An intramolecular hydrogen bond with large proton polarizability within the head group of phosphatidylserine

An infrared investigation

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ABSTRACT Films of O-phospho-L-serine-P-ethylester (PSE) were studied by infrared spectroscopy. PSE films were studied pure and as 1:1 mixture with LiOH, NaOH, KOH, and $Ca(OH)_2$ as a function of the degree of hydration. The same investigations were performed if $(L\text{-glu})_n$ was added to the system (ratio 1:1, PSE/glu residue).

In the PSE molecules an intramolecular (I) $\mathrm{COOH}\cdots{}^{-}\mathrm{OP} \rightleftharpoons \mathrm{COO}^{-}\cdots\mathrm{HOP}$ (II) hydrogen bond is present. In this bond a double minimum proton potential occurs and it shows large proton polarizability. This hydrogen bond is relatively stable as shown by the neutralization experiments. At low degree of hydration the cations are present at the phosphate groups. The Li ions polarize the intramolecular hydrogen bonds much more than the other cations, i.e., the weight of the proton-limiting structure $\mathrm{COOH}\cdots{}^{-}\mathrm{OP}$ is increased by Li ions.

Regarding these results one has to assume that such a hydrogen bond is also present in the phosphatidylserine head groups. It is discussed that such hydrogen bonds could be part of a lateral charge-conducting system in the polar surfaces of biological membranes. Such systems could connect proton-creating and proton-consuming centers at the membrane surface and conduct positive charge at an extremely high rate.

I. INTRODUCTION

Some years ago we studied aqueous solutions of the molecules O-phospho-L-serine-P-methylester (PSM) and O-phosphoethanolamine-P-methylester (PEM) (1) using infrared (IR) spectroscopy. These molecules are models of the head groups of the phospholipid molecules phosphatidylserine and phosphatidylethanolamine.

In references 2 and 3 the influence of the head groups on the structure of the diacylglycerol residues was studied. Such information can, of course, not be obtained with these models. These model molecules have, however, the advantage that they are soluble in water, and furthermore, no diacylglycerol residue bands mask bands of the head groups. In the case of the PSM molecule, if it is present as zwitterion, an intramolecular (I) $\mathrm{COOH}\cdots{}^{-}\mathrm{OP} \rightleftharpoons \mathrm{COO}^{-}\cdots\mathrm{HOP}$ (II) hydrogen bond was observed (1) showing large proton polarizability (4–6) as indicated by an IR continuum in the region 3,000–1,600 cm^{-1}.

In the cristae mitrochondriales as well as in the thylakoid membranes of chloroplasts protons are pumped on one side of the membranes and flow in the opposite direction, supplying the energy for the ATP synthesis. The protons migrate from the pumping center to the ATPase. The question arose whether the protons must go out in the bulk phase or whether they can migrate in the polar surfaces of the biological membranes. This problem was studied recently by various authors with different techniques (7–14). All these authors conclude that lateral conduction within the polar surface of phopholipid layers may occur. These results were criticized, recently, on the basis of kinetic measurements of the proton transfer between the surfaces and the bulk phase (15).

A necessary precondition of a fast conduction of protons in the membrane surface would be the presence of a network of hydrogen bonds with large proton polarizability within this surface. Many different hydrogen bonds with large proton polarizability formed between side chains in polar regions of membrane proteins could be involved in such a conducting system (16). In this and in a following paper we hypothesize whether also hydrogen bonds with large proton polarizability between phospholipid molecules or between phospholipid molecules and side chains may be part of such a proton-conducting system.

Under this aspect films of O-phospho-L-serine-P-ethylester (PSE) are studied as a function of the degree of hydration. Furthermore, 1:1 mixtures of PSE and LiOH, NaOH, KOH, and $Ca(OH)_2$ are investigated. Herewith information is obtained about which protons of the PSE zwitterions are neutralized and how the various cations influence the systems. Finally, these systems are studied in the presence of $(L\text{-glu})_n$.

II. METHOD

O-Phospho-L-serine-P-ethylester was prepared as described in the following: 2 g O-phospho-L-serine (purchased from Sigma Chemical Co., St. Louis, MO) were suspended in 40 ml thoroughly dried ethanol. The

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suspension was heated and, during 1 h, an equimolar amount of freshly distilled thionylchloride was added dropwise to the refluxing suspension. By reaction with thionylchloride one yields soluble products. The light yellow solution was cooled to room temperature. Immediately after cooling the solution was evaporated in vacuum yielding a white gummy residue. This residue was treated with 50 ml ether, and after standing for some hours for crystallization, the precipitation was filtered. After washing twice in 50 ml ether/acetone (1:1) the white crystalline product was dried at 80°C for 1 h yielding 2.1 g (60%) O-phospho-L-serine-P-ethylester$\cdot 2\,H_2O$.

(L-glu)$_n$ was purchased from Miles GmbH (Munich, FRG) with a mean chain length of $n = 275$. LiOH, NaOH, KOH, and $Ca(OH)_2$ were purchased from E. Merck (Darmstadt, FRG) and Fluka (Neu-Ulm, FRG), respectively.

All systems were prepared as films on silicon supports (6 μval substance per film). PSE films were prepared from 0.03 N aqueous solution, and (L-glu)$_n$ films from a 0.03 N, 2% pyridine-containing aqueous solution. If desired, equimolar amounts of the respective aqueous solution of PSE and the metal hydroxides were added.

The precipitation of the films was performed with the centrifugation–drying procedure described in reference 17, using an ultracentrifuge. Like the IR cells described in reference 18, the cells used could be evacuated, permitting adjustment of a well defined humidity at the films by use of saturated aqueous solutions of different salts. The IR measurements were performed with an IR spectrophotometer (model 325; Bodenseewerk Perkin-Elmer, Überlingen, FRG).

III. RESULTS AND DISCUSSION

A. O-Phospho-L-serine-P-ethylester (PSE)

Fig. 1 shows the IR spectra of the zwitterion in the region 4,000–1,700 cm^{-1} as a function of the degree of hydration. In Fig. 2, *a* and *b* IR spectra of the same system are presented in the region 1,800–450 cm^{-1}. In Fig. 3 spectra of PSE after H/D exchange are shown. In Table 1 the assignments of the bands are given.

Fig. 1 shows that above 1,700 cm^{-1} a continuous absorption is observed. As in reference 1 it indicates the presence of the intramolecular (I) $COOH\cdots{}^{-}OP \rightleftharpoons COO^{-}\cdots HOP$ (II) hydrogen bonds with large proton polarizability (4–6). The intensity of this continuum decreases with increasing degree of hydration caused by the interaction of these polarizable hydrogen bonds with their hydration water environment.

