Deuterium NMR study of head-group deuterated phosphatidylserine in pure and binary phospholipid bilayers

Interactions with monovalent cations Na^+ and Li^+

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Head-group deuterated 1,2-dimyristoyl-*sn*-glycero-3-phosphorylserine (DMPS) was synthesized. ^2H NMR spectra reflect the ionic strength-dependent polymorphism of DMPS aqueous dispersions. Results obtained with pure DMPS and mixed bilayers with phosphatidylcholine or phosphatidylethanolamine at various NaCl or LiCl concentrations indicate that interactions with Na^+ and Li^+ have very different effects upon the head-group quadrupole splittings.

Phosphatidylserine *^2H-NMR* *Deuterated phospholipid* *Membrane-ion interaction* *Polymorphism*

1. INTRODUCTION

Phosphatidylserine (PS) is a negatively charged phospholipid and thus plays an important role in membrane surface interactions with positively charged ions [1] and proteins [2].

The behavior of PS in aqueous solutions is very different from those of zwitterionic phospholipids. At high water content and in the absence of salt, large amounts of water are incorporated between adjacent charged bilayers because of repulsive forces, leading to large vesicular structures [3,4]. Monovalent cations such as Na^+ and K^+ shield the negatively charged surface, and the water layer is reduced [5]. An even larger bilayer dehydration effect occurs with divalent cations, and strong high-melting (155°C) complexes are obtained with Ca^{2+} [6,7]. Interaction of Li^+ with PS appears to be distinct from that of other monovalent cations with respect to its ability to form strong highly dehydrated PS complexes similar to those observed for divalent cations [8]. It has been argued that this particular behavior may relate to the clinical efficiency or toxic side effects of this psychopharmacologically potent ion [8].

Here, ^2H NMR has been used to monitor salt-induced phase changes of head-group deuterated PS bilayers. ^2H NMR spectra also provide data at the molecular level upon head-group interactions with the monovalent cations Na^+ and Li^+. As pointed out recently, ^2H NMR allows precise structural studies of cation-lipid interactions over a wide concentration range [9,10].

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; DMPS, dimyristoylphosphatidylserine; DSC, differential scanning calorimetry

2. MATERIALS AND METHODS

For simplification of discussion the following nomenclature is employed for the phosphoserine head-group segment:

O(O)P(O)O-C**D**$_2$-CH-(NH$_2$)COOH	α-deuterated DMPS
O(O)P(O)O-CH$_2$-C**D**-(NH$_2$)COOH	β-deuterated DMPS

DMPC and DMPE were purchased from Sigma.

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Head-group deuterated DMPS (free acid form) was synthesized by using a modified version of a previous synthesis [11]: 1,2-dimyristoyl-*sn*-glycero-3-phosphoric acid (DMPA) was condensed with *N*-carbobenzoxybenzyl-L-serine using triisopropylbenzenesulfonyl chloride (TPS), and protective groups were removed by hydrogenolysis on platinum oxide. *N*-Carbobenzoxybenzyl-L-serine was prepared according to Baer and Maurukas [12]. Deuterated serine was prepared by enzymatic optical resolution [13] of acetyl-DL-serine with hog acylase. Crude deuterated acetyl-DL-serine was obtained according to King [14] and purified by cation-exchange chromatography [15]. DMPA was prepared using phospholipase D as in [16].

DMPS was used as the monosodium salt which was obtained by Folch washing [17] of acidic DMPS with 1 N Hepes buffer, 100 mM EDTA (pH 7.5). Lipid mixtures with DMPC or DMPE were dissolved in $CHCl_3$/MeOH (9:1), and solvents removed under N_2. The residues were dried under a high vacuum and lyophilized from pure water dispersions. For DSC measurements, lipids (2 mg) were dispersed above T_c in 1 ml of 100 mM NaCl, 1 mM EDTA, 10 mM Hepes buffer (pH 7) and degassed under vacuum. Samples were analyzed in a Microcal MC-1 high-sensitivity differential scanning calorimeter at a temperature scan rate of 20°C/h. For NMR experiments two types of sample were prepared. For the first type of sample (method 1), lipids (50 mg) were dispersed in excess (10 ml) buffer (10 mM Hepes, 1 mM EDTA, pH 7) and centrifuged (40000 rpm, 2 h) after 3 freeze-thaw cycles. The pellet was transferred into an NMR tube and the volume adjusted to 0.5 ml. Increasing amounts of salt were added stepwise into the NMR tube from a 5 N stock solution, and 3 freeze-thaw cycles were applied to the sample to obtain correct equilibration of salt among the lipid bilayers. A second set of samples (method 2) was prepared as for method 1, except membranes were preformed in small amounts of buffer (100 μl/50 mg lipid) before dispersion in excess buffer. The buffer used in method 2 was 10 mM Hepes, 1 mM EDTA, 100 mM NaCl (pH 7) and higher salt concentrations were obtained as described in method 1.

