Eur Biophys J (1991) 19:317–322

European Biophysics Journal

^{31}P-NMR study of pig intestinal brush-border membrane structure: effect of zinc and cadmium ions

F. Tacnet, P. Ripoche, M. Roux, and J.-M. Neumann

Département de Biologie Cellulaire et Moléculaire, Centre d'Etudes Nucléaires de Saclay, F-91191 Gif sur Yvette, France

Received October 16, 1990/Accepted in revised form February 18, 1991

Abstract. ^{31}P-NMR experiments on intact pig small intestine brush-border membrane vesicles (BBMV) and detergent-solubilized membranes gave direct insights into the organization of the phospholipids (PL) and their interaction with zinc and cadmium ions. Various endogenous PL were identified from well resolved BBM micelle spectra. These experiments revealed a strong interaction of Zn^{2+} and Cd^{2+} with the negatively charged phosphatidylinositol and phosphatidylserine. In BBM micelles, a progressive time-dependent PL degradation occurred in the absence of ions and indicated the presence of active phospholipases. The presence of zinc inhibited the degradation process whereas cadmium had the opposite influence. ^{31}P spectra of BBMV were carefully characterized. Neither zinc nor cadmium affected the PL bilayer structural organization. A degradation of PL, monitored by the increase of the inorganic phosphate (P_i) signal, also occurred in vesicles but to a lesser extent than in micelles. A 2/3 internal, 1/3 external PL asymmetry was observed in the absence and presence of ions.

Key words: ^{31}P-NMR – Phospholipid – Zinc – Cadmium – Brush-border membrane vesicles

Introduction

A wide variety of biological and model membranes have been investigated using phosphorus Nuclear Magnetic Resonance (NMR) experiments to get information about the dynamic and structural properties of the phospholipids (PL) (Seelig 1978; Cullis and De Kruijff 1979; Smith and Ekiel 1984). In particular, such experiments have been used to study the role of metallic cations in the structure and function of biomembranes. These cations are able to specifically change the PL polar head group conformation (Roux and Bloom 1990 and references herein) and induce a phase transition of PL in model membranes (Hope and Cullis 1980).

Offprint requests to: P. Ripoche

Among these ions, zinc, a major trace element, plays a critical role in membrane structure (Bettger and O'Dell 1981). Zinc ions have a protective effect against lipid peroxidation, which causes membrane distortion (Chvapil et al. 1972). The membrane stabilizing effect of zinc has been attributed to specific interactions of the cation with functional groups of intrinsic components of the plasma membrane, such as membrane proteins and PL (Chvapil 1976).

In the present study, we have used ^{31}P-NMR experiments to investigate interactions of zinc with the PL component of the pig intestinal membrane. The choice of the intestinal brush-border membrane (BBM) is justified by its specific role in zinc metabolism: the luminal membrane is the first physiological barrier for dietary zinc and the main site of zinc homeostasis (Cousins and Failla 1980). The cation has also been shown to strongly interact with this membrane (Hoadley and Cousins 1988; Watkins et al. 1989) and to extensively bind pig small intestine brush-border membrane vesicles (BBMV) (Tacnet and Ripoche 1990).

On the other hand, it has been shown that cadmium, a toxic homologue of zinc and a potent competitive inhibitor of intestinal zinc uptake (Tacnet et al. 1990), has the same affinity for the vesicular Zn^{2+} binding sites as zinc itself (Tacnet and Ripoche 1990). Thus, we studied and compared the effects of both cations on the intestinal membrane structure.

A first set of experiments were devoted to BBM in micelles (obtained by Triton X100 solubilization of BBMV) in order to characterize the endogenous PL signals and to observe their changes upon addition of Zn^{2+} and Cd^{2+} ions. Then, metal ion effects were studied on intact BBMV, the phosphorus spectrum of which monitors the lipid organization in membranes.