Information on the position of the (I) $COOH\cdots{}^{-}OP \rightleftharpoons COO^{-}\cdots HOP$ (II) equilibria is obtained from $\nu(C = O)$ at ~1,745 cm^{-1}, from $\nu_{as}(PO_2^-)$ in the region 1,250–1,218 cm^{-1} (proton-limiting structure I), from $\nu_s(CO_2^-)$ in the region 1,423–1,395 cm^{-1}, and from $\nu[P - (OH)]$ in the region 960–936 cm^{-1} (structure II). In the case of pure PSE in Fig. 2 all these bands are intense and thus, they show that both proton-limiting structures have considerable weight, indicating that a double minimum proton potential is present in this intramolecular hydrogen bond. In the spectrum of the deuterated sample $\nu_{as}(CO_2^-)$ is observed as an intense band at 1,618 cm^{-1} (Fig. 3 *b*) (this band is masked in the H_2O-hydrated system by $\delta_{as}(NH_3^+)$ and $\delta(H_2O)$). The large intensity of this band in the deuterated sample is not caused by a deuteration-dependent shift of the proton transfer equilibrium since $\nu_s(CO_2^-)$ at ~1,411 cm^{-1} shows the same intensity in the H_2O and D_2O hydrated sample. This result confirms that proton-limiting structure (II) also has considerable weight.

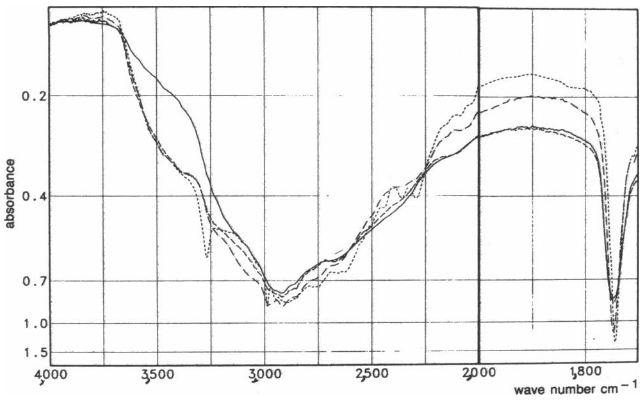


FIGURE 1 IR spectra of a PSE film at 23°C ——, 11% r.h.; ---, 29% r.h.; ------, 57% r.h.; · · · ·, 75% r.h.

The broad shoulder of $\nu(OH)$ of H_2O in the region 3,600–3,300 cm^{-1} shows that up to 29% relative humidity of the air (r.h.) weakly bonded water molecules are adsorbed to the sample. The quantity of this water increases only slightly up to 85% r.h. Only a few water molecules are attached, since the ammonium groups are cross-linked with the phosphate groups via strong $NH^{+}\cdots{}^{-}OP$ hydrogen bonds. The formation of these bonds is indicated by the relatively high position of $\delta_s(NH_3^+)$, which is observed with the samples below 57% r.h. at 1,540 cm^{-1} (Fig. 2 *a*). The stretching vibrations of the NH_3^+ groups are the main part of the broad band complex in the region 3,350–2,250 cm^{-1}. The CH_2 groups are fixed in the *trans* position because their $\delta(CH_2)$ vibration is observed as a sharp band at 1,467 cm^{-1} (21, 22).

Going up to 57% r.h. (· · · · · ·, spectrum in Fig. 2 *a*) some pronounced changes in the spectrum are observed. $\delta_s(NH_3^+)$ shifts from 1,540 to 1,519 cm^{-1} and $\delta_{as}(NH_3^+)$ appears now as a separated band at 1,615 cm^{-1}. Both findings show that the $NH_3^{+}\cdots{}^{-}OP$ interactions are slightly loosened. The band of $\nu_{as}(PO_2^-)$ shifts from 1,238 to 1,218 cm^{-1} and its intensity slightly increases. $\nu[P - (OH)]$ at 938 cm^{-1} shifts slightly toward higher wave numbers gaining doublet structure. The band of ν_s(RO-P-OEt) (R = serine) at 1,028 cm^{-1} increases strongly, indicating that the symmetry of this group is strongly disturbed. Furthermore, at 547 cm^{-1} the PO_2^-

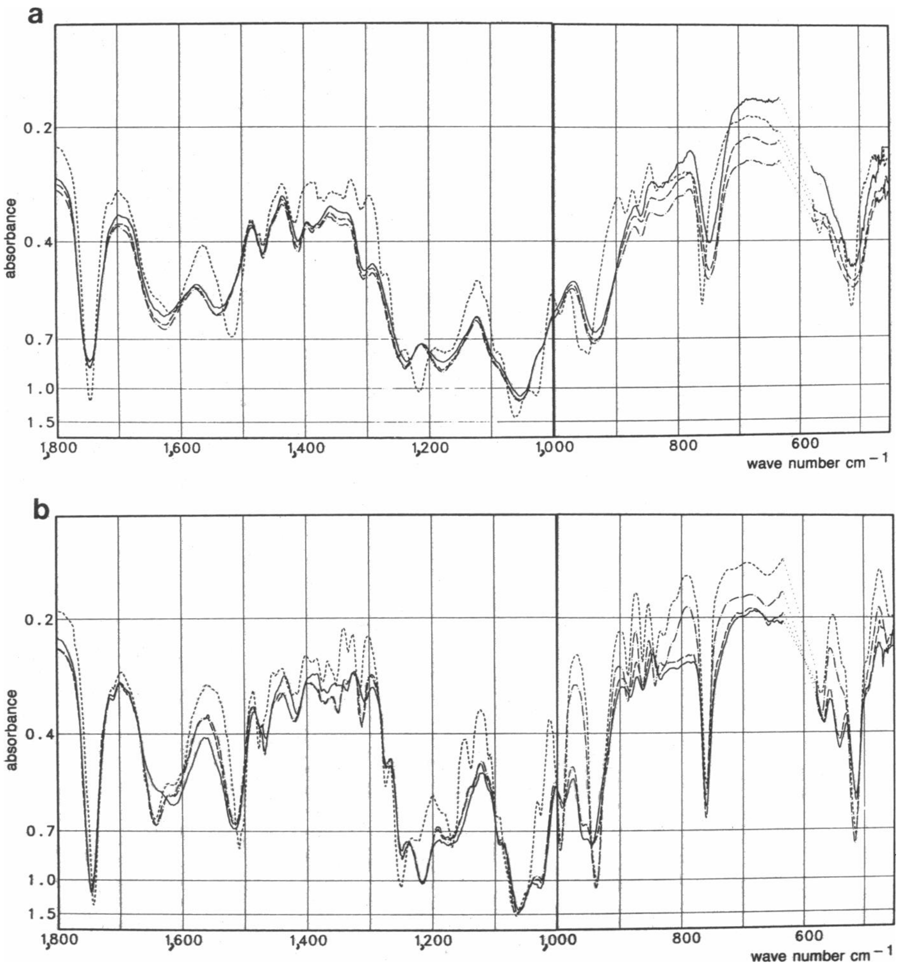


FIGURE 2 (*a*) IR spectra of a PSE film at 23°C ——, 11% r.h.; ---, 29% r.h.; ----, 44% r.h.; ····, 57% r.h. (*b*) IR spectra of a PSE film at 23°C ——, 66% r.h.; ---, 75% r.h.; ----, 85% r.h.; ····, 91% r.h.

