, D. (1966), *Clin. Chem. 12,* 369.
Robertson, J. D. (1964), *in* Cellular Membrane in Development, Locke, M., Ed., New York, N. Y., Academic Press, p 1.
Rottem, S., Hubbell, W. L., Hayflick, L., and McConnell, H. M. (1970), *Biochim. Biophys. Acta 219,* 104.
Seelig, J. (1970), *J. Amer. Chem. Soc. 92,* 3881.
Singer, S. J., and Nicolson, G. L. (1972), *Science 175,* 720.
Ulmer, D. D., Vallee, B. L., Gorchein, A., and Neuberger, A. (1965), *Nature (London) 206,* 825.
Urry, D. W., and Krivacic, J. (1970), *Proc. Nat. Acad. Sci. U. S. 65,* 845.
Urry, D. W., Mednieks, M., and Bejnarowicz, E. A. (1967), *Proc. Nat. Acad. Sci. U. S. 57,* 1043.
Waggoner, A. S., Kingzett, T. L., Rottschaefer, S., and Griffith, O. H. (1969), *Chem. Phys. Lipids 3,* 245.
Wallach, D. F. H., and Gordon, A. (1968), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 27,* 1263.
Wallach, D. F. H., and Zahler, H. P. (1966), *Proc. Nat. Acad. Sci. U. S. 56,* 1552.
Wilkins, M. H. F., Blaurock, A. E., and Engleman, D. M. (1971), *Nature (London), New Biol. 230,* 72.
Yu, K. Y. (1972), Ph.D. Dissertation, University of Pittsburgh, Pittsburgh, Pa.

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

Chin-An Chang‡ and Sunney I. Chan*

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

† Contribution No. 4795 from the Arthur Amos Noyes Laboratory of Chemical Physics, California Institute of Technology, Pasadena, California 91109. *Received November 19, 1973.* This work was supported by U. S. Public Health Service Grant GM-14523 from the National Institute of General Medical Sciences.

‡ Present address: Lawrence Berkeley Laboratory, University of California, Berkeley, Calif. 94720.

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

of phosphatidylcholine vesicles. In addition, upon complexing with vesicles, the conformation of poly(L-glutamic acid) was noted to change from a highly ordered structure to a more random state between pH 4.5 and 7.7 at an ionic strength less than 0.01.

The present work was undertaken to further elucidate the interaction between phosphatidylcholine vesicles and poly(L-glutamic acid). In view of the conflicting results which have been obtained, it would seem helpful to use a technique in these studies which will provide information on the interaction mechanism as well as the ensuing structural changes in the participants. Proton magnetic resonance (pmr) spectroscopy presents itself, and has been demonstrated to be a powerful tool in the study of the structural and dynamic properties of both the surface and the interior of lecithin vesicles (Sheetz and Chan, 1972; Seiter and Chan, 1973). This sensitivity has recently been exploited to monitor the interaction of various oligopeptides with lecithin multilayers and lecithin vesicles (Finer *et al.*, 1969; Hsu and Chan, 1973; Lau and Chan, 1974). This same approach has therefore been chosen in our present study on interactions between lecithin vesicles and poly(L-glutamic acid).

Experimental Section

L-α-Dipalmitoyllecithin (phosphatidylcholine) was purchased from General Biochemicals. This lecithin was purified using the silicic acid column with an eluting solution composition of 400 ml of $CHCl_3$, 400 ml of $CHCl_3$–CH_3OH, 9:1, and 1000 ml of $CHCl_3$–CH_3OH, 1:9. Spectroquality chloroform and methanol were distilled before use, and the purified lecithin was collected from the last 1000 ml elutions. Both the purified and unpurified lecithins showed a single spot with thin-layer chromatography. Poly(L-glutamic acid) (Na salt), poly(Glu), with molecular weight 50,000–80,000, was purchased from Pierce Chemicals. It was dialyzed and lyophilized before use. The solutions of pure poly(Glu) in D_2O at the normal concentration used in this study had an apparent pH of 6.9, or a pD of 7.3 (Appel and Yang, 1965).