Materials and methods

Brush-border membrane vesicles (BBMV)

The brush-border membrane was isolated from pig jejunum epithelial cells according to the modified Kessler

procedure (Kessler et al. 1978) as previously described (Mg^{2+} replacing Ca^{2+}) (Tacnet et al. 1990). Resulting vesicles are relatively homogeneous with respect to size (from freeze fracture pictures, a mean diameter of 200 ± 6 nm ($n = 1946$) was measured, data not shown) and remain right side out oriented to more than 90% (Klip et al. 1979). The intravesicular medium contained 100 m*M* mannitol, 10 m*M* N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes) adjusted to pH 7.4 with KOH.

For NMR experiments, BBMV were centrifuged at $28\,000 \times g$ and the pellets were then suspended in a small volume of the above buffer to obtain a protein concentration of around 60 mg/ml. Protein concentrations were determined by the Bio Rad protein assay according to Bradford (1976) with bovine serum albumin as standard. 400 µl of vesicle suspension (H_2O : D_2O, 80 : 20) were injected into 5 mm NMR tubes. The final mixture pH was equal to 7.2.

Brush-border membrane (BBM) micelles

BBMV were centrifuged at $28\,000 \times g$. The pellet was then suspended in 400 µl of a 100 m*M* mannitol, 50 m*M* Hepes buffer, pH 7.4 containing 1 m*M* EDTA in order to chelate paramagnetic species and thus increase the NMR spectral resolution. Protein concentration in the pellet was 25 mg/ml. 10% Triton X100 (weight/volume) was added to the vesicular suspension. Micelles thus obtained were eluted on a Sephadex G25M column (Pharmacia), previously equilibrated in a 100 m*M* mannitol, 50 m*M* Hepes, 0.1 m*M* EDTA buffer pH 7.4. 400 µl of micellar eluate (5 mg protein/ml) containing 20% D_2O was injected into 5 mm NMR tubes.

Exogenous phospholipids (PL)

Triton X100-solubilized exogenous PL used for spectral assignment were prepared in the 100 m*M* mannitol, 50 m*M* Hepes, 0.1 m*M* EDTA buffer containing 4% Triton X100 (weight/volume). Egg yolk phosphatidylcholine (PC) was purchased from Avanti Polar Lipids, Birmingham, USA; egg yolk phosphatidylethanolamine (PE) was purchased from Sigma Chemical Company, St Louis, USA; bovine brain phosphatidylinositol (PI), phosphatidylserine (PS) and sphingomyelin (SM) were obtained from Koch-Light Laboratories LTD, Bucks, England.

NMR experiments

121.5 MHz ^{31}P-NMR experiments were performed on a Bruker MSL 300 spectrometer at 20 °C with a two level proton decoupling. BBMV phosphorus spectra were recorded using a ($\pi/2$-τ-π-τ) dipolar echo sequence with a $\pi/2$ pulse length of 4 µs, a refocusing delay τ of 20 µs and a dwell time of 10 µs. BBM micelle phosphorus spectra were acquired using single pulse experiments with a dwell time of 62 µs. 4 000 scans per Free Induction Decay (FID) were usually accumulated. Chemical shifts were measured relative to external phosphoric acid. PL concentrations were estimated with respect to an external reference spectrum of a known concentration of inorganic phosphate. Zinc and cadmium were added as $ZnCl_2$ and $CdCl_2$.

The effect of the paramagnetic probe tempamine (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amine, Molecular Probes, Eugene, USA), frequently used as a spin label in Electron Spin Resonance experiments (Daveloose et al. 1983; Morse 1985) and in some NMR experiments (Seigneuret et al. 1990), was quantified on both exogenous PL micelles and intact BBMV. Preliminary experiments revealed that tempamine significantly broadened the charged PL signals such as PI and PS, and weakly affected the resonance of free inorganic phosphate in solution. As regards BBMV, tempamine was used to detect eventual distinct pools of compounds accessible to the paramagnetic probe.