wagging vibration arises. According to Shimanouchi et al. (29) the changes of the phosphate bands indicate a conformational change of the phosphate groups of the PSE molecule probably from *gauche-gauche* to *gauche-antiplanar*. This conformation change is probably induced by a tightly bound structural water molecule. The stretching vibration of these molecules is found in Fig. 1 in the dashed spectrum as broad absorption in the region 3,250–3,050 cm^{-1}. The CH_2 groups remain fixed in the *trans* position.

At r.h. larger than 85% the spectral feature changes again (······ spectrum in Fig. 2 *b*). $\delta_s(NH_3^+)$ shifts from 1,519 to 1,509 cm^{-1} and is now a relatively narrow band. $\nu_{as}(PO_2^-)$ shifts back from 1,218 to 1,250 cm^{-1} and $\nu[P-(OH)]$ is observed as an intense band at 936 cm^{-1}. Finally, the intensity of ν_s(RO-P-OEt) decreases. Hence the phosphate conformation changes again to *gauche-gauche*. These changes of the spectrum show that the $NH_3^+\cdots{}^-OP$ bonds are broken and the structural water molecule is removed. NH_3^+ and phosphate groups are now hydrated by water molecules bound via weak hydrogen bonds. Macroscopically, this structural change is reflected in the fact that the film becomes now fluid with further increasing r.h. Due to the breaking of the $NH_3^+\cdots{}^-OP$ bonds the transition moment of ν(C-N) at 992 cm^{-1} is strongly increased. Furthermore, at 2,991 cm^{-1} a sharp band appears (Fig. 1). The analogous band is found in the D_2O hydrated sample (Fig. 3 *a*). This band is probably ν(CH) of the methene group. The transition moment of this band increases also due to the breaking of the $NH_3^+\cdots{}^-OP$ bonds. A weak narrow band arises at 3,270 cm^{-1} (Fig. 1). In the deuterated sample this band is observed at 2,422 cm^{-1} (Fig. 3 *a*). Regarding the isotope effect of 1.34 this band should be the stretching vibration of hydrogen bond donor groups involved whenever in relatively weak hydrogen bonds. The CH_2 groups are no

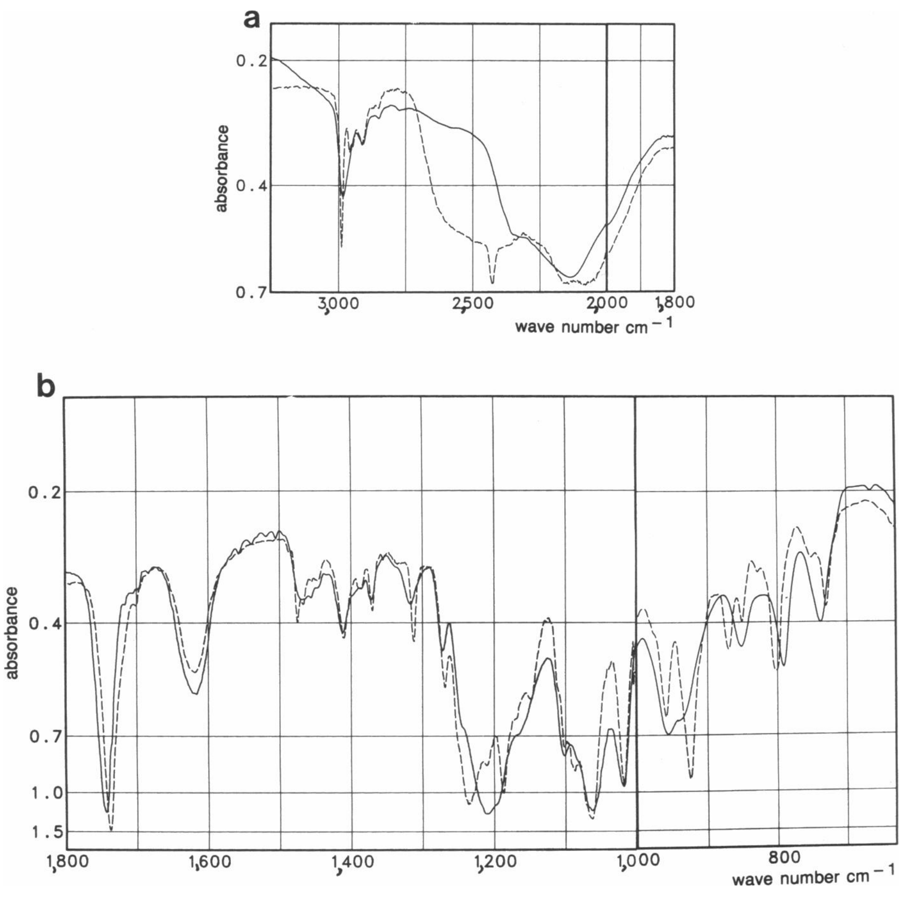


FIGURE 3 (*a* and *b*) IR spectra of deuterated PSE films at 23°C ——, 11% r.h.; ----, 75% r.h.

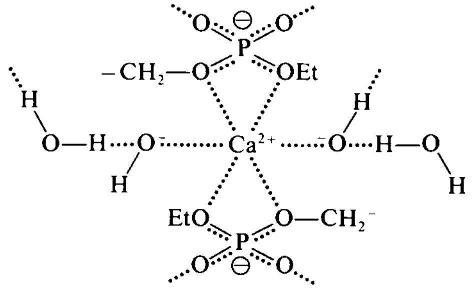
longer fixed in the *trans* position but randomly distributed as indicated by $\delta(CH_2)$, which has vanished at 1,467 cm^{-1} and is now found at 1,477 cm^{-1} (Fig. 2 *b*).

B. Influence of metal hydroxides

LiOH, NaOH, KOH, and $Ca(OH)_2$ films with PSE (1:1) were prepared to study the neutralization of protons of the PSE zwitterion as well as to study the specific influences of these cations in such systems. As shown in the scheme neutralization of the intramolecular hydrogen-bonded proton as well as of the NH_3^+ proton is possible.

Regarding the pK$_a$ values, it seems probable that the NH_3^+ proton is not affected. But we shall see that this is not true since the pK$_a$ of groups is only well defined for diluted aqueous solutions and cannot be used in other systems.