Dispersions of lecithins (*ca.* 2% wt) in D_2O (99.5%) were sonicated for 15 min at power level 4 with a Bronson sonifier, Model S-75. The sonicated solutions were centrifuged at 13,000 rpm in a Sorvall RC-2 centrifuge for 20 min, and the upper clear solutions were used for studies. In the present study, poly(Glu) was mixed with the sonicated lecithins at a concentration range of 1–100 amino acid residues per 100 lecithin molecules. These mixtures had an apparent pH of 6.9–7.1.

Pmr measurements were performed using a Varian HR-220 superconducting nmr spectrometer. Probe temperature was measured with ethylene glycol which was either in a sealed capillary immersed in the sample solution or in a separate nmr tube; the two measurements agreed to within 0.5°. Chemical shifts were measured relative to an external capillary of 1% Me_4Si in $CDCl_3$. The intensities of the resonance signals were measured against a 2,2-diphenyl-1-picrylhydrazyl doped $CHCl_3$ capillary which was calibrated with tetrabutylammonium bromide solution.

Electron microscopy studies were performed with a Phillips 300 electron microscope. Within a few hours after sample preparations, drops of solutions were applied to 200 mesh copper grids and fixed with phosphotungstic acid solution (0.5%).

Results

The pmr spectra of sonicated lecithins consisted of three peaks with chemical shifts of 3.21, 1.27, and 0.85 ppm, as measured downfield from the reference Me_4Si. These peaks are assigned to the choline methyl (Ch-CH_3), methylene ($(CH_2)_n$), and terminal methyl (T-CH_3) protons, respectively. In general, for the sonicated lecithin vesicles only the Ch-CH_3 peak was observed at low temperatures, the T-CH_3 and $(CH_2)_n$ peaks were observable only at temperatures close to and above the phase transition temperature (42°) of the lecithin vesicles. The sonicated lecithin vesicles studied in this work had an average diameter of 450–800 Å, as revealed by electron microscopy studies.

Effect of Purification on the Stability of Lecithin Vesicles. Although both the purified and unpurified lecithins showed a single spot with thin-layer chromatography, the sonicated vesicles of these two forms of lecithins differed significantly in stabilities toward clustering and fusing. For example, an increasingly heavy turbidity was developed in a clear solution of purified lecithin vesicles in 3 days while storing at room temperature (24°). On the other hand, solutions containing unpurified lecithin vesicles remained clear for more than a month. Concomitant with this difference in turbidity development, the pmr spectral intensities of the Ch-CH_3 protons of purified lecithin vesicles decreased by about 50% at 17° and above the phase transition temperature to a lesser extent. The unpurified lecithins, however, showed only slight decrease in its Ch-CH_3 pmr intensities in a month both below and above the phase transition temperature.

These observations indicated much less stability of the purified lecithin vesicles. According to Sheetz and Chan (1972) the pmr intensities of the Ch-CH_3 protons of sonicated lecithin vesicles depended on the size of the vesicles studied. The pmr spectra of the Ch-CH_3 groups of small vesicles (average diameter 300 Å) were nearly 100% observable at room temperature, while that of the larger vesicles (average diameter 1000 Å) were hardly observable at this temperature and became 100% observable only above the phase transition temperature. A comparison of the $ChCH_3$ intensities observed at ~20° with those above the phase transition temperature could thus provide an approximate estimate of the distribution of lecithin molecules in small vesicles. Furthermore, the room temperature pmr intensity of the Ch-CH_3 protons should be sensitive to changes in the size distribution of the vesicles and could be used to indicate fusing of small vesicles into larger ones. Accordingly, our observed differences in the Ch-CH_3 pmr intensities cited above indicated possible fusing of purified lecithin vesicles into larger ones in 3 days. The unpurified lecithin vesicles, however, showed much higher stabilities against such change in vesicle sizes. This process of vesicle fusion is presently under study in this laboratory (N. O. Petersen and S. I. Chan, manuscript in preparation).