Results and discussion

^{31}P-NMR study of BBM micelles

Phosphorus spectra of BBM micelles ([1, −1] ppm region of Fig. 1, 1A, 2A, 3A) display three major peaks (90% of the total signal) and one minor resonance. From signal area, micelle PL concentration was estimated to be around 2 m*M*. According to standard chemical shift data (Dennis and Plückthun 1984), the three major resonances correspond to different PL species. Signal assignment was achieved by adding various detergent solubilized exogenous PL (see Methods): PE, PI, PS, SM, and PC. For example, addition of PC micelles increases the highfield resonance. In summary and with respect to the decreasing chemical shifts, the three main signals are assigned to the following lipids: *1)* 0.28 ppm: PE+SM; *2)* 0.13 ppm: PI+PS; *3)* −0.37 ppm: PC. The minor resonance exhibits a chemical shift value of −0.14 ppm, corresponding to a large variety of phosphoryl compounds (Dennis and Plückthun 1984), and was not identified. An inorganic phosphate (P_i) resonance is also detected at 2.2 ppm, a value which corresponds to the P_i chemical shift at a pH of 7.4 (Prigent et al. 1980) in agreement with our experimental conditions.

The relative PL concentrations determined from the three major resonance areas are 42% (PE+SM), 26% (PI+PS) and 32% (PC), (average values over the three experiments shown in Fig. 1, 1A, 2A, 3A). The proportions of PE vs SM on the one hand, and PS vs PI on the other hand, were obtained by chemical analysis (Geurts Van Kessel et al. 1977; Christon et al. 1989): HPLC of BBM phospholipids gave a PL composition of 31% PE, 6.5% SM, 25% PS, 6.5% PI and 31% PC. A comparable PL composition was reported in rabbit jejunum (Barsukov et al. 1986) and in pig ileum (Christiansen and Carlsen 1981).

As a control, the time-dependence of the BBM micelle spectrum in the absence of metal ions is shown in Fig. 1,