^{2}H NMR spectra were recorded on a Bruker WM-500 apparatus at 76.8 MHz with the single-pulse mode. The pulse width was 60 μs, and recycling delay 50 ms. Phosphorus NMR measurements were made at 36.4 MHz under proton-decoupling conditions on a Bruker WH-90 instrument.

3. RESULTS AND DISCUSSION

3.1. *Ionic strength-dependent polymorphism of pure DMPS aqueous dispersion*

Fig.1 shows ^{2}H NMR spectra of pure α-deuterated DMPS aqueous dispersions in the fluid state at 45°C (for pure head-group deuterated DMPS, T_c was found to be 37°C by DSC), formed using method 1 (see section 2) and recorded at various NaCl concentrations. At low ionic strength, a relatively narrow resonance overlapped by a broader signal can be seen. Upon increasing the NaCl concentration, the narrow signal disap-

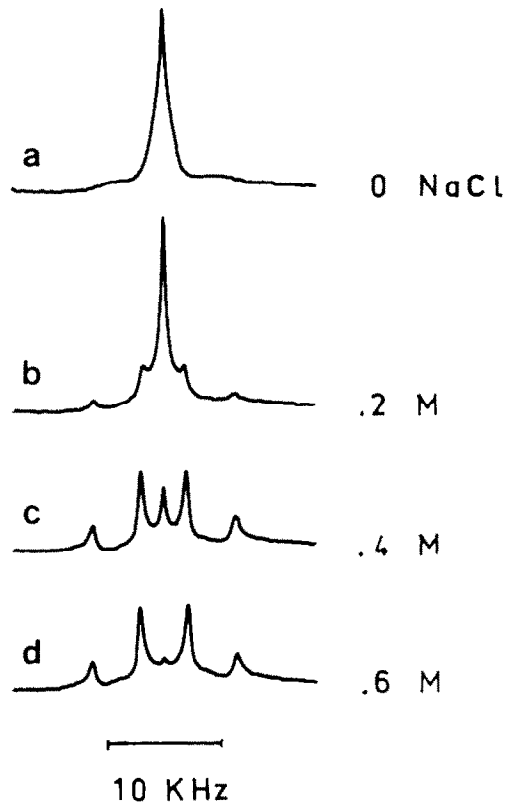

Fig.1. ^{2}H NMR spectra (76.7 MHz; single-pulse method) of α-deuterated DMPS (Na^+ salt) buffered dispersion (10 mM Hepes, 1 mM EDTA, pH 7), with various NaCl concentrations (see preparation method 1). Measuring temperature 45°C (20000 scans).

pears, whereas two well-resolved quadrupole splittings appear, yielding spectra similar to those reported by Browning and Seelig [18] in previous ^2H NMR studies of head-group deuterated DMPS. These authors assumed that the occurrence of two signals is due to the non-equivalence of the two deuterons of the serine α-CD_2. ^{31}P NMR experiments carried out on the same samples yield an isotropic resonance (120 Hz at half-width) in the absence of salt (fig.2a) and a multilamellar powder-type spectrum at 1 M NaCl (fig.2b).

From X-ray scattering and freeze-fracture experiments, Atkinson et al. [4] reported that in dilute aqueous dispersions of low ionic strength, PS bilayers exhibit a pronounced swelling (i.e. continuous hydration), leading to the formation of spherical particles, while at high ionic strength multilamellar structures are observed. Our data are in agreement with these results. Indeed, the intense isotropic signal obtained in the absence of NaCl can be attributed to motional averaging of the quadrupole splittings induced by fast phospholipid lateral diffusion along the curved spherical bilayer and rapid tumbling of the vesicular structures on the NMR time scale [19]. The broader component seen on the ^2H NMR spectra obtained in the absence of salt may be due to a distribution of lipidic particles whose sizes and bilayer packing properties are intermediate between those of the vesicular population (characterized by the isotropic resonance), and those of the multilamellar liposomes which give well-defined quadrupole splittings.