The differences observed between the purified and unpurified lecithin vesicles could very possibly be due to impurities which were removed upon column purification. Supporting this possibility were the following observations. (1) In one case, solution containing unpurified lecithin vesicles was dialyzed after storing at room temperature for 3 weeks. The resultant solution was found to develop a heavy turbidity after storing at room temperature for another 2 days. (2) The unpurified lecithin showed an unknown pmr peak which was located at lower field than the choline

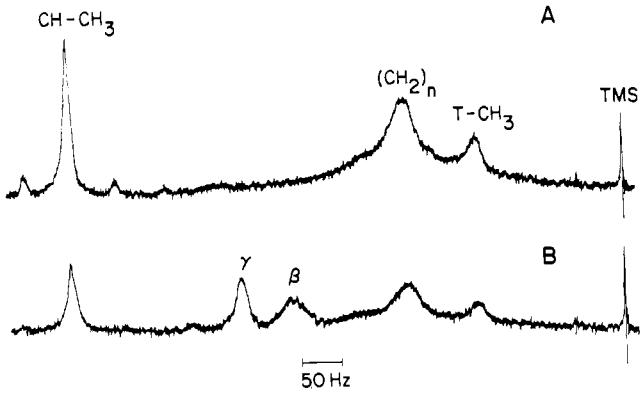

FIGURE 1: Pmr spectrum (220 MHz) at 44° of (A) sonicated lecithin vesicles 2.5 wt % in D_2O, (B) sonicated lecithin vesicles 2.5 wt % in D_2O containing 5.0 wt % poly(Glu). The two small peaks on each side of the Ch-CH_3 peak in (A) are spinning side bands.

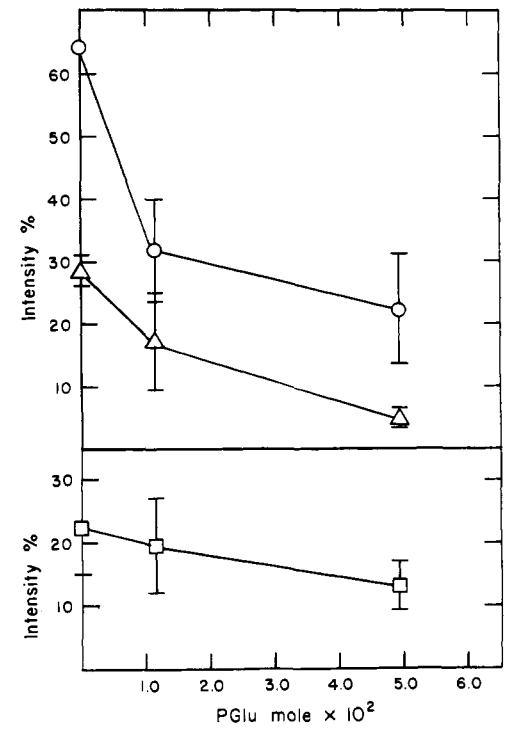

FIGURE 2: Effect of poly(Glu) on the pmr intensities of the lecithin proton signals observed for 2.5 wt % sonicated lecithin vesicles in D_2O. (○) Ch-CH_3 protons at 44°; (△) Ch-CH_3 protons at 17°; (□) T-CH_3 + $(CH_2)_n$ protons at 44°. Poly(Glu) concentrations are expressed in units of amino acid residues.

methyl peak, both in the sonicated form in D_2O and in a dissolved form in chloroform. This peak was absent for the purified lecithins. (3) Conductivity measurements of the solution used to dialyze the unpurified lecithins from several sources revealed an ion content of 5 ± 3 mol % after dialysis (G. Feigenson, private communication).

