Reconstitution of brush border membrane proteins in phosphatidylcholine vesicles

Biochemical and functional characterization

Maryse BOUDOUARD,*† Jean GIUDICELLI,* Christian VANNIER† and Pierre SUDAKA*

*Laboratoire de Biochimie, Faculté de Médecine, Avenue de Vallombrose, F-06034 Nice Cedex, France, and †Centre de Biochimie, Parc Valrose, F-06034 Nice Cedex, France

Horse kidney brush border membrane proteins were incorporated into phosphatidylcholine vesicles. Structural analysis of proteoliposomes prepared with various lipid:protein ratios showed that: (a) only a few of the proteins present in the crude brush border extract are integrated, (b) all known membrane hydrolases are integrated, and (c) these proteoliposomes are homogeneous vesicles. Papain solubilization of brush border membrane hydrolases, i.e. aminopeptidase M, neutral α -glucosidase, γ -glutamyltransferase and alkaline phosphatase, performed in parallel on native membrane vesicles and proteoliposomes, revealed similar kinetics. Analysis of membrane vesicles and proteoliposomes on sucrose density gradients either without any treatment, or after papain treatment showed that: (a) in proteoliposomes, neutral α -glucosidase is associated with radiolabelled phosphatidylcholine, and (b) papain-treated vesicles and proteoliposomes released enzyme activity in the same way. These results suggest that the integration mechanism of brush border membrane proteins may be similar in proteoliposomes and native membrane vesicles. Transport experiments under equilibrium exchange conditions showed that the uptake properties of proteoliposomes are similar to those of brush border membrane vesicles.

INTRODUCTION

Renal and intestinal brush border membrane proteins have been reconstituted in artificial or natural lipid bilayers for several purposes. One aim was to gain a better knowledge of the membrane architecture (Pattus *et al.*, 1976; Wacker *et al.*, 1976; Brunner *et al.*, 1978, 1979; Hussain *et al.*, 1981; Kenny & Maroux, 1982; Kenny *et al.*, 1983; Hussain, 1985). Such reconstitutions have been performed to confirm the amphipathic properties of hydrolases, since only amphipathic forms of these proteins have been shown to be capable of reassociating within a lipid bilayer. In these cases, the reconstitution experiment is a tool allowing us to verify the asymmetrical incorporation of purified hydrolases into the phospholipid bilayer, just as in the native membrane. The second aim was attempts to reconstitute solute transport systems (Kinne & Faust, 1977), particularly the Na⁺-dependent D-glucose transporter of kidney cortex (Crane *et al.*, 1976a,b, 1978; Fairclough *et al.*, 1979; Ducis & Koepsell, 1983; Koepsell *et al.*, 1983). The reconstitutions were performed either with crude brush border membrane (Crane *et al.*, 1976a,b, 1978; Fairclough *et al.*, 1979) or partially purified extracts (Ducis & Koepsell, 1983; Koepsell *et al.*, 1983; Neeb *et al.*, 1985). To date, attempts to purify only one protein component responsible for a transport phenomenon and incorporate it into liposomes have been unsuccessful. Hitherto, in transport studies on reconstituted systems, the structural organization of the membrane proteins in the phospholipid vesicles was left aside, the authors restricting their work to demonstration of the incorporation or enrichment of a protein or a

protein component thought to participate in transport phenomenon.

However, it has never been demonstrated that such reconstituted systems possessed structural properties similar to those of native membrane vesicles. Our aim was to assess whether a reconstitution system of renal brush border proteins exhibiting transport properties could be characterized by the structural and functional properties of native membrane vesicles. Thus the brush border membrane reconstitution system described here has been characterized by analytical studies demonstrating its homogeneity and vesicular nature. Furthermore, the mode of integration of membrane proteins into phospholipid vesicles was found to be similar to that of native brush border membrane vesicles. The suitability of our system for transport studies was shown by L-alanine uptake experiments.

MATERIALS AND METHODS

Materials

Triton X-100 was purchased from Serva, L- α -phosphatidylcholine (type VE) from Sigma, L-3-phosphatidylcholine 1,2-di-[¹⁴C]palmitoyl (CFA 604), from Amersham, Biobeads SM 2 from Bio-Rad, papain from NBC, and L-[¹⁴C]alanine from CEA. All other reagents were of the best available grade.

Preparation of brush border membrane vesicles

Brush border membrane vesicles from horse kidney cortex were prepared according to the method described

† To whom correspondence should be addressed.

for pig kidney (Vannier *et al.*, 1976) with some adaptations (Poirée *et al.*, 1978).