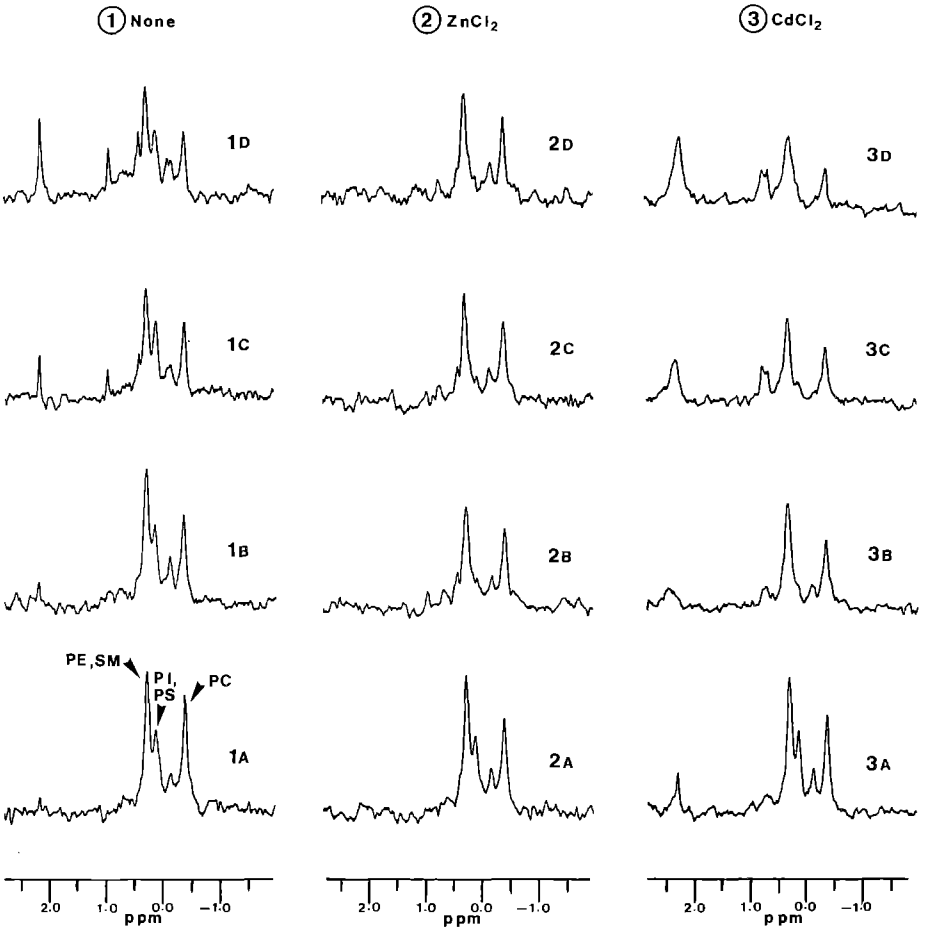

Fig. 1. ^{31}P-NMR spectra of Triton X100-solubilized BBMV. *1)* BBM micelle spectra without any cation added. *2)* BBM micelle spectra before and after Zn^{2+} addition. *3)* BBM micelle spectra before and after Cd^{2+} addition. *A, B, C, D:* Spectra recorded 3 · 5 h (*A*), 7 h (*B*), 14 h (*C*) and 21 h (*D*) after the beginning of the acquisition. $ZnCl_2$ or $CdCl_2$ were added after the first spectrum. Temperature of experiment was 20 °C

1 B, 1 C and 1 D. A progressive decrease of PL signals is observed and accompanied by an increase of the P_i resonance and the appearance of lysoPL peaks (−0.12, 0.41 and 0.95 ppm). The PC resonance is first affected (Fig. 1, 1 B), then the (PE + SM) signal is altered (Fig. 1, 1 C) and lastly, the (PI + PS) resonance decreases (Fig. 1, 1 D). These results reflect a continuous PL degradation achieved by various enzymes known to hydrolyze PL (Subbaiah and Ganguly 1970; Verger et al. 1982; Ehle et al. 1985; Gassama-Diagne et al. 1989), particularly in the presence of detergent (El-Sayed and Roberts 1985). The presence of increasing lysoPL and P_i signals indicates that these enzymes achieve a large and non-specific hydrolysis of BBM phospholipids, which could be more precisely analysed by biochemical techniques such as TLC.

Figure 1, 2 B and 3 B shows the phosphorus spectra of BBM micelles after addition of 1.5 m*M* $ZnCl_2$ and $CdCl_2$ respectively. In both cases, the (PI + PS) resonance is totally broadened beyond detection whereas a slight increase of the (PE + SM) and PC linewidth is observed. Such an effect on the (PI + PS) signal after addition of either Zn^{2+} or Cd^{2+} was also obtained on a mixture of reconstituted PL micelles, where the percentage of each exogenous PL mimics the endogenous composition (data not shown). These results indicate a strong electrostatic interaction of both Zn^{2+} and Cd^{2+} only with the negatively charged PL.

In the presence of Zn^{2+} ions, time-dependence of the BBM micelle signal (Fig. 1, 2 C and 2 D) shows that no further change of the spectrum observed in Fig. 1, 2 B occurs after the addition of zinc: (PE + SM) and PC signals remain unchanged. While the detailed investigation of the enzymatic activities involved is not the concern of the present study, ^{31}P-NMR data unambiguously reveal the complete efficiency of high $ZnCl_2$ concentrations in preventing PL hydrolysis. In contrast, the presence of Cd^{2+} ions induces a marked PL alteration during the same time interval (Fig. 1, 3 C and 3 D). In addition, the PL degradation achieved in the presence of Cd^{2+} ions is three times greater than in the absence of ions (compare the P_i signal area in spectra 1 D and 3 D of Fig. 1). The chemical shifts of the resulting PC and PE lysoderivatives (0.65 and 0.75 ppm) are slightly different from that obtained in the absence of ion.

^{31}P-NMR spectra of BBM micelles gave a direct insight on zinc and cadmium effects on PL: both ions exhibit the same interaction with the negatively charged PL and exert opposite effects on the enzymatic PL degradation.