Other X-ray scattering studies [5] of DMPS aqueous dispersions show that the salt-induced exclusion of water from adjacent lipid bilayers leads to a more packed multilamellar arrangement. It is shown that upon increasing the NaCl concentration, the lamellar periodicity of a 50% water dispersion of PS is reduced down to a minimum value of 62 Å which is reached at 0.5 M salt. Here, complete disappearance of the central isotropic peak occurs at 0.5 M NaCl in good agreement with the X-ray experiments.

Previously, Browning [20] also noticed the presence of an isotropic resonance in ^2H and ^{31}P NMR spectra of acidic phospholipid membranes, and reported that preforming bilayers in a small amount of buffer (see method 2) minimize this signal. This method allows us to obtain DMPS deuterium spectra without an isotropic signal, even at low ionic strength. It is important to note that under such conditions quadrupole splittings measured at the various NaCl concentrations were nearly identical to those obtained with samples in which the isotropic component occurred.

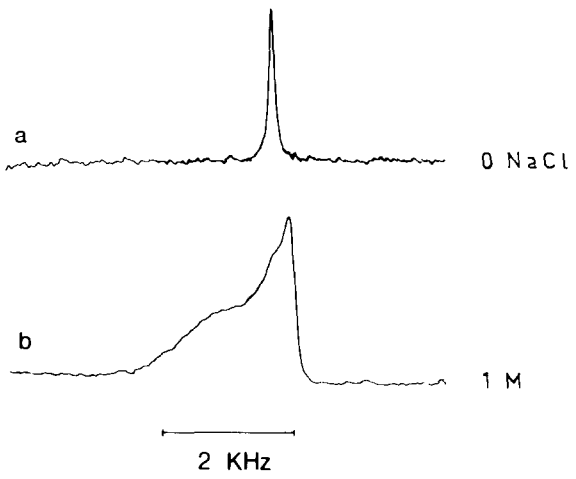

Fig.2. ^{31}P NMR spectra (36.4 MHz, proton-decoupled) of DMPS (Na^+ salt) buffered dispersion (10 mM Hepes, 1 mM EDTA, pH 7) (see preparation method 1): (a) no salt (18000 scans), (b) 1 M NaCl (70000 scans). Measuring temperature 45°C.

3.2. *DMPS head-group interactions with monovalent cations Na^+ and Li^+, in pure and binary phospholipid bilayers*

Comparative studies of α-deuterated DMPS interactions with Na^+ and Li^+ were carried out with 3 types of dispersions: (i) pure DMPS, (ii) 25% DMPS in DMPC, (iii) 25% DMPS in DMPE. For the binary mixtures, high-sensitivity DSC experiments show a single transition occurring at 26 or 49°C, respectively, for mixed dispersions with DMPC or DMPE, concurring with previous DSC studies [21] indicating complete miscibility of DMPS in DMPC and DMPE bilayers.

To avoid the occurrence of the isotropic signal observed at low ionic strength, membranes were preformed in small amounts of 100 mM NaCl buffer according to method 2 (see section 2). The resulting spectra, recorded in the fluid state 8°C above T_c, are shown in fig.3. The two quadrupole splittings observed with pure DMPS bilayers (3.8 and 14 kHz) are also detected on the spectra