Extraction of brush border membrane proteins

Extractions were performed using Triton X-100 at 2.5% (w/v) final concentration as surfactant. Brush border membrane vesicles (8 mg/ml), equilibrated in 10 mM-Tris/150 mM-NaCl (pH 8.0), were solubilized by adding a 25% (w/v) Triton X-100 solution in the same buffer for 1 h at room temperature or for 18 h at 4 °C; the suspension was then centrifuged at 105000 *g* for 60 min, and the supernatant constituted the 'Triton X-100 extract' (Vannier *et al.*, 1981) used in further reconstitution experiments.

Reconstitution experiments

These were performed according to Gerritsen *et al.*, (1978) with slight modifications. L- α -Phosphatidylcholine was dried from chloroform under a stream of N₂ and dispersed by vortexing in a Triton X-100 buffer in the presence of varying amounts of freshly prepared Triton X-100 extract. The buffer used was either 18 mM-sodium phosphate containing 0.5% (w/v) Triton X-100 (pH 8.0), or 10 mM-Tris/150 mM-NaCl containing 0.5% (w/v) Triton X-100 (pH 8.0). The final concentration was 4 mg of phosphatidylcholine/ml of buffer; the protein concentration ranged from 0.1 to 0.8 mg/ml of buffer. The dispersion was then treated with Biobeads SM 2 according to Holloway (1973) for 2 h at 4 °C with gentle shaking, in order to remove the surfactant. This technique is faster than the centrifugation or filtration methods used in other reconstitution protocols (Ducis & Koepsell, 1983; Da Cruz *et al.*, 1983). The efficiency of Biobeads SM 2 can be easily checked by measuring after this treatment the decrease of the absorbance at 280 nm due to the Triton X-100. Biobeads were removed from the suspension by filtration through a syringe fitted with a glass frit, the eluate was diluted twice with a Triton X-100 buffer and centrifuged for 10 min at 3000 *g*. This step was performed to eliminate the non-liposomal aggregates (consisting of the excess of phospholipid not involved in liposome formation) which can occur with high lipid: protein ratio and especially when liposomes are prepared without proteins. The supernatant was carefully removed by aspiration and sedimented at 105000 *g* for 2 h. The pellet was resuspended in an appropriate buffer after washing at a protein concentration between 2 and 5 mg/ml. Liposomes without proteins were prepared in the same manner.

Sucrose density gradients

Sucrose gradients [0–12% (w/w) or 0–50% (w/w)] in 25 mM-Tris/HCl (pH 7.4) were prepared with an ISCO gradient former (model 570). Isopycnic centrifugations were made in a SW 27 rotor (Beckman) at 85000 *g* and 4 °C for 37 h. After centrifugation, each gradient was collected from the bottom and the fractions obtained were assayed for enzymes and phosphatidylcholine content. In this case, radiolabelled phosphatidylcholine in trace amounts was added to the reconstitution experiment.

Protein and enzyme assays

Proteins were measured according to Lowry *et al.* (1951) with 3% SDS as described by Gerritsen *et al.*

(1978). Enzymic activities were determined as described by Poirée *et al.* (1978)

Electrophoresis

SDS/polyacrylamide-gel electrophoresis was performed following the procedure described by O'Farrell (1975). Samples (80 μ g) were treated with 2% (w/v) SDS and boiled for 90 s. The running gel was 5–12% (w/v) polyacrylamide gel. Electrophoresis was carried out at 9 mA for 16 h at 6 °C in a LKB 2001 vertical electrophoresis unit. Proteins were then stained with 0.05% Coomassie Blue. After photography, the slab gel was dried using a LKB 2003 gel slab dryer and scanned with a Vernon densitometer.

Papain digestion

Papain treatments were made according to Louvard *et al.* (1975). Proteoliposomes and membrane vesicles samples were incubated with an activated papain suspension (5.62 mg/ml) in 50 mM-KH₂PO₄ containing 750 μ M-cysteine and 22.5 μ M-dithiothreitol (pH 6.2) at 37 °C. Papain was used at a final concentration of 3% (w/w) with respect to the total proteins of the samples. A time-course of papain digestion was performed by removing aliquots which were transferred to test-tubes containing *N*-ethylmaleimide at a final concentration of 6 mM. Aliquots were then centrifuged for 60 min at 13000 *g* in the angular rotor of a Hettich microliter centrifuge. The released enzyme activities in the supernatant were expressed as a percentage of the activities of an untreated sample.