^{31}*P-NMR study of BBMV*

In the absence of Zn^{2+} or Cd^{2+} ions, BBMV phosphorus spectra (Fig. 2, 1 A, 2 A and 3 A) exhibit a well known

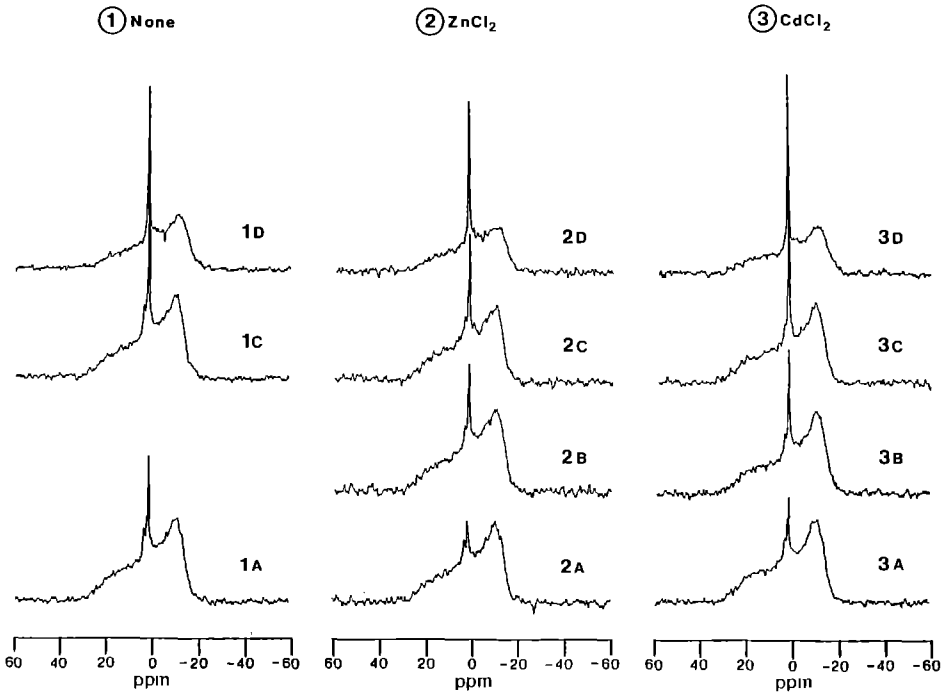

Fig. 2. ^{31}P-NMR spectra of intestinal BBMV. 1) BBMV spectra without any cation added. 2) BBMV spectra before and after Zn^{2+} addition. 3) BBMV spectra before and after Cd^{2+} addition. *A*, *B*, *C*, *D*: Spectra recorded 2 h (*A*), 4 h (*B*), 12 h (*C*) and 14 h (*D*) after the beginning of the acquisition. $ZnCl_2$ or $CdCl_2$ were added after the first spectrum. (*B*: 1 m*M* final ion concentration; *C*: 2 m*M* final ion concentration; *D*: addition of 50 m*M* tempamine). Temperature of experiment was 20 °C

axially symmetric powder pattern characteristic of PL bilayer structures (Cullis and De Kruijff 1979). Vesicle PL concentration was estimated to be 25 m*M*. The chemical shift anisotropy (CSA) of the PL bilayer signal is about 35 ppm. Two isotropic resonances superimposed on the large bilayer signal are also detected. Signal integration indicates that the BBM phospholipids in the bilayer represent more than 90% of the total phosphorus signal.

Similar isotropic resonances were also observed in the phosphorus spectra of BBM isolated from rabbit intestine (Vallet-Strouve et al. 1985) and rat kidney (Elgavish and Elgavish 1985), and it was suggested that the high-field signal could be due to rapid tumbling PL (inverted micelles or small vesicles). The following experiments enable us to precisely identify the isotropic resonances of pig intestinal BBMV.