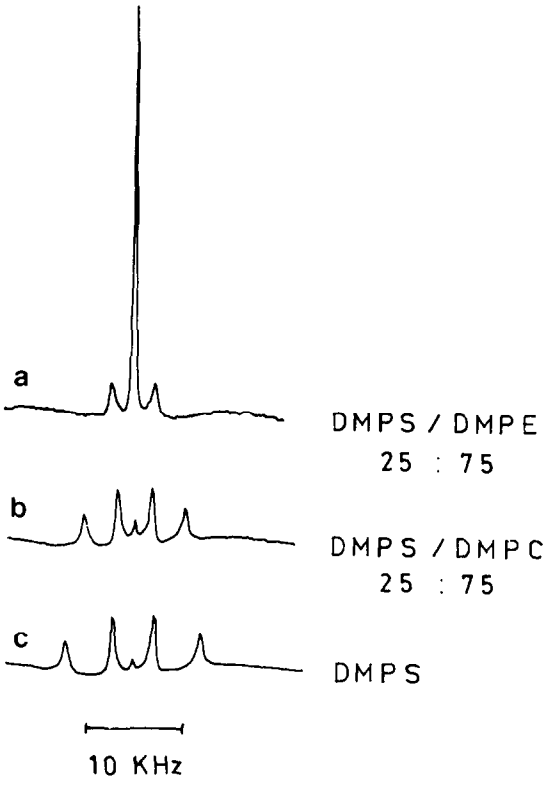

Fig.3. ^{2}H NMR spectra (76.7 MHz) of α-deuterated DMPS buffered dispersion (10 mM Hepes, 1 mM EDTA, 100 mM NaCl, pH 7) (see preparation method 2): (a) 25% DMPS in DMPE (57°C), (b) 25% DMPS in DMPC (34°C), (c) pure DMPS (45°C); 20000 scans.

recorded from mixed bilayers with DMPC or DMPE, although their values are decreased. This decrease in quadrupole splittings is more pronounced in mixtures with DMPE than with DMPC bilayers. On a reduced temperature scale [$T = T_c(1 + K)$, giving 31.7 and 59.8°C, respectively, for DMPC and DMPE-containing membranes and 45°C for pure DMPS with $K = 0.22$], the differences are more pronounced. In DMPE-containing membranes the smaller quadrupole splitting is nearly zero (single resonance).

It is interesting to note that although intermolecular bonding of PS is possible with PE bilayers but cannot occur with PC, more important quadrupole splitting reductions are observed in PE-containing bilayers. Thus, the difference may rather be explained as being due to lower steric hindrance of the serine moiety in the DMPS/DMPE bilayer, considering that the PE head group is smaller than that of PC.

Fig.4 displays spectra of DMPS/DMPC bilayers at high NaCl or LiCl concentration. It can be seen that 2 M NaCl does not affect significantly the outer quadrupole splitting, while a slight increase of the inner signal is observed. On the other hand, after addition of 2 M LiCl to the 100 mM NaCl DMPS/DMPC dispersion, the external quadrupole splitting is reduced by almost a factor of 2, whereas the internal signal is rather unmodified. Similar results are observed with DMPS/DMPE mixed bilayer (not shown). In the case of the pure DMPS dispersion at 45°C, addition of increasing amounts of LiCl leads to a ^{2}H NMR signal which becomes broadened beyond detection, at a salt concentration in the range 50–150 mM. This can be explained by the considerable augmentation of

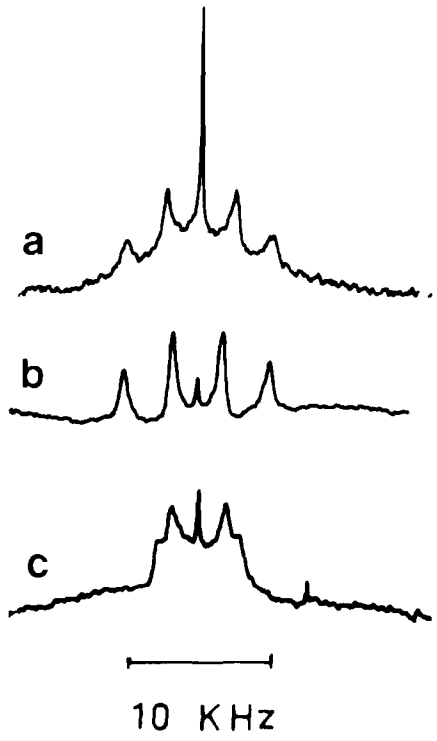

Fig.4. ^{2}H NMR spectra (76.7 MHz) of 25% α-deuterated DMPS in DMPC, dispersed in 100 mM NaCl, dispersed in buffer (10 mM Hepes, 1 mM EDTA, 100 mM NaCl, pH 7) (see preparation method 2): (a) 2 M NaCl, (b) 100 mM NaCl, (c) 2 M LiCl (+ 100 mM NaCl). Measuring temperature 34°C (20000 scans).