The comparison of Fig. 4 *a* (Li^+ as example for all other cations) with Fig. 1 shows that $\nu(OH)$ of the H_2O molecules is much more intense under comparable conditions. This is true for all systems if hydroxides are added.



(III)

PSE + LiOH

Fig. 4 shows the IR spectra of a 1:1 PSE/LiOH film as a function of the degree of hydration. The continuum is shown in the dashed line spectrum in Fig. 8 *a*.

The comparison of the intensity of $\nu(C = O)$ at 1,735 cm^{-1} in Fig. 4 with that of the pure PSE system in Figs. 1 and 2 shows that at least 50% of the intramolecular (I) $COOH\cdots{}^-OP \rightleftharpoons COO^-\cdots HOP$ (II) bonds are destroyed due to neutralization in the film hydrated at

11–57% r.h. If the degree of hydration increases further the number of intramolecular hydrogen bonds decreases as indicated by a decrease of the intensity of this band.

The position of the (I) $COOH\cdots{}^{-}OP \rightleftharpoons COO^{-}\cdots HOP$ (II) equilibrium in the remaining hydrogen bonds can be estimated from $\nu(C = O)$ at 1,735 cm^{-1} and from $\nu[P - (OH)]$ at ~930 cm^{-1}. $\nu_s(CO_2^-)$ and $\nu_{as}(PO_2^-)$ cannot be used since neutralized groups also contribute to these bands. $\nu[P - (OH)]$ is only observed as very weak band at 930 cm^{-1}. Thus, the proton transfer equilibrium is strongly shifted in favor of proton-limiting structure (I). Hence the Li ions interact strongly with the

TABLE 1 Assignment of the bands of O-phospho-L-serine-P-ethylester (PSE), in brackets the wave number value of the deuterated PSE

Wave number			Vibration	Reference
	cm^{-1}			
11 (11) % r.h.	57 % r.h.	85 (75) % r.h.	%	
3,400	3,400	3,400	$\nu(H_2O)$	
		3,270	(?)	
	3,200–3,100		$\nu(OH)$, Strongly hydrogen-bonded water molecules (structural water)	
3,250–2,250	3,250–2,250	3,250–2,250	Broad $\nu(NH_3^+)$ band	19
		2,991	$\nu(C - H)$	20
2,951 (2,956)	2,951	2,951 (2,956)	$\nu_{as}(CH_3)$	20
2,920 (2,919)	2,920	2,920 (2,919)	$\nu_{as}(CH_2)$	20
2,870 (2,870)	2,870	2,870 (2,870)	$\nu_s(CH_3)$	20
2,856 (2,852)	2,856	2,856 (2,852)	$\nu_s(CH_2)$	20
		(2,700-2,250)	$\nu(D_2O)$	
		(2,422)	(?)	
(2,136)		(2,212)	$\nu_{as}(ND_3^+)$	
		(2,072)		
1,747	1,747	1,742	$\nu(C = O)$	
1,625 (1,618)	1,640	1,640 (1,618)	$\nu_{as}(CO_2^-) + \delta(H_2O)$	
Broad	1,615	1,613	$\delta_{as}(NH_3^+)$	
1,540	1,519	1,509	$\delta_s(NH_3^+)$	
	1,477	1,477 (1,475)	$\delta(CH_2)$ random	21
1,467	1,467	1,467 (1,470)	$\delta(CH_2)$ *trans*	22
1,447	1,447	1,447	$\delta_{as}(CH_3)$	
1,409 (1,411)	1,417	1,423 (1,411)	$\nu_s(CO_2^-)$	
		1,415		
1,387	1,377	1,376		
1,377	1,369	1,356		
1,344	1,351	1,354	Vibrations with $\nu(C - O)$ and $\delta(OH)$ character	23
1,305	1,310	1,315		
	1,338	1,332	$\nu(C - O)$	
Masked	1,276	1,274	$\nu(P = O)$	
1,238	1,250 (w)	1,250 (s)	$\nu_{as}(PO_2^-)$	24, 25
Broad	1,218 (s)	1,224 (1,233)		29
1,180	1,180	1,185	$\nu_{as}(RO - P - OEt)$*	26
	1,110	1,110		
	1,092	1,092		
1,054 (1,062)	1,065	1,065 (1,062)	$\nu_s(PO_2^-)$	
	1,028	1,028	$\nu_s(RO - P - OEt)$	
1,020	1,020	1,020	$\nu(C - NH_2)$	27
	990	992	$\nu(C - NH_3^+)$	
(953)		(956)	$\nu(C - ND_3^+)$	
	959			
938 (933)	946	936 (924)	$\nu[P - (OH)]$	28
(552)	567	570 (557)		
	543		$\delta(O - P - O)$	29
514 (504)	514	514 (504)		

The numbers in parentheses are wave number values of deuterated PSE.
*R, serine.