First, for accurately measuring their chemical shifts, echo spectra were recorded by using a longer refocusing delay and a longer dwell time (1 ms and 62 μs respectively, instead of 20 μs and 10 μs). These conditions lead to the disappearence of the bilayer signal (the transverse relaxation time of which is less than 1 ms) and to a better resolution. The highfield isotropic resonance is located at 2.05 ± 0.05 ppm (average over the three experiments shown in Fig. 2, 1 A, 2 A and 3 A) and thus corresponds to P_i ions at $pH = 7.25 \pm 0.05$ (Prigent et al. 1980). The low-field peak exhibits a chemical shift of 3.75 ppm, similar to that obtained for phosphoryl-ethanolamine or phosphoryl-serine (Dennis and Plückthun 1984).

In a second experiment, the time-dependence of the BBMV phosphorus signal was investigated as in the case of BBM micelles. The spectrum 1 C shown in Fig. 2, recorded 12 hours after the first acquisition (Fig. 2, 1 A), indicates that during this time interval, the bilayer structure is conserved whereas the P_i signal intensity is increased by a factor of 2. According to the data obtained for BBM micelles, P_i increase results from PL degradation due to the presence of active enzymes. Other degradation compounds such as lysoPL are not detected probably because these amphiphilic compounds remain anchored in the bilayer so that their resonances cannot be distinguished from the main signal.

Finally, in a third experiment, a paramagnetic tempamine solution (50 m*M* final concentration) was added to the sample tube at the end of the last acquisition (Fig. 2, 1 D) to try to discriminate distinct pools of PL and/or P_i. Addition of tempamine does not affect the P_i resonance and leads to a 35% decrease of the bilayer signal intensity. Further addition of tempamine does not modify the spectrum. The interpretation of these data is quite difficult since although we observed a weak paramagnetic effect of tempamine on the P_i resonance in solution (see Materials and methods) we also observed that the constant increase of the P_i signal due to lipid degradation still occurs during the acquisition-time. Consequently, we cannot distinguish, in these conditions, the internal or external P_i compartments. As regards the PL signal, our data could indicate an asymmetry in the distribution of PL and be related to the highly asymmetrical PL distribution found in BBM enterocytes (Barsukov et al. 1986; Patton JS, personal communication). PL asymmetry can be explained by the important fraction of glycolipids present at the external cell surface of intestinal BBM (Forstner and Wherrett 1973; Christiansen and Carlsen 1981). The asymmetry could be also related to the protein composition of the BBMV: the majority of proteins are external components of the membrane (Louvard et al. 1975).

Taking into account the above results and our prior data relative to BBM micelles, it appears that neither of

the two isotropic peaks is consistent with a signal of PL in micelles or very small vesicles. Therefore, the extended bilayer is the only structural organization detected for the endogenous PL of BBMV.

Figure 2, 2B and 3B shows the BBMV phosphorus spectra after addition of 1 m*M* $ZnCl_2$ and 1 m*M* $CdCl_2$ respectively. Whatever the cation added, the initial CSA value (35 ppm) is conserved. Further addition of zinc or cadmium ions (2 m*M* final ion concentration) and time-evolution (over 12 h) (Fig. 2, 2C and 3C) do not change this result. This indicates that no vesicle fusion, leading to the formation of larger species, occurs (Burnell et al. 1980). It has been also previously shown by freeze fracture analysis that $ZnCl_2$ does not promote any aggregation of BBMV (Watkins et al. 1989). In our vesicular system, neither Zn^{2+} nor Cd^{2+} ions induce a lipid phase transition such as the change from a bilayer to a hexagonal (H_{II}) phase observed on PS model membranes in the presence of Ca^{2+} (Hope and Cullis 1980).