Transport experiments

L-Alanine uptake in proteoliposomes was measured under equilibrium exchange conditions. Proteoliposomes (50 μ g) were preincubated for 1 h at 25 °C in 100 mM-NaCl/100 mM-mannitol/10 mM-Hepes/Tris (pH 7.4) or in 100 mM-KCl/100 mM-mannitol/10 mM-Hepes/Tris (pH 7.4) containing 0.1 mM-L-alanine. Uptake was started by mixing 0.1 mM radiolabelled L-alanine in the same medium as the preincubation buffer. Transport was stopped with 1 ml of cold solution consisting of 300 mM-mannitol/10 mM-Hepes/Tris (pH 7.4). The proteoliposomes were then immediately applied to 0.65 μ m cellulose nitrate filters (Sartorius) presoaked in stop solution and washed with 4 ml of stop solution. The filters were then assayed for radioactivity after addition of scintillation medium.

RESULTS AND DISCUSSION

Preparation of proteoliposomes

Reconstitution experiments with brush border membrane proteins were performed in the same manner as for the integration of the band 3 protein from human erythrocyte membranes into phospholipid vesicles (Gerritsen *et al.*, 1978). In these experiments, two different buffers were used: 18 mM-sodium phosphate (pH 8.0) and 10 mM Tris/150 mM-NaCl (pH 8.0) without any difference being found in the integration: approx. 20% of the total protein amount present at the beginning of the reconstitution process and 10–25% of the enzymic activities (i.e. neutral α -glucosidase, γ -glutamyltransferase, alkaline phosphatase, aminopeptidase M) were incorporated into the proteoliposomes and 30–55% of the

Table 1. Yields of recovery of phosphatidylcholine and brush border membrane proteins reconstituted in proteoliposomes

The values are means $\pm$ S.D. obtained from six different preparations of proteoliposomes.

Proteoliposome lipid:protein ratio (w/w)	[14 C]Phosphatidylcholine reconstituted (%)	Proteins reconstituted (%)	Enzyme activity reconstituted (%)			
			γ -Glutamyl-transferase	Alkaline phosphatase	Amino-peptidase M	Neutral α -glucosidase
0	23.26 $\pm$ 0.52	—	—	—	—	—
5:1	55.78 $\pm$ 1.65	21.69 $\pm$ 1.60	23.07 $\pm$ 2.23	19.63 $\pm$ 4.31	18.60 $\pm$ 0.98	18.67 $\pm$ 1.59
15:1	44.91 $\pm$ 1.47	18.85 $\pm$ 4.20	19.25 $\pm$ 0.97	10.01 $\pm$ 1.80	15.56 $\pm$ 0.54	26.25 $\pm$ 2.40
40:1	31.78 $\pm$ 0.66	17.39 $\pm$ 4.97	12.79 $\pm$ 1.89	10.46 $\pm$ 1.23	10.70 $\pm$ 1.26	15.53 $\pm$ 1.80

initial phospholipid content was recovered in the proteoliposomal fraction. Yields of integration of these markers are presented in Table 1. This procedure is thus suitable for brush border membrane incorporation into artificial phospholipid vesicles, since it is exceptionally simple and allows rapid preparation of proteoliposomes. The material incorporated consisted of a Triton X-100 extract of horse renal brush border membrane vesicles. Although the chosen detergent and the extraction conditions may seem rather drastic compared with those of others (Kinne & Faust, 1977; Ducis & Koepsell, 1983; Koepsell *et al.*, 1983; Ferguson *et al.*, 1982), they are necessary to obtain nearly complete solubilization of alkaline phosphatase and neutral α -glucosidase which are not released by milder extraction conditions (J. Giudicelli, unpublished work). Furthermore, this treatment does not affect the transport properties of extracted material since Na^+ -dependent L-alanine transport is preserved in the proteoliposomes.

