NMR OBSERVATION OF THE INTERACTION OF SMALL OLIGOPEPTIDES WITH PHOSPHOLIPID VESICLES

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SUMMARY: Using proton spin-lattice relaxation times, the interaction of small oligopeptides with sonicated vesicles of synthetic β-γ-dimyristoyl L-α-lecithin has been monitored at 29°C in D_2O. The measured relaxation times for the lecithin choline methyl, alkyl chain, and terminal methyl protons were observed to shorten markedly with increasing concentration of peptide, the relaxation remaining exponential. Noticeable resonance broadening was observed at the highest peptide concentration studied. The data reported are for the effect of the pharmacologically active pentapeptide methionine-enkephalin. Similar results have been observed for the effect of tetraglycine. The relaxation of the observable resonances of the added peptide appear to be unaffected. The results are discussed in terms of peptide-vesicle interactions.

INTRODUCTION: A considerable body of literature exists which describes the use of well defined phospholipid bilayer vesicles as model membrane systems. In particular the behavior of NMR* signals has been extensively used to study these vesicles and their interaction with oligopeptides such as poly (L-glutamic acid) (1) and the antibiotics valinomycin (2) and alamethicin (3). These oligopeptides range in molecular weight from ∿1,100 to ∿80,000 D, and the effects of smaller peptides on the NMR spectra of vesicle model membrane systems appears to have been ignored. The influence of smaller peptides on model membranes is of considerable current interest, however, in view of the increasing number which are being found to exhibit pharmacological activity on the central nervous system.

*nuclear magnetic resonance

Recently we became interested in the biochemistry and biophysics of the opiate peptide, methionine-enkephalin (4) and have determined some features of its conformation in aqueous and non-aqueous solution (5). This pentapeptide (H-Tyr-Gly-Gly-Phe-Met-OH, MW = 573 D) acts as an opiate agonist in the guinea pig myenteric plexus (4) and has an anatomical distribution in mammalian central nervous systems similar to that of the opiate receptor (6). The question of whether such peptides act as neurotransmitters or neurohormones is under study, but it is clear that a possibility exists that they interact directly with the lipid component of synaptic membranes. To evaluate this possibility we have performed and describe below the obvious experiment of monitoring changes in the NMR parameters of phospholipid vesicles in aqueous solution induced by the addition of methionine-enkephalin.

MATERIALS AND METHODS: The vesicles used in this study were prepared from synthetic β-γ-dimyristoyl L-α-lecithin obtained from Calbiochem (lot# 400488). This material showed a single spot (R_f = 0.21) when visualized after thin layer chromatography (chloroform-methanol-water, 80:25:3) and was not further purified. We note, however, that an impurity contributing to vesicle stability is thought to be present in synthetic dipalmitoyllecithin which shows a single spot on thin layer chromatography (1). The vesicles were formed by sonication (45 minutes) to opalescence in 100% D_2O using a power level of 40 W with a model W 185 D sonicator, Heat Systems-Ultrasonics, Inc.. Equivalent lecithin monomer concentration was 15.8 ± 1.2 mM. Individual preparations were carried out for each peptide concentration used. Each vesicle preparation was divided in half to provide a control without peptide for each sample to which peptide was added. The preparation, purification, and analytical examination of the methionine-enkephalin has been described elsewhere (5). Tetraglycine was obtained from R-Plus, Inc., migrated on paper chromatography (phenol-water, 5:1) as a single spot (R_f = 0.54) and was not further purified. The pH was adjusted with DCl or NaOD to p D = 3.23 ± 0.09 (7). Special care was taken to use the minimum necessary amount of DCl or NaOD in order to minimize the amount of salt present, which can affect the NMR spectra of the vesicles (1). The samples were unbuffered and no attempt was made to remove dissolved oxygen. However, our data is obtained with control samples to which no peptide is added, and we have previously measured the proton relaxation of methionine-enkephalin in carefully degassed and treated solutions (5). These latter solutions act as controls for effects of possible paramagnetic impurities. Samples generally were used immediately after preparation and always within 24 hours and kept at 30°C when not in use.

Proton NMR spectra were obtained in the Fourier transform mode at 100MHz and bandwidth of 1.0 KHz (8192 data points) using the JEOL-PFT-100 spectrometer of the New England Area Research Resource. The resonance from residual HDO was suppressed (8). Temperature was controlled to ± 1°C with the standard JEOL temperature controller. Spin-lattice relaxation times T_1 were determined with the 180°-τ-90°τ pulse sequence (9) with t at least six times the longest measured T_1. Two or four parameter non-linear regression analysis was used to obtain T_1 from the single exponential or weighted biexponential decay functions (not their logarithms). Peak resonance intensities were used in determining T_1.