However, time-dependence of the ^{31}P-NMR spectra in the presence of zinc and cadmium reveals that the intensity of the highfield isotropic resonance progressively increases. Accurate chemical shift measurements (performed by spin echo experiments mentioned above), clearly indicate that, as in the absence of added cations, P_i ions are the only source of the highfield resonance enhancement. The relative increase of the P_i signal (with respect to the first acquired spectrum) is similar for both Zn and Cd ions and close to that obtained in the absence of added ions. Comparison of these results with the data of BBM micelles indicates that PL degradation occurs to a much lower extent in BBMV than in micelles. Two explanations are possible: 1 – The bilayer organization is less favorable for enzyme attack. 2 – Various endogenous divalent cations able to influence the enzyme activities are still present in the BBMV sample whereas, in order to obtain high resolution, EDTA was added to the BBM micelle sample (see experimental section). Another difference with BBM micelles is that no protective zinc effect against PL alteration was observed in BBMV. This could be due to the lower zinc/PL concentration ratio used in BBMV experiments. As observed in the absence of added ions, the presence of tempamine leads to a 35% decrease of the bilayer signal and apparently does not affect the P_i resonance (Fig. 2, 2D, 3D).

Our data indicate that whereas Zn^{2+} and Cd^{2+} strongly interact with endogenous PI and PS, they neither affect the PL bilayer organization nor induce the appearance of other PL structures such as micelles, inverted micelles, small vesicles or hexagonal phases. Our data also show that the PL concentration is probably larger in the inner than in the outer monolayer. This can be related to our previous report showing a zinc binding asymmetry in pig jejunum brush-border (Tacnet and Ripoche 1990).

Acknowledgements. The authors thank Dr. Raymond Christon (INRA, Jouy en Josas, France) for HPLC and phosphorus analysis of the brush-border membrane phospholipids.

References

Barsukov LI, Bergelson LD, Spiess M, Hauser H, Semenza G (1986) Phospholipid topology and flip-flop in intestinal brush-border membrane. Biochim Biophys Acta 862:87–99

Bettger WJ, O'Dell BL (1981) A critical physiological role of zinc in the structure and function of biomembranes. Life Sci 28:1425–1438

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal Biochem 72:248–254

Burnell EE, Cullis PR, De Kruijff B (1980) Effects of tumbling and lateral diffusion on phosphatidylcholine model membrane ^{31}P-NMR lineshapes. Biochim Biophys Acta 603:63–69

Christiansen K, Carlsen J (1981) Microvillus membrane vesicles from pig small intestine. Purity and lipid composition. Biochim Biophys Acta 647:188–195

Christon R, Even V, Daveloose D, Léger CL, Viret J (1989) Modification of fluidity and lipid-protein relationships in pig intestinal brush-border membrane by dietary essential fatty acid deficiency. Biochim Biophys Acta 980:77–84

Chvapil M (1976) Effect of zinc on cells and biomembranes. Med Clin North Am 60:799–812

Chvapil M, Ryan JN, Zukoski CF (1972) Effects of zinc on lipid peroxidation in liver microsomes and mitochondria. Proc Soc Exp Biol Med 141:150–153

Cousins RJ, Failla ML (1980) Cellular and molecular aspects of mammalian zinc metabolism and homeostasis. In: Nriagu JO (ed) Zinc in the environment, Part II: Health effects, chap 6. Wiley, New York, pp 121–135

Cullis PR, De Kruijff B (1979) Lipid polymorphism and the functional roles of lipids in biological membranes. Biochim Biophys Acta 559:399–420

Daveloose D, Fabre G, Berleur F, Testylier G, Leterrier F (1983) A new spin label method for the measurement of erythrocyte internal microviscosity. Biochim Biophys Acta 763:41–49

Dennis EA, Plückthun A (1984) Phosphorus-31 NMR of phospholipids in micelles. In: Gorenstein DG (ed) Phosphorus-31 NMR. Principles and applications, chap 14. Harcourt Brace Jovanovich; Academic Press, London, pp 423–446

Ehle H, Müller E, Horn A (1985) Alkaline phosphatase of the calf intestine hydrolyzes phospholipids. Febs Lett 183:413–416

Elgavish A, Elgavish GA (1985) In vitro ethanol effects on the transport properties of isolated renal brush-border membrane vesicles. J Membr Biol 88:123–130

El-Sayed MY, Roberts MF (1985) Charged detergents enhance the activity of phospholipase C (*Bacillus cereus*) towards micellar short-chain phosphatidylcholine. Biochim Biophys Acta 831:133–141