Characterization of proteoliposomes

Reconstitutions with various lipid: protein ratios were studied in order to select the most convenient one for structural and functional studies. Proteoliposomes prepared with 0.1–0.8 mg of Triton X-100 extract for 4 mg of phosphatidylcholine (containing a trace amount of radiolabelled phosphatidylcholine) at ratios ranging from 5:1 to 40:1, a sample of liposomes not containing proteins and a Triton X-100 extract were analysed by isopycnic centrifugation on a 0–12% linear sucrose gradient. After centrifugation, each gradient was collected in 650 μl fractions which were assayed for enzyme and phospholipid content (Fig. 1). For all the ratios studied, the enzymic activities present in the protein extract were recovered under the same peak as phosphatidylcholine and only one band was obtained for each ratio tested

Fig. 1. Isopycnic centrifugation of proteoliposomes on 0–12% (w/w) linear sucrose gradients

(a) Liposomes without proteins; (b) proteoliposomes at a lipid:protein ratio of 40:1; (c) proteoliposomes at a lipid:protein ratio of 5:1. Radioactivity and enzyme activities in each fraction are expressed as a percentage of the total activities recovered along the gradient. Three separate experiments gave the same results. —, Radiolabelled phosphatidylcholine; ----, aminopeptidase M; - - - - , neutral α -glucosidase; $\triangle$ — $\triangle$, γ -glutamyl-transferase. The top of the gradient is to the left.

(except for the ratio 40). The proteoliposome densities for ratios, 5, 15 and 40 were respectively 1.0570, 1.0477 and 1.0230; the liposome density was 1.0115. Interestingly, enzymic activities associated with the Triton X-100 extract sedimented at the bottom of the sucrose gradient. These results showed that proteoliposomes were homogeneous vesicles, since only one kind of population was found for all ratios tested except the ratio 40:1 where two populations were present. The lightest one was not taken into account because it did not incorporate all the enzymic markers of renal brush border membrane, in particular neutral α -glucosidase. These results clearly demonstrate, that, in proteoliposomes, regardless of the ratio, the membrane proteins are truly integrated into the proteoliposomes and are not involved in a reversible lipid-protein association or a binding phenomenon at the surface of the phospholipid vesicles, otherwise they would sediment in the same way as the proteins of the Triton X-100 extract.

SDS/polyacrylamide-gel electrophoresis was carried out on the different proteoliposome preparation (Fig. 2). Comparison of the electrophoretic patterns of the three kinds of proteoliposomes with that of the Triton X-100 extract revealed that only a few protein species are integrated into proteoliposomes, namely those with an M_r higher than 75000. By contrast, proteins with a lower M_r almost never seem to be integrated. The selective

protein incorporation exhibited by these different kinds of proteoliposomes concurs with the data obtained in other reconstitution systems (Ducis & Koepsell, 1983; Kinne & Faust, 1977; Poirée *et al.*, 1983). The results presented here show that, among the membrane proteins, hydrolases are integrated in proteoliposomal structures. In addition to having amphipathic properties (Kenny & Maroux, 1981), hydrolases have a rather high molecular weight and are considered to be hydrophobic intrinsic proteins. The vesicular character of our proteoliposome preparation, proved by sucrose density gradient analysis, was confirmed by electron micrographs, which show a large, homogeneous unilamellar population with very few multilamellar vesicles and practically no aggregated material (results not shown).

Study of integration of brush border membrane proteins into proteoliposomes

Once proteoliposomes were obtained, the next step was determination of how membrane proteins are incorporated into proteoliposomes. Only the lipid:protein ratio of 5 was used in this study because it gave the highest protein incorporation and was the most suitable for transport studies, as shown by preliminary experiments. The mode of integration of proteins in proteoliposomes could occur in several ways: (1) with a random orientation of the hydrolases in the leaflet, (2) with an entrapment of a part of hydrolases within the proteoliposomes, (3) with an asymmetric orientation of proteins in the lipid bilayer. The two first propositions would result in a higher amount of enzyme activities after detergent solubilization which will allow the substrates access to the active site of the enzymes. The effect of Triton X-100 on release of enzyme activities is shown in Table 2. The fact that there is no increase of enzyme activities after Triton X-100 treatment is in a good agreement with the third proposition and with the data obtained for endopeptidase (Kenny *et al.*, 1983). An interesting point is the poor solubilization of alkaline phosphatase from the proteoliposomes, suggesting different kinds of interactions between phospholipid and this protein and an inability of the detergent to disrupt these interactions.

Kinetics of papain solubilization of membrane hydrolases (neutral α -glucosidase, γ -glutamyltransferase, alkaline phosphatase and aminopeptidase M) were studied in parallel on the same amount (1 mg) of horse renal brush border membrane vesicles and proteoliposomes. Solubilization curves were nearly identical for hydrolases released from either membrane vesicles or proteolipo-

Fig. 2. Densitometric scanning of SDS/polyacrylamide-gel electrophoresis

(a) Brush border membrane extract; (b) proteoliposomes at a lipid:protein ratio of 5:1; (c) proteoliposomes at a lipid:protein ratio of 15:1; (d) proteoliposomes at a lipid:protein ratio of 40:1. M_r markers (Pharmacia) were ($\times 10^{-3}$): 220, ferritin subunit; 67, bovine serum albumin; 60, catalase subunit; 30, carbonic anhydrase; 20.1, soybean trypsin inhibitor. Loadings: 80 μ g of protein.