T_c observed in the presence of this ion (T_c = 45°C at 100 mM LiCl [5]). Since such effects are not observed in the DMPS mixed bilayers with DMPC or DMPE, this suggests that no significant lateral segregation and freezing of the DMPS molecules occur in these systems at LiCl concentrations up to 2 M.

The salt dependence of α-deuterated DMPS quadrupole splittings is summarized in fig.5. Determination of DMPS quadrupole splittings becomes difficult when the values are close to zero. For mixed bilayers with DMPE at high LiCl concentrations, the internal and external signal could not be measured with accuracy. The outer quadrupole splitting reduction observed in fig.5 with increasing LiCl concentrations may be due to either a conformational change or an increase in motional freedom of the DMPS head group. The concomitant slight increase of the internal signal leads us to believe that salt addition to the lipid bilayer might induce a conformational change of the DMPS polar head group.

Similar results and conclusions were made by Akutsu and Seelig [22] in ^{2}H NMR studies of headgroup deuterated DPPC ionic interactions, although they suggested that the ion-induced conformational change should be small. On the other hand, these authors thought that the quantitative differences they observed on DPPC deuterium NMR spectra in the presence of various cations reflect only different binding affinity and that ion-induced conformational changes were qualitatively similar for all mono-, di- and trivalent ions investigated.

In the framework of this interpretation, a crude approximation of the apparent binding constants K_{Li} and K_{Na} on the basis of a simple Langmuir adsorption interpretation [22] of our ^{2}H NMR binding data (fig.5) would give a value of 200 for K_{Li}/K_{Na}. Considering previous binding studies of monovalent cations to PS membranes, this ratio appears to be quite high [23,24]. This suggests that the differences observed between Na^+ and Li^+ effects may at least in part be related to the nature