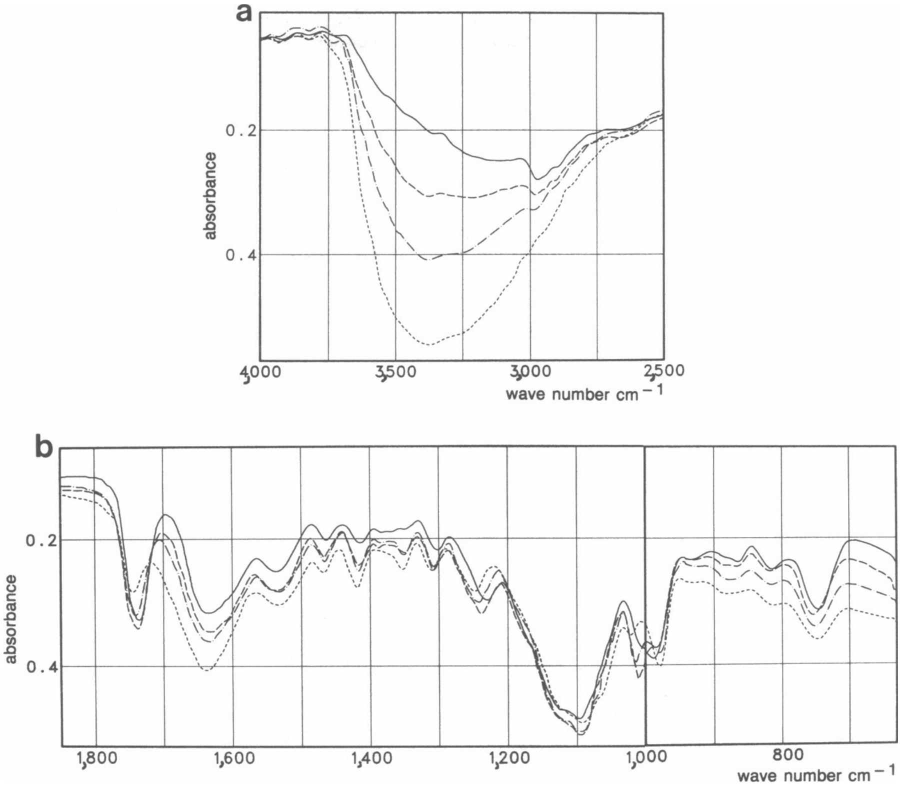


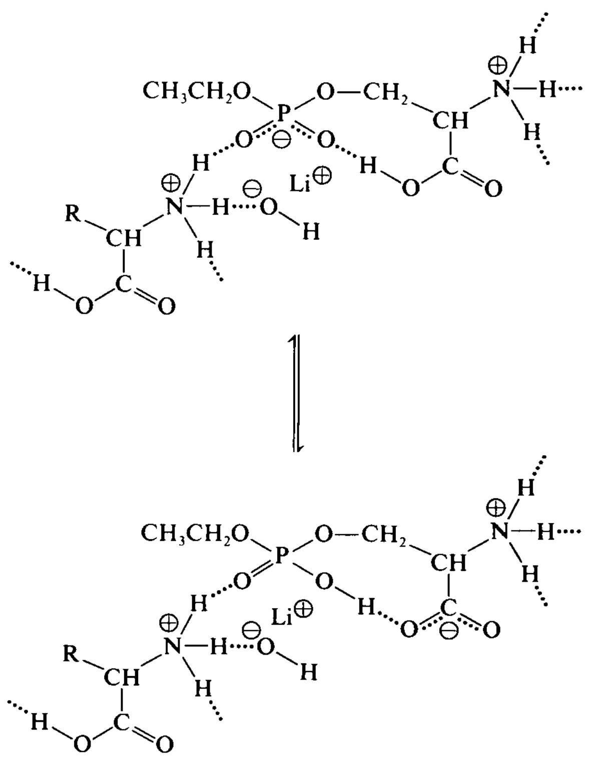
FIGURE 4 (*a* and *b*) IR spectra of a film prepared from PSE + LiOH (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, 11% r.h.; ---, 57% r.h.; -·-·, 75% r.h.; ·····, 85% r.h.

phosphate groups if not too much hydration water is present and polarize the intramolecular hydrogen bonds in this way.

The interaction of the Li ions with the PO_2^- groups is indicated by $\nu_{as}(PO_2^-)$. At 11% r.h. this band is observed at 1,234 cm^{-1}. It shifts with increasing degrees of hydration (loosening of the Li^+-phosphate bond) toward larger wave numbers.

The intensity of the continuum in the region 2,250–1,600 cm^{-1} is weak with this system (Fig. 8 *a*) since the (I) $COOH\cdots{}^-OP \rightleftharpoons COO^-\cdots HOP$ (II) equilibrium is shifted to a large extent to the left-hand side.

The deprotonation of the amino groups can be studied using $\nu(C-N)$. In the case of $C-NH_3^+$ groups this band is observed at 988 cm^{-1} and with $C-NH_2$ groups, at 1,019 cm^{-1}. Fig. 4 *b* shows that already at 11% r.h. a shoulder is observed at 1,019 cm^{-1}. With increasing r.h. up to 57% this band increases strongly and decreases again if the r.h. further increases. At 85% r.h. the band at 988 cm^{-1} is again intense and at 1,019 cm^{-1} only a weak band remains. These results show that already at 11% r.h. some NH_3^+ groups are neutralized, at 57% r.h. the number of neutralized groups is very large and decreases again strongly with increasing degree of hydration. The


(I)

(II)

above formula illustrates this hydration degree dependent neutralization. At low degree of hydration the Li ion polarizes the $NH^+ \cdots {}^-OH$ hydrogen bond so strongly that the proton cannot transfer to the OH^-. If the Li ion is loosened from the OH^- the proton may transfer in the $NH^+ \cdots {}^-OH \rightleftharpoons N \cdots HOH$ bond and thus, NH_3^+ groups are neutralized. If the degree of hydration increases further the NH_2 groups are again reprotonated by water molecules. From the literature (30) it is known that Li ions interact strongly with NH_2 groups. Thus, also an interaction of the Li ions with the NH_2 groups may favor the deprotonation.

PSE + NaOH system

Fig. 5 shows the IR spectra of a 1:1 PSE/NaOH film as a function of the degree of hydration. The continuum is shown in the spectrum in Fig. 8 *a*.

Also in the Na^+ system the comparison of the intensity of $\nu(C = O)$ at 1,740 cm^{-1} with that in the case of pure PSE shows that already at 11% r.h. at least 50% of the intramolecular hydrogen bonds are destroyed due to neutralization. The number of destroyed bonds increases in the Na^+ system with increasing degree of hydration, this occurs especially in the step from 57 to 75% r.h.

At 11% r.h. the Na ions interact with the PO_2^- groups as shown by $\nu_{as}(PO_2^-)$ which is found at 1,234 cm^{-1} shifting with increasing degree of hydration toward higher wave numbers since the Na ions are loosened from the phosphate groups. The influence of the Na ions on the position of the (I) $COOH \cdots {}^-OP \rightleftharpoons COO^- \cdots HOP$ (II) equilibrium is, however, much less than that of Li ions as indicated by the marked band of the $\nu[P - (OH)]$ vibration at 920 cm^{-1}. It shows that proton-limiting structure II has considerable weight.

The continuum in the region 2,250–1,600 cm^{-1} is much more intense in the Na^+ compared with the Li^+ system (Fig. 8 *a*). This result confirms that the intramolecular hydrogen bond is less strongly polarized by the cation field. The proton polarizability is larger as indicated by the more intense continuum. Both proton-limiting structures have considerable weight.

The band of $\nu(C - N)$ of $C - NH_3^+$ groups is found at 974 cm^{-1}. Only a weak shoulder due to the respective vibration of $C - NH_2$ groups is found at ~1,020 cm^{-1}. This result can be explained as follows: At low degrees of hydration the electrostatic field of the Na ions at the $NH_3^+ \cdots {}^-OH$ bonds is still enough strong that only few NH_3^+ groups are neutralized by transfer of the H^+ to OH^-. At higher degrees of hydration the deprotonated NH_3^+ groups are instantaneously reprotonated due to the excess of water molecules.

PSE + KOH system

The situation is completely different in the case of the 1:1 PSE + KOH system. The IR spectra of this system are shown in Fig. 6. The continuum is shown in the dashed line spectrum in Fig. 8 *b*.