Table 2. Release of enzyme activities from proteoliposomes after an 18 h, 2.5% (w/v) Triton X-100 solubilization

Results are means $\pm$ S.D. of three different experiments.

Component	Release (%)
[14 C]Phosphatidylcholine	61.77 $\pm$ 2.32
Proteins	68.05 $\pm$ 4.45
γ -Glutamyltransferase	73.18 $\pm$ 5.67
Alkaline phosphatase	10.84 $\pm$ 1.54
Aminopeptidase M	90.87 $\pm$ 10.41
Neutral α -glucosidase	99.94 $\pm$ 4.24

somes, whereas the protein solubilization curves were different. Since after 1 min incubation a large amount of enzymes was solubilized, earlier time-points of incubation (between 10 s and 1 min) were also made in order to assess whether the vesicle populations were homogeneous. A linear kinetic pattern for release of enzymes was found, supporting this hypothesis (results not shown). The parallelism between the papain treatment release kinetics for native brush border membrane vesicles and proteoliposomes demonstrates that the hydrophilic part of the hydrolases exhibit the same accessibility towards the active site of papain in brush border membrane vesicles and proteoliposomes. This result indicates a correct integration of membrane hydrolases in the phospholipid layer of proteoliposomes, since papain treatment does not affect the integrity of the lipidic core of native or reconstituted vesicles. The higher release of total proteins from proteoliposomes than from membrane vesicles reflects the fact that, among the bulk of proteins present in a Triton X-100 extract, only hydrophobic intrinsic proteins such as hydrolases, which enter into strong interactions with phospholipids, are incorporated into reconstituted vesicles.

The effect of papain treatment on proteoliposomes and membrane vesicles was analysed by isopycnic centrifugation on a 0–12% linear sucrose gradient and a 5–50% sucrose gradient respectively. Untreated samples of proteoliposomes and membrane vesicles (0.3 mg) or the same amount of proteoliposomes and membrane vesicles digested for 1 h with 3% (w/w) papain were layered on sucrose gradients. After centrifugation, fractions were collected for measurement of neutral α -glucosidase, and phospholipid content (for proteoliposomes). In proteoliposomes, neutral α -glucosidase copurified with radiolabelled phosphatidylcholine, as mentioned above, in one vesicle population banding at a density of 1.0570. After papain treatment, the enzyme is entirely located at the same density, whereas the radiolabelled phosphatidylcholine is found under a peak at lighter fractions of the gradient ($d = 1.0309$) without enzyme activity being detected at this place. In brush border membrane vesicles, the enzyme activity copurified with proteins in one vesicle population ($d = 1.2295$); papain treatment resulted in release of neutral α -glucosidase in lighter fractions of the 5–50% sucrose gradient at a density, 1.0600, very close to the value found for the enzyme released from the proteoliposomes. These results supported the above mentioned data on the correct integration of hydrolases in proteoliposomes.

Transport experiments

Fig. 3 illustrates the time course of L-alanine uptake by proteoliposomes prepared at a lipid:protein ratio of 5, in the presence of NaCl or KCl under equilibrium exchange conditions. L-Alanine uptake was faster in the presence of NaCl than in the presence of KCl: the equilibrium level of the labelled L-alanine was almost reached after only 1 min incubation with NaCl medium, whereas attainment of the equilibrium value required 3 h incubation with KCl medium. These results suggest that incorporation of a crude Triton X-100 extract into phospholipid vesicles leads to the formation of vesicles which exhibit functional properties as demonstrated by Na^+ -dependent L-alanine uptake. These results suggest that the architecture and orientation of the molecular entity involved in this

Fig. 3. Uptake of L-alanine by proteoliposomes

Proteoliposomes were incubated for 1 h at 25 °C in 100 mM-KCl/10 mM-Hepes/Tris/100 mM-mannitol (pH 7.4) or 100 mM-NaCl/10 mM-Hepes/Tris/100 mM-mannitol (pH 7.4), both containing 0.1 mM-L-alanine. Isotope exchange was initiated by addition of radioactive L-alanine in the same buffer. All measurements were performed in triplicate. Results are means $\pm$ S.E.M. of two separate experiments. $\circ$, NaCl medium; $\triangle$, KCl medium.

phenomenon are similar in native and reconstituted vesicles, as can be shown for amphipathic hydrolases.