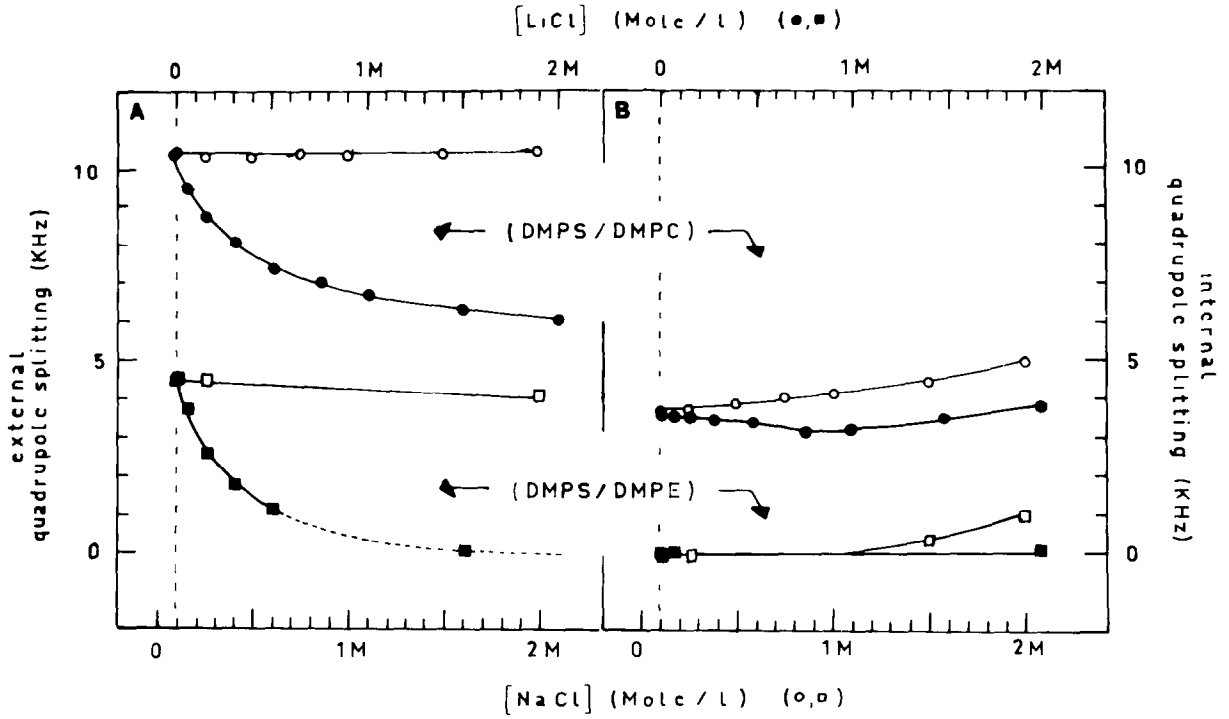

Fig.5. External and internal quadrupole splitting (kHz) of α-deuterated DMPS in mixed bilayers with DMPC (○, ●) or DMPE (□, ■) plotted vs total salt concentration: (○, □) NaCl; (●, ■) LiCl. (A) External quadrupole splitting, (B) internal quadrupole splitting. Samples were prepared as described for fig.4.

of the conformational change induced by these two ions. Furthermore, the curves obtained for the inner signal (fig.5B) indicate that the changes in quadrupole splittings induced by ions are rather complex and probably due to the combination of different phenomena occurring simultaneously, such as electrostatic energy reduction by cation screening of the negatively charged bilayers and cation-specific adsorption on the membrane surface.

A more detailed analysis of these effects, including those of the divalent cations Ca^{2+} and Mg^{2+}, is now underway with the DMPS headgroup deuterated at either the α- or β-position, and should provide us with more detailed information on the PS polar group ionic interactions in model membranes.

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REFERENCES

[1] Papahadjopoulos, D. (1978) Cell Surf. Rev. 5, 765–790.
[2] Devaux, P.F. and Seigneuret, M. (1985) Biochim. Biophys. Acta 822, 63–125.
[3] Hauser, H. and Phillips, M.C. (1973) J. Biol. Chem. 248, 8585–8591.
[4] Atkinson, D., Hauser, H., Shipley, G.G. and Stubbs, J.M. (1974) Biochim. Biophys. Acta 339, 10–29.
[5] Hauser, H. and Shipley, G.G. (1983) Biochemistry 22, 2171–2178.
[6] Hauser, H. and Shipley, G.G. (1984) Biochemistry 23, 34–41.
[7] Papahadjopoulos, D., Vail, W.J., Jacobson, K. and Poste, G. (1975) Biochim. Biophys. Acta 394, 483–491.
[8] Hauser, H. and Shipley, G.G. (1981) J. Biol. Chem. 256, 11377–11380.
[9] Altenbach, C. and Seelig, J. (1984) Biochemistry 23, 3913–3920.
[10] Borle, F. and Seelig, J. (1985) Chem. Phys. Lipids 36, 263–283.
[11] Aneja, R., Chadha, J.S. and Davies, A.P. (1970) Biochim. Biophys. Acta 218, 102–111.
[12] Baer, E. and Maurukas, J. (1954) J. Biol. Chem. 212, 25–38.
[13] Akabori, S., Otani, T.T., Marshall, R., Winitz, M. and Greenstein, J.P. (1959) Arch. Biochem. Biophys. 83, 1–9.
[14] King, J.A. (1947) J. Am. Chem. Soc. 69, 2738–2741.
[15] Narita, K. (1958) Biochim. Biophys. Acta 30, 352–359.
[16] Roux, M., Huynh-Dinh, T., Igolen, J. and Prigent, Y. (1983) Chem. Phys. Lipids 33, 41–45.
[17] Folch, J., Lees, M. and Stanley, G.H. (1957) J. Biol. Chem. 226, 497.
[18] Browning, J.L. and Seelig, J. (1980) Biochemistry 19, 1262–1270.
[19] Stockton, G.W., Polnaszek, C.F., Tulloch, A.P., Hasan, F. and Smith, I.C.P. (1976) Biochemistry 15, 954–966.
[20] Browning, J.L. (1981) Biochemistry 20, 7123–7133.
[21] Silvius, J.R. and Gagne, J. (1984) Biochemistry 23, 3232–3240.
[22] Akutsu, H. and Seelig, J. (1981) Biochemistry 20, 7366–7373.
[23] Puskin, J.S. (1977) J. Membrane BIol. 35, 39–55.
[24] Eisenberg, M., Gresalfi, T., Riccio, T. and McLaughlin, S. (1979) Biochemistry 18, 5213–5223.