The spectrum of the sample at 11% r.h. drawn with a solid line in Fig. 6 shows the following: At this degree of hydration also in the K^+ system at least 50% of the intramolecular hydrogen bonds are destroyed by neutralization. In the remaining (I) $COOH \cdots {}^-OP \rightleftharpoons COO^- \cdots HOP$ (II) bonds limiting structure II has considerable weight as shown by $\nu[P - (OH)]$ at 920 cm^{-1}. Hence also in the K^+ system these bonds show considerable proton polarizability as indicated by the continuum in the dashed line spectrum in Fig. 8 *b*.

The behavior of the system changes, however, completely if the degree of hydration is increased by a raise of the r.h. to 29%. $\delta_s(NH_3^+)$ at 1,550 cm^{-1} has vanished to a large extent and instead $\delta(NH_2)$ is observed at 1,572

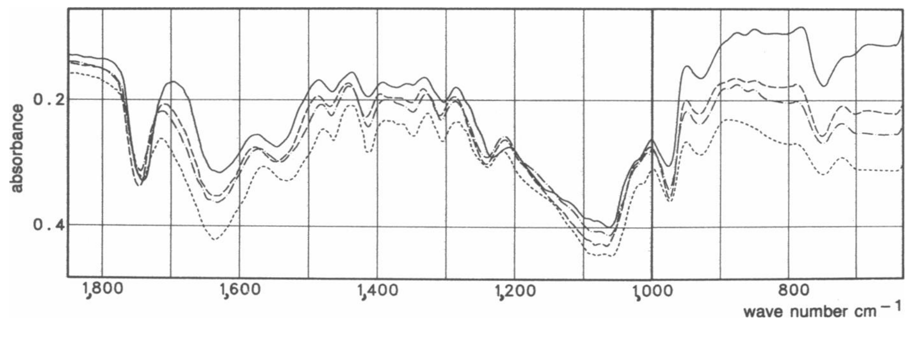


FIGURE 5 IR spectra of a film prepared from PSE + NaOH (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, 11% r.h.; ---, 44% r.h.; ----, 57% r.h.; ····, 75% r.h.

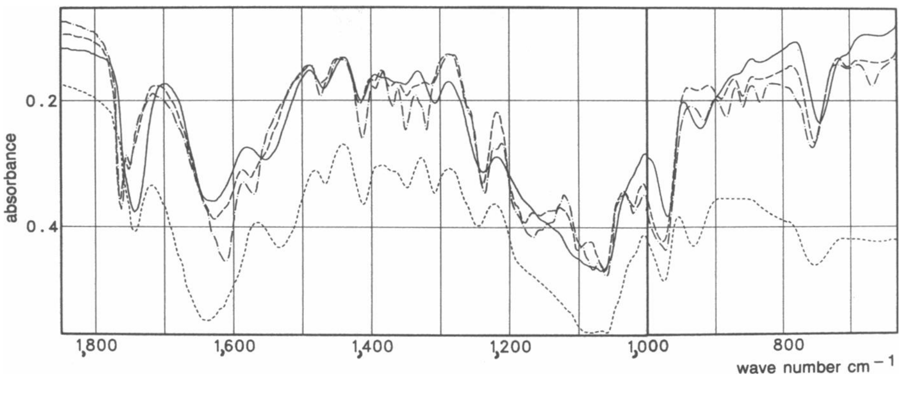


FIGURE 6 IR spectra of a film prepared from PSE + KOH (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, 11% r.h.; ---, 29% r.h.; ----, 75% r.h.; ····, 85% r.h.

cm^{-1}. The relatively weak electrical field of the K ions allows the transfer of the charge in the $NH^+ \cdots {}^-OH$ bonds and thus, neutralization of the NH_3^+ groups. Instantaneously, instead of $\nu(C=O)$ at 1,742 two $\nu(C=O)$ bands arise, a more intense band at 1,763 cm^{-1} and a less intense one at 1,749 cm^{-1}. The band at 1,763 cm^{-1} demonstrates that a large number of the intramolecular hydrogen bonds are broken. The weaker $\nu(C=O)$ band at 1,742 cm^{-1} may be caused by the carbonyl groups in structure I and the relatively weak $\nu[P-(OH)]$ band at 910 cm^{-1} by structure II in molecules in which the intramolecular hydrogen bond is still formed. The $\nu(C-N)$ band also shows that the protons are removed from the NH_3^+ groups. With the hydration step from 11 to 29% r.h. a weak band at 1,021 cm^{-1} and a relatively broad intense band at 985 cm^{-1} arises. The band at 1,021 cm^{-1} is $\nu(C-N)$ from $C-NH_2$ groups in molecules in which the intermolecular bond is formed and the band at 985 cm^{-1} the analogous vibration of molecules in which the intramolecular hydrogen bond is broken.

With increasing degree of hydration in the step from 75 to 85% r.h. at the sample the NH_2 groups are reprotonated to a large extent by the water molecules. This is shown since in the dashed spectrum in Fig. 6 instead of $\delta(NH_2)$, $\delta_s(NH_3^+)$ is observed again. Furthermore, in this spectrum instead of bands of the $C-NH_2$ groups, $\nu(C-N)$ of $C-NH_3^+$ groups is found again at 970 cm^{-1}. Intramolecular (I) $COOH \cdots {}^-OP \rightleftharpoons COO^- \cdots HOP$ (II) bonds are formed again as shown by $\nu(C=O)$ at 1,742 cm^{-1} and $\nu[P-(OH)]$ at 920 cm^{-1}. Both proton-limiting structures have considerable weight since $\nu(C=O)$ as well as $\nu[P-(OH)]$ is relatively intense. Thus, the remaining intramolecular hydrogen bonds are largely symmetrical. This is confirmed by the intense continuum which extends also toward smaller wave numbers (dashed line spectrum in Fig. 8 *b*). It demonstrates that the intramolecular hydrogen bond shows particularly large proton polarizability.

The PSE + $Ca(OH)_2$ system

The IR spectra of this system are shown in Fig. 7. The continuum is found in the ····· spectrum in Fig. 8 *b*. The comparison of the intensity of the $\nu(C=O)$ band at 1,745 cm^{-1} in Fig. 7 with those of the respective band in pure PSE in Fig. 2 shows that $Ca(OH)_2$ does not destroy the intramolecular (I) $COOH \cdots {}^-OP \rightleftharpoons COO^- \cdots HOP$ (II) bonds. The same is true with regard to the NH_3^+ groups since only the $\nu(C-N)$ band of $C-NH_3^+$ groups is found at 981 cm^{-1}. Thus, no groups are neutralized by the OH ions.

Both proton-limiting structures of the intramolecular hydrogen bond have considerable weight as indicated by $\nu(C=O)$ at 1,745 cm^{-1}, $\nu_{as}(PO_2^-)$ at 1,232 cm^{-1}, $\nu_s(CO_2^-)$ at 1,410 cm^{-1}, and $\nu[P-(OH)]$ at 936 cm^{-1}.