TABLE 1

PROTON SPIN-LATTICE RELAXATION TIMES[a] FOR SONICATED DIMYRISTOYLLECITHIN VESICLES[b] AT 29°C AND pD = 3.23

Concentration (mM)[c]	$N^+(CH_3)_3$		$-(CH_2)_n-$		$T-CH_3$	
	Vesicles	Vesicles + Met-Enkephalin	Vesicles	Vesicles + Met-Enkephalin	Vesicles	Vesicles + Met-Enkephalin
0.155	0.295 ± 0.002	0.293 ± 0.004	0.297 ± 0.004	0.298 ± 0.007	0.399 ± 0.010	0.397 ± 0.006
3.67	0.296 ± 0.002	0.282 ± 0.003	0.301 ± 0.005	0.293 ± 0.005	0.403 ± 0.008	0.388 ± 0.009
7.75	0.288 ± 0.007	0.240 ± 0.003	0.297 ± 0.005	0.281 ± 0.003	0.385 ± 0.011	0.372 ± 0.006
29.2	0.298 ± 0.005	0.179 ± 0.007	0.296 ± 0.007	0.263 ± 0.004	0.391 ± 0.011	0.336 ± 0.013
83.5	0.283 ± 0.004	0.0897[d]	0.292 ± 0.005	0.242 ± 0.004	0.391 ± 0.011	0.291 ± 0.006

a In sec.. The ± figures denote approximate 95% confidence limits (∿ two standard deviations), as determined by computer analysis.
b Lecithin monomer concentration is 15.8 ± 1.2 mM.
c The concentration given is for amino acid residues. Thus the concentration of met-enkephalin is 20% of the given concentration.
d Average T_1 calculated from the four parameters characterizing the weighted biexponential relaxation decay function.

RESULTS AND DISCUSSION: The spectra obtained were typical of sonicated lecithin vesicles (10) and showed resonances from the choline methyl and fatty acid alkyl chain and terminal methyl protons. A shoulder was just barely (but reproducibly) discernable on the upfield side of the choline methyl resonance and is due to the effect of vesicle surface curvature in distinguishing the inner from the outer choline methyls (3, 11). It should be noted that the two choline methyl resonances were not sufficiently resolved to allow the measurement of separate T_1's. The data discussed below are for the most intense, more downfield choline methyl resonance and because of the partial overlap of the outer and inner resonances reflect a weighted average.

Table 1 summarizes the measured T_1's. Exponential relaxation was observed within experimental error for all cases but one. At the highest concentration of methione-enkephalin interference from the Tyr and Phe β resonances of the peptide resulted in a measurable departure of the choline methyl relaxation from exponentiality. In view of the fact that the peptide resonances involved have longer T_1's than the vesicle resonance the average T_1 given in Table 1 is an upper-bound for the true value and thus does indicate appreciable peptide vesicle interaction.

Fig. 1 displays the effect of increasing concentration of methionine-enkephalin in shortening the vesicle T_1's. Considering the correlation times observed in such systems (12) this shortening reflects a decrease in motional freedom of the lipid protons. To facilitate comparison with the behavior of larger peptide additives (1-3, 13) the concentration is given in terms of amino acid residues. Normally the NMR parameter monitored in demonstrating the influence of an additive peptide is resonance intensity or width, and not T_1. Thus, comparison with the literature can be made only in general terms. Such comparison reveals that the effect seen in Fig. 1 arises at relative lecithin-amino acid residue concentrations equivalent to those found for alamethicin (3) and poly(L-glutamic acid)(1) in sonicated vesicles.

Fig. 1 also indicates that the greatest effect and also the effect seen at

lowest peptide concentrations is for the choline methyl protons. It is significant, however, that a definite reduction is seen for the alkyl chain and terminal methyl proton T_1's at the higher peptide concentration. The influence on the terminal methyl protons is especially significant in view of the fact that these protons are located in the center of the bilayer. The implication is that either the peptide has penetrated the bilayer or a surface effect has been propagated into the bilayer center. The difference in NMR behavior of unsonicated dipalmitoyllecithin and dimyristoyllecithin upon addition of valinomycin has led to the conclusion (2) that the antibiotic is incorporated into the less stable dimyristoyllecithin structure. Furthermore it has been pointed out (2) that the effect of valinomycin in apparently penetrating sonicated phospholipid bilayers (13) is consistent with the significantly looser molecular packing in sonicated versus unsonicated phospholipids.