The most likely impurity responsible for these observations is palmitic acid (Robles and Van den Berg, 1969). Palmitic acid when incorporated into the lecithin vesicles would make the vesicle surface charged. This charged surface would then cause repulsion among the vesicles and prevent them from clustering and fusing. In fact, 1–5% palmitic acid is frequently added to dipalmitoyllecithin preparations in order to stabilize sonicated vesicles. Removal of these impurities, either by column or by dialysis, reduces such surface charges and enhances the rate of clustering and fusing, as observed for the purified lecithin vesicles.

Effect of Poly(L-*glutamic acid*) *on the Lecithin Vesicles.* Addition of poly(L-glutamic acid) to the sonicated lecithin vesicles created turbidity in the solutions as well as lowered the pmr intensities of the latter. These changes were observed for both the purified and unpurified lecithins, thus ruling out the possibility that the above mentioned impurities were responsible for the observed changes. However, the instability of the purified lecithin vesicles made a detailed study on this form of lecithin difficult. Therefore, the unpurified lecithin was used in the bulk of our studies of the interactions between lecithin vesicles and poly(L-glutamic acid).

The pmr spectra of the lecithin vesicles with and without added poly(Glu) are shown in Figure 1. Dependence of the lecithin pmr intensities upon the added poly(Glu) is shown in Figure 2. It is noted that a large decrease in pmr intensity with added poly(Glu) is observed for the Ch-CH_3 protons above the phase transition temperature of the lecithin vesicles, and to a lesser degree below the phase transition temperature. The decrease in intensity is the least for the T-CH_3 and $(CH_2)_n$ protons. It is also observed that, without poly(Glu), the Ch-CH_3 proton intensities were quite reproducible among different sonicated samples. However, those of the poly(Glu)-containing solutions were sensitive to how well the lecithin solutions were sonicated. For example, the poly(Glu)-containing solutions prepared from a less clearly looking sonicated lecithin solution gave higher lecithin pmr intensities than those with the corresponding poly(Glu) contents from a clearer lecithin solution. This gives the rather large range of the observed pmr intensities shown in Figure 2. It is shown later that, although the sonifier used failed to provide a better quantitative consistency among the different sonicated samples, the observed decrease in pmr intensities is large enough for our concern of the interactions between the lecithin vesicles and poly(L-glutamic acid).

The pmr intensities of these solutions were found to decrease rather slowly with time when the solutions were stored at room temperatures; the decrease was less than 10% 1 week after the preparation of the samples.

Because the sodium salt of poly(Glu) is used in the present study as well as in the other workers' studies, the possible effect of salt on the pmr intensities of the lecithin vesicles has also been studied. NaCl was chosen for this purpose. Analytical Reagent grade NaCl from Mallinckrodt Chemical Co. was added to the sonicated lecithin solutions. The mixed solution showed immediate development of homogeneous turbidities which increased with increasing amount of NaCl added. Pmr spectra were measured at 17 and 44 °, and the results are shown in Figure 3.

It is seen that an abrupt drop in intensities at 0.01 M NaCl concentration was observed for the Ch-CH_3 spectra at both 17 and 44°, and for the T-CH_3 + $(CH_2)_n$ spectra at 44°. However, within the NaCl concentration range studied here, the intensities remained nearly constant with the NaCl concentration. After storing at room temperature for a week, all the NaCl-containing solutions showed another slight decrease of the lecithin pmr intensities at both 17 and 44°.