Fig. 8 *b* (····· spectrum) shows that the continuous absorption is not only observed in the region above 1,700 cm^{-1}. In the Ca^{2+} system not only the intramolecular bonds show large proton polarizability and contribute to the continuum. The OH ions form with water $H_3O_2^-$ groups, which are present in the hydrate structure network. The hydrogen bond of these groups is so strongly polarized by the electrical field of the Ca ions that no PSE protons can transfer to these groups and neutralize the negative charges. But the hydrogen bond in these $H_3O_2^-$ groups shows proton polarizability and contributes to the continuum (4, 5, 31).

$\nu_{as}(PO_2^-)$ is found at 1,232 cm^{-1}, i.e., shifted toward smaller wave numbers, and this result is independent of the degree of hydration. Hence, the phosphate groups

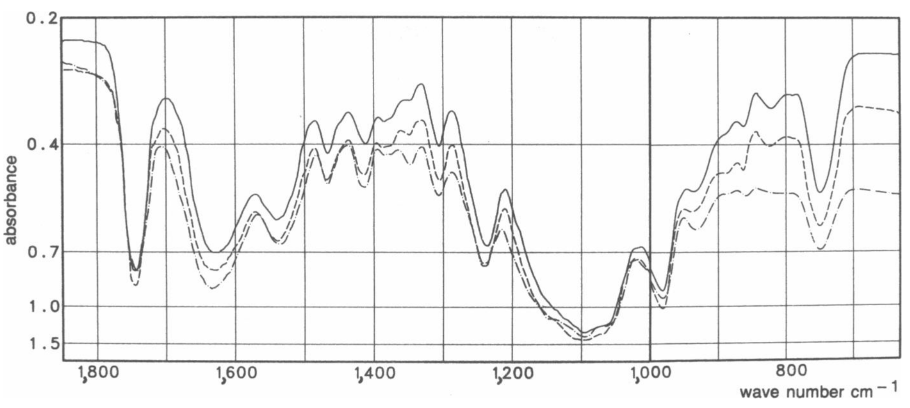
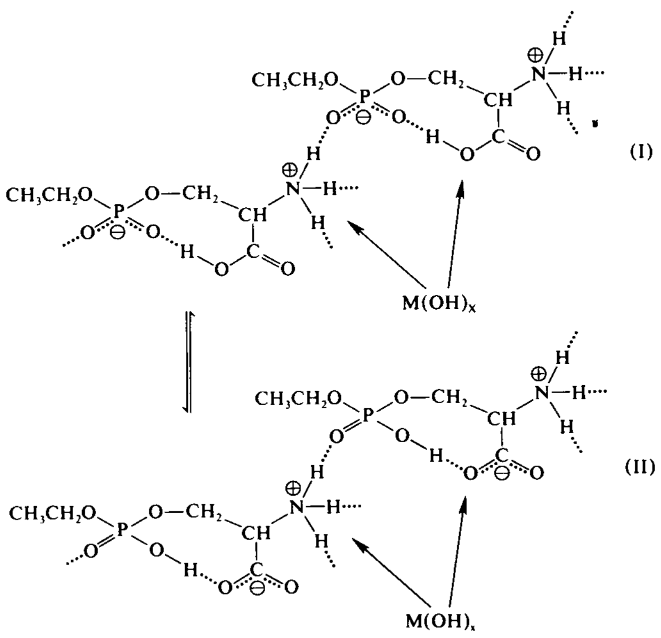


FIGURE 7 IR spectra of a film prepared from PSE + $Ca(OH)_2$ (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, 11% r.h.; ---, 44% r.h.; -·-·-, 85% r.h.



interact also with the Ca ions. Thus, an arrangement as shown in Scheme III is probably present.

Such an arrangement would explain the rigidity of phosphatidylserine headgroups in the presence of Ca ions observed by Browning and Seelig (32) using NMR spectroscopy.

C. (L-glu)$_n$ + PSE systems

The backbone of (L-glu)$_n$ is present as an α-helix (33–37). If the carboxylic acid groups are deprotonated at large degrees of hydration the backbone is also α-helical. With a decreasing degree of hydration the structure changes to antiparallel β-structure (35, 37). The system (L-glu)$_n$ + n-propylamine changes if it is dried also from α-helical to antiparallel β-structure (35).

The conformation of the backbone can be determined from the amide bands (38, 39). α-Helix is indicated by a relatively sharp amide I band at 1,650 cm^{-1} and an amide II band at ~1,545 cm^{-1}, whereas antiparallel β-structure is indicated by an amide I band at 1,630 cm^{-1} with a weak shoulder at 1,690 cm^{-1} and an amide II band at 1,525 cm^{-1}.

(L-glu)$_n$ + PSE, 1:1

The PSE bands of the (L-glu)$_n$ + PSE 1:1 system are identical with the bands in the pure PSE system in Fig. 2. This is true with the dry system as well as with regard to the changes, which are observed with increasing degree of hydration. No indication is found with regard to an interaction between (L-glu)$_n$ and PSE.

(L-glu)$_n$ + PSE + LiOH, NaOH, KOH, and $Ca(OH)_2$ 1:1 system

Fig. 9 shows the IR spectra of these systems in the region 1,850–1,475 cm^{-1} as a function of the degree of hydration. These figures show that the amide I band at 1,650 cm^{-1}, characteristic for α-helical structure, vanishes with increasing degree of hydration and instead of this band an intense amide I band at 1,630 cm^{-1} arises together with a weak band at 1,690 cm^{-1}, characteristic for antiparallel β-structure. Analogous changes of the spectra are observed in the region of the amide II band. This band change is, however, superimposed on the band of $\nu_{as}(CO_2^-)$ of glutamate residues arising with increasing degree of hydration.