This system for reconstitution of brush border membrane proteins into phosphatidylcholine vesicles is particularly fast and simple, and allows preparation of proteoliposomes with structural and functional properties similar to those of native membrane vesicles. Furthermore, the procedure is very suitable for transport studies, and could represent a preliminary step in identifying a transporter.

We wish to gratefully acknowledge the expert technical assistance of Miss Nicole Limare and Mrs Sylvia Morgan for the revision of this manuscript. This work was supported by grants from the I.N.S.E.R.M. (contrat de recherche externe no. 84-1011).

REFERENCES

- Brunner, J., Hauser, H. & Semenza, G. (1978) *J. Biol. Chem.* **253**, 7538–7546
- Brunner, J., Hauser, H., Braun, H., Wilson, K. J., Wacker, H., O'Neill, B. & Semenza, G. (1979) *J. Biol. Chem.* **254**, 1821–1828
- Crane, R. K., Malathi, P. & Preiser, H. (1976a) *FEBS Lett.* **67**, 214–216
- Crane, R. K., Malathi, P. & Preiser, H. (1976b) *Biochem. Biophys. Res. Commun.* **71**, 1010–1016
- Crane, R. K., Malathi, P., Preiser, H. & Fairclough, P. (1978) *Am. J. Physiol.* **334**, E1–E5
- Da Cruz, M. E. M., Kinne, R. & Lin, J. T. (1983) *Biochim. Biophys. Acta* **732**, 691–698
- Ducis, I. & Koepsell, H. (1983) *Biochim. Biophys. Acta* **730**, 119–129
- Fairclough, P., Malathi, P., Preiser, H. & Crane, R. K. (1979) *Biochim. Biophys. Acta* **553**, 295–306
- Ferguson, D. R., Koepsell, H. & Menuhr, H. (1982) *J. Physiol. (London)* **327**, 94P–96P
- Gerritsen, W. J., Verkley, A. J., Zwaal, R. F. A. & Van Deenen, L. L. M. (1978) *Eur. J. Biochem.* **85**, 255–261

- Holloway, P. W. (1973) *Anal. Biochem.* **53**, 304–308
- Hussain, M. M. (1985) *Biochim. Biophys. Acta* **81**, 306–312
- Hussain, M. M., Trandum-Jensen, J., Norén, O., Sjöström, H. & Christiansen, K. (1981) *Biochem. J.* **199**, 179–186
- Kenny, A. J. & Maroux, S. (1982) *Physiol. Rev.* **62**, 91–128
- Kenny, A. J., Fulcher, I. S., McGill, K. A. & Kershaw, D. (1983) *Biochem. J.* **211**, 755–762
- Kinne, R. & Faust, R. G. (1977) *Biochem. J.* **168**, 311–314
- Koepsell, H., Menuhr, H., Ducis, I. & Wismuller, T. F. (1983) *J. Biol. Chem.* **258**, 1888–1894
- Louvard, D., Maroux, S., Vannier, C. & Desnuelle, P. (1975) *Biochim. Biophys. Acta* **375**, 236–248
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Neeb, M., Fasald, H. & Koepsell, H. (1985) *FEBS Lett.* **182**, 139–144
- O'Farrell, P. H. (1975) *J. Biol. Chem.* **250**, 4007–4021
- Pattus, F., Verger, R. & Desnuelle, P. (1976) *Biochem. Biophys. Res. Commun.* **69**, 718–723
- Poirée, J. C., Vannier, C., Sudaka, P. & Fehlman, M. (1978) *Biochimie*, **60**, 645–651
- Poirée, J. C., Starita-Geribaldi, M. & Sudaka, P. (1983) *Biochem. Biophys. Res. Commun.* **112**, 444–449
- Vannier, C., Louvard, D., Maroux, S. & Desnuelle, P. (1976) *Biochim. Biophys. Acta* **455**, 185–199
- Vannier, C., Boudouard, M., Giudicelli, J., Starita-Geribaldi, M. & Sudaka, P. (1981) *Biochimie* **63**, 375–387
- Wacker, H., Müller, F. & Semenza, G. (1976) *FEBS Lett.* **68**, 145–152

Received 22 July 1985/14 October 1985; accepted 25 November 1985