Effect of Lecithin Vesicles on the Poly(L-*glutamic acid*). The pmr spectrum of poly(Glu) of the poly(Glu)-containing lecithin solution is shown in Figure 1. In order to study the conformation change of poly(Glu) upon mixing with the lecithin vesicles, we compare the pmr spectra of poly(Glu) in the mixture with those of pure poly(Glu) at different ex-

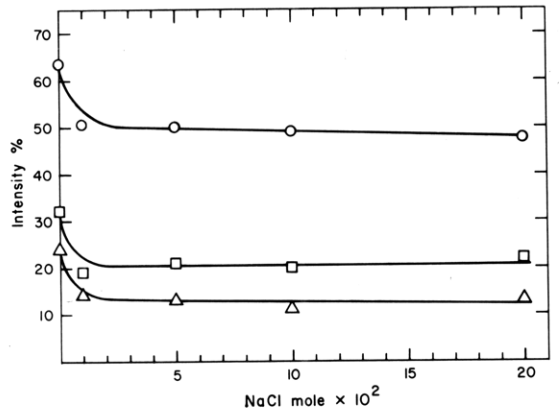

FIGURE 3: Effect of NaCl on the pmr intensities of lecithin proton signals for 2.5 wt % sonicated lecithin vesicles in D_2O. (○) Ch-CH_3 protons at 44°; (△) Ch-CH_3 protons at 17°; (□) T-CH_3 + $(CH_2)_n$ protons at 44°.

TABLE I: pD and Temperature Dependences of the Chemical Shifts and Line Widths of Poly(L-glutamic acid) in D_2O.

pD	Temp (°C)	Chemical Shifts (ppm)			Line Width (Hz)		
		α	β	γ	α	β	γ
7.3	17	3.975	1.666	1.984	20	40	20
7.3	39	3.946	1.659	1.939	19	40	20
7.3	52	3.936	1.650	1.923	18	43	20
5.4	17	3.816	1.882	2.229	24	48	63
5.4	39	3.855	1.809	2.141	?	29	42
5.4	56	3.861	1.756	2.091	19	26	24
4.9	17	3.796	1.964	2.255	30	60	64
4.9	39	3.811	1.884	2.225	?	41	48
4.9	60	3.799	1.806	2.145	20	26	27

tents of helicity. Poly(Glu) is known to exist in the helical form below pD 4.0 and in the random coil form above pD 7.0 with a transition point at pD 5.4 (Appel and Yang, 1965). The pmr spectra of poly(Glu) were measured at pD 7.3, 5.4, and 4.9; these corresponded to 100% random, *ca.* 50% helix and *ca.* 90% helix, respectively. In addition, because the lecithin vesicles were studied over a certain temperature range, similar temperature dependence of the helicity content of poly(Glu) was also studied. The pmr spectra of poly(Glu) at different pD's were taken at 17, 39, 52–60°, as shown in Figure 4. Only the spectral region of the β and γ peaks are shown here, as it is this region which we shall be concerned with in the comparison to be described below.

It is seen that significant differences in line widths and chemical shifts exist among the pmr spectra of these three samples. Table I lists the observed pD and temperature dependences of the line widths and chemical shifts of these spectra. The following observations are noted: (1) the pD 7.3 sample retains its line shapes for the β and γ peaks throughout the temperature range studied; (2) for the acid solutions, at low temperature the γ peak becomes broader than the β peak; at higher temperatures both peaks become narrower, and have about the same line width at the highest temperature studied.

Comparing the poly(Glu) spectra of Figure 1 with that of the random coil form of Figure 4, it is clear that, in neutral solutions, poly(Glu) retains its random coil form upon mixing with the lecithin vesicles, both at the low and high temperatures studied.

Electron Microscopy Study. The electron microscopy study revealed drastic changes of the lecithin vesicles upon mixing with the poly(Glu). The poly(Glu)-free lecithin solutions showed isolated vesicles of varying sizes, while the poly(Glu)-containing ones showed clusters of vesicles. Typical electron micrographs are shown in Figure 5.