Forstner GG, Wherret JR (1973) Plasma membrane and mucosal glycosphingolipids in the rat intestine. Biochim Biophys Acta 306:446–459

Gassama-Diagne A, Fauvel J, Chap H (1989) Purification of a new calcium-independent, high molecular weight phospholipase A2/lysophospholipase (phospholipase B) from guinea pig intestinal brush-border membrane. J Biol Chem 264:9470–9475

Geurts Van Kessel WSM, Hax WMA, Demel RA, De Gier J (1977) High performance liquid chromatographic separation and direct ultraviolet detection of phospholipids. Biochim Biophys Acta 486:524–530

Hoadley JE, Cousins RJ (1988) Regulatory mechanisms for intestinal transport of zinc and copper. In: Essential and toxic trace elements in human health and disease, vol 18. Liss, New York, pp 141–155

Hope MJ, Cullis PR (1980) Effects of divalent cations and pH on phosphatidylserine model membranes: a ^{31}P-NMR study. Biochem Biophys Res Commun 92:846–852

Kessler M, Acuto O, Storelli C, Murer H, Muller M, Semenza G (1978) A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush-border membranes. Biochim Biophys Acta 506:136–154

Klip A, Grinstein S, Semenza G (1979) Transmembrane disposition of the phlorizin binding protein of intestinal brush borders. Febs Lett 99:91–96

Louvard D, Maroux S, Vannier CH, Desnuelle P (1975) Topological studies on the hydrolases bound to the intestinal brush border membrane. I. Solubilization by papain and triton X-100. Biochim Biophys Acta 375:236–248

Morse PD (1985) A comparison of the spin labels MAL-3 and TEMPAMINE for measuring the internal microviscosity of human erythrocytes. Biochim Biophys Acta 844:337–345

Prigent Y, Trandinh S, Gary Bobo CM (1980) Proton gradient across membranes of lecithin vesicles as measured by ^{31}P-NMR: asymmetrical behaviour of internal and external layers. Biochem Biophys Res Commun 95:1218–1223

Roux M, Bloom M (1990) Ca^{2+}, Mg^{2+}, Li^{+}, Na^{+}, and K^{+} distributions in the headgroup region of binary membranes of phosphatidylcholine and phosphatidylserine as seen by deuterium NMR. Biochemistry 29:7077–7089

Seelig J (1978) ^{31}P nuclear magnetic resonance and the head group structure of phospholipids in membranes. Biochim Biophys Acta 515:105–140

Seigneuret M, Neumann JM, Levy D, Rigaud JL (1990) High resolution ^{13}C-NMR study of the topography and dynamics of methionine residues in detergent-solubilized bacteriorhodopsin. Biochemistry (in press)

Smith ICP, Ekiel IH (1984) Phosphorus-31 NMR of phospholipids in membranes. In: Gorenstein DG (ed) Phosphorus-31 NMR. Principles and applications, chap 15. Harcourt Brace Jovanovich; Academic Press, London, pp 447–475

Subbaiah PV, Ganguly J (1970) Studies on the phospholipases of rat intestinal mucosa. Biochem J 118:233–239

Tacnet F, Ripoche P (1990) Brush-border zinc uptake and binding in pig small intestine. Z Gastroenterol 28:435

Tacnet F, Watkins DW, Ripoche P (1990) Studies of zinc transport into brush-border membrane vesicles isolated from pig small intestine. Biochim Biophys Acta 1024:323–330

Vallet-Strouve C, Tellier C, Poignant S, Boucrot P (1985) Effect of micellar lipids on rabbit intestinal brush-border membrane phospholipid bilayer integrity studied by ^{31}P-NMR. J Membr Biol 84:73–79

Verger R, Ferrato F, Mansbach CM, Pieroni G (1982) Novel intestinal phospholipase A2: purification and some molecular characteristics. Biochemistry 21:6883–6889

Watkins DW, Chenu C, Ripoche P (1989) Zinc inhibition of glucose uptake in brush-border membrane vesicles from pig small intestine. Pflügers Arch 415:165–171