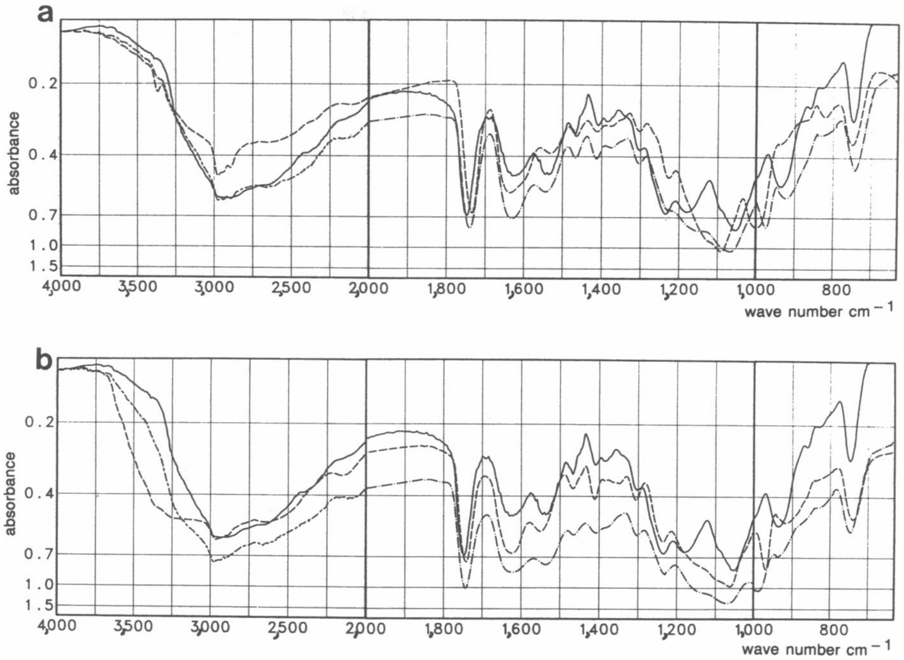


FIGURE 8 (*a*) IR spectra of films prepared from PSE + LiOH, PSE + NaOH (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, PSE; ---, PSE + LiOH; ----, PSE + NaOH. (*b*) IR spectra of films prepared from PSE + KOH, PSE + $Ca(OH)_2$ (ratio 1:1) at 25°C (same amount of PSE as in the pure PSE films). ——, PSE; ---, PSE + KOH, ----, PSE + $Ca(OH)_2$.

The broad band at ~1,730 cm^{-1} is a superposition of $\nu(C = O)$ of COOH groups of PSE and $(L\text{-glu})_n$. The conformational change is induced by a deprotonation of $(L\text{-glu})_n$ (35, 37). Thus, the comparison of the decrease of $\nu(C = O)$ at 1,745 cm^{-1} with the degree of the conformational change suggests that preferentially the carboxylic acid groups of $(L\text{-glu})_n$ are deprotonated and not the proton in the intramolecular bonds in the PSE molecule.

If one compares in Fig. 9 the LiOH, NaOH, and KOH systems at comparable r.h. the following result is obtained: the neutralization of the carboxylic acid groups of $(L\text{-glu})_n$ and the conformational change to antiparallel β-structure is most proceeded with the K^+, a little less with the Na^+, and much less with the Li^+ system. In the case of the $Ca(OH)_2$ system, also at high degree of hydration, considerable parts of the backbone are still α-helical.

All these results taken together demonstrate that OH^- neutralizes carboxylic acid groups of $(L\text{-glu})_n$. Caused by the formation of the intramolecular (I) $COOH\cdots{}^-OP \rightleftharpoons COO^-\cdots HOP$ (II) bond with proton polarizability in PSE, the proton in this bond is so strongly stabilized that instead of these protons the glutamic acid protons are neutralized. A different situation is expected with $(L\text{-his})_n$ + PSE and $(L\text{-lys})_n$ + PSE systems.

IV. CONCLUSIONS

In the O-phospho-L-serine-P-ethylester (PSE) molecules an intramolecular (I) $COOH\cdots{}^-OP \rightleftharpoons COO^-\cdots HOP$ (II) hydrogen bond with double minimum proton potential and large proton polarizability is present. In the films this hydrogen bond is relatively stable as shown by the neutralization experiments. This is confirmed by the fact that if PSE and $(L\text{-glu})_n$ are present, not PSE but $(L\text{-glu})_n$ is preferentially neutralized. At low degrees of hydration the cations are attached to the phosphate groups. The Li ions polarize the intramolecular hydrogen bonds much more than the other cations, i.e., the weight of the proton-limiting structure $COOH\cdots{}^-OP$ is increased by Li ions.

With regard to these results one has to assume that such a hydrogen bond is also present in the head group of phosphatidylserine in biological membranes. Thus, these head groups could be part of a lateral proton-conducting system in the polar surfaces of biological membranes. Due to the large proton polarizability of such systems local lateral electrochemical gradients could easily shift positive charge with a very high rate from the proton-creating to the proton-consuming centers at the surface.

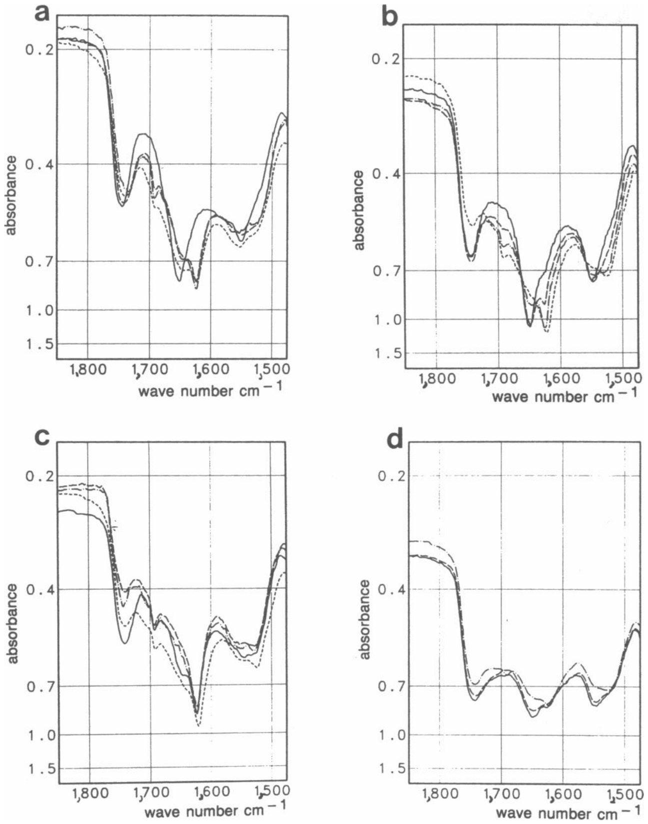


FIGURE 9 IR spectra of films prepared from (*a*) $(glu)_n$ + PSE + LiOH: ——, 30% r.h.; ---, 66% -·-·, 75% r.h.; ······, 85% r.h.; (*b*) $(glu)_n$ + PSE + NaOH: ——, 30% r.h.; ---, 47% r.h.; -·-·, 57% r.h.; ······, 85% r.h.; (*c*) $(glu)_n$ + PSE + KOH: ——, 30% r.h.; ---, 66% r.h.; -·-·, 75% r.h.; ······, 91 r.h.; (*d*) $(glu)_n$ + PSE + $Ca(OH)_2$: ——, 30% r.h.; ---, 44% r.h.; -·-·-; 75% r.h.

As in ice (40) after each charge transport the hydrogen-bonded system must be regenerated by reorientation of the hydrogen bond donor groups.

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