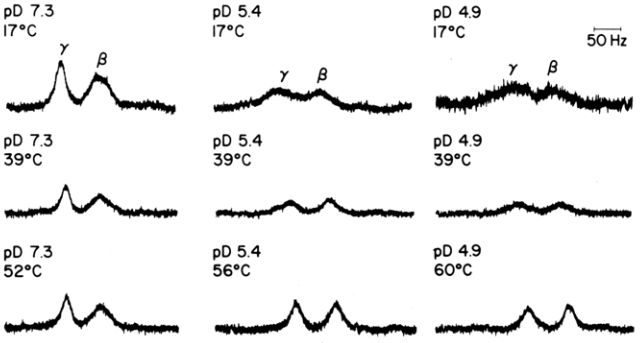

FIGURE 4: Pmr spectra (220 MHz) of *ca.* 6.0 wt % poly(Glu) in D_2O at different pD's and temperatures.

Discussion

Interactions of Lecithin Vesicles and Poly(Glu). Our present study of the interaction of lecithin vesicles with poly(Glu) shows that poly(Glu) does complex and interact with lecithin vesicles. The pronounced decrease in the pmr intensities of the choline methyl group signals clearly indicates that this interaction is confined near the surface of the bilayer vesicles. This reduction in the pmr intensity no doubt arises from immobilization of the choline methyl groups, either as a result of some specific interaction of the poly(Glu) residues with the polar head groups, or one of a less specific nature involving electrostatic interactions of the negatively charged carboxyl groups of the glutamic acid side chains with the electric double layer potential of the bilayer surface. This conclusion is augmented by the results of a similar study of the interaction of lecithin vesicles with poly(L-lysine) (poly(Lys)) (C. A. Chang and S. I. Chan, unpublished). Here the side chains of the polypeptide bear positive instead of negative charges, and, as expected, the poly(Lys) study revealed only a slight decrease in the pmr intensities of the choline methyl signals upon the addition of poly(Lys) to a sonicated lecithin solution. Whereas the observed choline methyl signal intensity in lecithin–poly(Glu) mixtures amounts to only 20–40% of that seen in the absence of the polypeptide, this signal retains 80–90% of its intensity in the case of poly(Lys).

The pmr intensities of the methylene protons were also

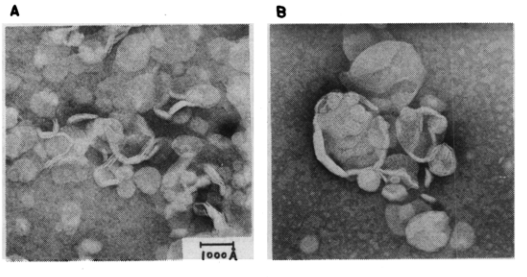

FIGURE 5: Electron micrographs of (A) sonicated lecithin vesicles in D_2O, and (B) sonicated lecithin vesicles in D_2O containing poly(Glu).

noted to decrease upon poly(Glu) binding to the lecithin vesicles. This intensity reduction, however, is much smaller than that noted for the choline methyl protons. This result constitutes further support of our contention that perturbations of the bilayer upon poly(Glu) binding are confined to the vicinity of the polar head groups. It might be that the interactions between poly(Glu) and the polar head groups lead to some lateral tightening of the bilayer. Such a lateral compression would render the fatty acid chains of the lecithin molecules less mobile than in the case of uncomplexed lecithin vesicles. Our conclusion here is in agreement with the work of Yu *et al.* (1974). These workers also propose such a lateral tightening of the bilayer with poly(Glu) binding on the basis of spin-label and glucose permeability measurements.

The above proposed strong interaction between poly(Glu) and lecithin vesicles should also be discussed in the light of the results of our salt effect studies described earlier. While the addition of NaCl did cause a decrease in the pmr intensity of the lecithin molecules, the effects here are too small to account for the much larger intensity reductions observed in the case of the lecithin vesicles-poly(Glu) system. The effect of NaCl on the lecithin pmr intensities is an interesting one, and arises from either osmotically induced shape changes in the lecithin vesicles and/or coagulation of the lecithin vesicles. Further study is now in progress in this laboratory in order to ascertain the effect of salt on the size distribution and the state of aggregation of sonicated lecithin vesicles.