We note that Fig. 1 reveals a greater effect on the terminal methyl as compared to the alkyl chain protons. It is difficult to interpret this difference in terms of peptide penetration into the bilayer since the initial motional states of these two groups of protons are doubtless different. The

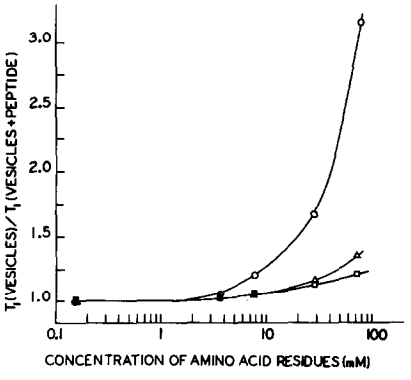

Figure 1-----The effect of methionine-enkephalin on the proton spin-lattice relaxation of sonicated dimyristoyllecithin vesicles (o-choline methyl protons, □ -alkyl chain protons, Δ -terminal methyl protons). Concentration of peptide is given in terms of amino acid residues (see footnote c to Table 1.) Solid lines are drawn only as an aid in visualizing the data.

greater shortening of the terminal methyl T_1 upon addition of peptide may be a reflection of an initial state of greater motional freedom.

We have also conducted preliminary experiments at 16°C which is below the transition temperature of ∿23°C characterizing peptide free vesicles (14) and have observed no shortening of the choline methyl T_1 upon addition of peptide. In view of the known sensitivity of the transition temperature of unsonicated dimyristoyllecithin bilayers to the concentration of valinomycin (2) interpretation of this result is deferred.

To evaluate the influence of the vesicles on methione-enkephalin the T_1's of the Phe and Tyr aromatic and Met methyl resonances of the peptide were measured. No significant differences were observed at 29°C from the T_1's measured in a carefully degassed and treated aqueous solution (5). Aside from indicating the absence of serious paramagnetic contaminants in our vesicle preparations, these results carry the following implication: either the exchange between vesicle bound and unbound peptide is slow on the time scale given by T_1 or the exchange is not slow and the concentration of bound peptide which is causing the effect on the vesicles is very small.

To provide a comparison with the results observed using methionine-enkephalin we have conducted similar experiments with tetraglycine, a peptide which is not known to exhibit pharmacological acitvity. At 29°C, a peptide amino acid residue concentration of 26.0 mM, and the same concentration of vesicles and pD used in Table 1 we observe the following results for T_1 (vesicles)/T_1 (vesicles + tetraglycine): choline methyl-1.30, alkyl chain-1.05, and terminal methyl-1.04. While these effects are smaller than those observed in Fig. 1, they nonetheless raise the possibility that the interaction between vesicles and oligopeptides of all sizes and charge characteristics may be a general phenomenon. This, outside of its own interest, may have practical consequences. It may mean, for example, that common buffers produce similar results.

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REFERENCES

1. Chang, C.A., and Chan, S.I. (1974) Biochemistry 13, 4381-4385.
2. Hsu, M., and Chan, S.I. (1973) Biochemistry 12, 3872-3876.
3. Lau, A.L.Y., and Chan, S.I. (1974) Biochemistry 13, 4942-4948.
4. Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A., and Morris, H.R. (1975) Nature 258, 577-579.
5. Bleich, H.E., Cutnell, J.D., Day, A.R., Freer, R.J., Glasel, J.A., and McKelvy, J.F. (1976), Proc. Natl. Acad. Sci., in press.
6. Kuhar, M.J., Pert, C.B., and Snyder, S.H. (1973), Nature 245, 447-450.
7. Glasoe, P.K., and Long, F.A. (1960) J. Phys. Chem. 64, 188-190.
8. Bleich, H.E., and Glasel, J.A. (1976) J. Magn. Resonance 18, 401-405.
9. Cutnell, J.D., Bleich, H.E., and Glasel, J.A. (1976) J. Magn. Resonance 21, 43-46.
10. Lee, A.G., Birdsall, N.J.M., Levine, Y.K., and Metcalfe, J.C. (1972) Biochim. Biophys. Acta 255, 43-56.
11. Bystrov, V.F., Dubrovina, N.I., Barsukov, L.I., and Bergelson, L.D. (1971 Chem. Phys. Lipids 6, 343-350.
12. Horwitz, A.F., Horsley, W.J., and Klein, M.P. (1972) Proc. Natl. Acad. Sci. 69, 590-593.
13. Finer, E.G., Hauser, H., and Chapman, D. (1969) Chem. Phys. Lipids 3, 386-392.
14. Phillips, M.C., Williams, R.M., and Chapman, D. (1969) Chem. Phys. Lipids 3, 234-244.