Conformation of Poly(Glu) in Lecithin-Poly(Glu) Mixtures. In monitoring the interaction of poly(Glu) with lecithin vesicles, we have also compared the pmr spectrum of poly(Glu) in mixtures of lecithin with those of pure poly(Glu) of various degrees of helicity in order to clarify the earlier conflicting results concerning the conformational changes of the polypeptide (Hammes and Schullery, 1970; Yu *et al.*, 1974). This study reveals that, within the sensitivity of our method, poly(Glu) remains in the random coil form upon mixing with lecithin vesicles over the pH range of 6.7–7.1. This result is in accord with the conspicuous lack of change in the helicity of the polypeptide previously reported by Hammes and Schullery (1970). The somewhat different conclusions obtained by Yu *et al.* (1974) most certainly arise from the fact that over the pH range of 4.5–7.7 employed in their studies, poly(Glu) existed as a mixture of both the helical and the random coil forms. Yu *et al.* reported that the addition of lecithin to poly(Glu) resulted in a conformational change of the poly(Glu) from a highly ordered structure of a more random state. This result might be anticipated if poly(Glu) complexes with lecithin vesicles in the random coil configuration.

Conclusions

We have studied the interactions between poly(Glu) and sonicated lecithin vesicles. A pronounced decrease in the pmr intensities of the lecithin proton signals indicated a rather strong interaction between the polypeptide and the bilayer surface. These reductions in the pmr intensities cannot be accounted for by alterations in the shape and size of the lecithin vesicles or changes in their state of aggregation. It is proposed that poly(Glu) binding to lecithin vesicles leads to lateral tightening of the bilayer membrane. This present pmr study clearly indicates that poly(Glu) remains in random coil configuration upon complexation with the lecithin vesicle. A previously reported discrepancy between two different groups of workers on this point has been resolved.

References

Appel, P., and Yang, J. T. (1965), *Biochemistry 4,* 1244.

Bangham, A. D. (1972), *Chem. Phys. Lipids 8,* 386.

Finer, E. G., Flook, A. G., and Hauser, H. (1972), *Biochim. Biophys. Acta 360,* 49, 59.

Finer, E. G., Hauser, H., and Chapman, D. (1969), *Chem. Phys. Lipids 3,* 386.

Griffith, O. H., Jost, P., Capaldi, R. A., and Vanderkooi, G. (1973), *Ann. N. Y. Acad. Sci. 222,* 561.

Guidotti, G. (1972), *Annu. Rev. Biochem. 41,* 731.

Hammes, G. G., and Schullery, S. E. (1970), *Biochemistry 9,* 2555.

Hsu, M.-C., and Chan, S. I. (1973), *Biochemistry 12,* 3872.

Huang, C.-H. (1969), *Biochemistry 8,* 344.

Kornberg, R. D., and McConnell, H. M. (1971a), *Biochemistry 10,* 1111.

Kornberg, R. D., and McConnell, H. M. (1971b), *Proc. Nat. Acad. Sci. U. S. 68,* 2564.

Lau, A., and Chan, S. I. (1974), *Biochemistry* (in press).

Lee, A. G., Birdsall, N. J. M., Levine, Y. K., and Metcalfe, J. C. (1972), *Biochim. Biophys. Acta 255,* 43.

Papahadjopoulos, D., and Watkins, J. C. (1967), *Biochim. Biophys. Acta 135,* 639.

Robles, E. C., and Van den Berg, D. (1969), *Biochim. Biophys. Acta 187,* 520.

Seiter, C. H. A., and Chan, S. I. (1973), *J. Amer. Chem. Soc. 95,* 7541.

Sheetz, M. P., and Chan, S. I. (1972), *Biochemistry 11,* 4573.

Trudell, J. R., Hubbell, W. L., and Cohen, E. N. (1973), *Ann. N. Y. Acad. Sci., 222,* 530.

Yu, K.-Y., Baldassare, J. J., and Ho, C. (1974), *Biochemistry 13,* 4375.