Reconstitution of the renal brush-border membrane sodium/phosphate co-transporter

Vincent VACHON,*†‡ Marie-Claude DELISLE,*† Raynald LAPRADE† and Richard BÉLIVEAU*†

*Laboratoire de Membranologie Moléculaire, Département de chimie-biochimie, Université du Québec à Montréal, P.O. Box 8888, Station A, Montreal, Quebec, Canada H3C 3P8, and †Groupe de Recherche en Transport Membranaire, Université de Montréal, P.O. Box 6128, Station A, Montreal, Quebec, Canada H3C 3J7

A simple and rapid procedure was developed for the reconstitution of Na⁺-dependent phosphate-transport activity from bovine kidney brush-border membranes. The phosphate transporter appears to be particularly sensitive to extraction conditions. To prevent its inactivation, the phosphate carrier was solubilized in a buffer containing its substrates, Na⁺ and phosphate, CHAPS, dithiothreitol, brush-border membrane lipids and glycerol. The uptake of phosphate by reconstituted vesicles was strongly stimulated by the presence of a transmembrane Na⁺ gradient. This stimulation was abolished when the Na⁺ gradient was dissipated by monensin. The affinity of the carrier for phosphate was similar in proteoliposomes and in brush-border membrane vesicles (apparent $K_t = 40 \mu\text{M}$). The transporter was also stimulated by the presence of a high concentration of phosphate on the *trans* side of the membrane. The reconstituted transport activity was inhibited by arsenate, a known inhibitor of phosphate transport. However, the bovine phosphate carrier, intact or reconstituted, was much less sensitive to inhibition by phosphonoformic and phosphonoacetic acids than were those of other species studied so far. SDS/PAGE revealed that only a small number of brush-border membrane proteins were incorporated into the proteoliposomes. This reconstitution procedure should be useful for the purification and identification of the carrier protein.

INTRODUCTION

The involvement of Na⁺-dependent transport systems in the reabsorption of phosphate and other solutes at the level of the proximal tubule of the nephron is well established (Mizgala & Quamme, 1985; Gmaj & Murer, 1986; Hammerman, 1986). The use of isolated brush-border membrane vesicles has contributed greatly to the characterization of this transport process (Hoffman *et al.*, 1976; Cheng & Sacktor, 1981; Murer & Burckhardt, 1983; Hammerman & Schwab, 1984; Bindels *et al.*, 1987; Béliveau & Strévey, 1987). Yet a full understanding of the mechanisms involved awaits the identification and isolation of the carrier molecule. The main problem associated with this approach stems from the fact that reconstitution of the fully functional phosphate carrier has proved difficult to achieve, despite several worthy attempts (Kinne & Faust, 1977; Lin *et al.*, 1984; Schäli & Fanestil, 1985; Schäli *et al.*, 1986).

The low Na⁺-dependent transport activities which were obtained in previous studies, including our own, appear to be due to the inactivation of the phosphate carrier during its extraction from the brush-border membrane and its reconstitution into proteoliposomes. Previous work on the phosphate-linked antiporter of *Streptococcus lactis* has demonstrated that such inactivation could be minimized by including membrane lipids, glycerol and phosphate in the extraction buffer (Ambudkar & Maloney, 1986). This strategy has also proved useful for the reconstitution of several transport proteins of prokaryotic and eukaryotic origin (Maloney & Ambudkar, 1989). In general, the best results were obtained with lipids purified from the same membrane as the carrier protein, but glycerol, which serves as a protein stabilant, could be replaced by a variety of other osmolytes (Ambudkar & Maloney, 1986; Maloney & Ambudkar, 1989).

In this paper, we describe a reconstitution protocol, based on this approach, for the renal Na⁺/phosphate symporter. The

reconstituted phosphate-transport activity is strongly Na⁺-dependent, and its main characteristics agree well with those found in isolated brush-border membrane vesicles.

MATERIALS AND METHODS

Preparation of membranes and lipids

Bovine kidneys were obtained from a local abattoir, perfused extensively with ice-cold 8.5% (w/v) NaCl immediately after evisceration, and carried in this solution to the laboratory. Brush-border membrane vesicles were prepared by the MgCl₂ precipitation method of Booth & Kenny (1974). The final pellet containing purified brush-border membranes was resuspended in 300 mM-mannitol/20 mM-Hepes/Tris, pH 7.5, and the following proteinase inhibitors: chymostatin A (10 $\mu\text{g/ml}$), bacitracin (10 $\mu\text{g/ml}$), pepstatin (10 $\mu\text{g/ml}$) and aprotinin (1.85 $\mu\text{g/ml}$). The membranes were stored in liquid N₂ and used within 1 month. Under these conditions, storage had no significant effect on the ability of the vesicles to transport phosphate. Alkaline phosphatase was enriched 7.4 $\pm$ 1.5-fold in the brush-border membrane preparations compared with the cortex homogenate.

Lipids were extracted from purified brush-border membrane vesicles or from homogenized kidney cortex by the method of Bligh & Dyer (1959) as modified by Kates (1972), and egg-yolk phospholipids were prepared as described by Dawson (1963). The lipid preparations were stored at -20°C under N₂.

Reconstitution procedure

A chloroform suspension of lipids (containing 20.4 μmol of phospholipids) was first dried to a thin film under a stream of N₂ in a 16 mm-wide glass tube and resuspended in 1.8 ml of 150 mM-Na₂HPO₄/NaH₂PO₄/5 mM-Hepes/Tris (pH 7.5)/1 mM-dithiothreitol/30% (w/v) glycerol/1.5% (w/v) CHAPS. The mixture was sonicated to clarity in a Bransonic water-bath sonicator filled with de-aerated water at 35 $^\circ\text{C}$. The suspension

‡ To whom correspondence should be addressed, at the Université du Québec à Montréal.

was cooled on ice before the addition of 200 μ l (approx. 11 mg of protein) of brush-border membrane vesicles. After a 10 min incubation on ice, the mixture was centrifuged for 30 min at 100000 g at 4 $^{\circ}C$, and the pellet was usually discarded. Lipids (containing 7.5 μ mol of phospholipids) were mixed with 250 μ g of cholesterol and resuspended as described above in 500 μ l of 150 mM- Na_2HPO_4/NaH_2PO_4 , pH 7.5. In most experiments, the lipid preparation used for both extraction of proteins and formation of the liposomes was a total lipid extract from purified brush-border membrane vesicles. Liposomes were prepared by mixing 500 μ l of supernatant containing solubilized proteins with 500 μ l of resuspended lipids. The mixture was passed through a Sephadex G-50 column (13.0 cm $\times$ 1.0 cm) which was, unless specified otherwise, equilibrated and eluted with 150 mM- $KNO_3/5$ mM-Hepes/Tris, pH 7.5, at 4 $^{\circ}C$. The turbid fractions eluted in the void volume were collected, pooled and frozen in liquid N_2 for at least 10 min. After thawing at room temperature, the liposomes were concentrated by centrifugation at 45000 g for 20 min at 4 $^{\circ}C$ and resuspended in 150 mM- $KNO_3/5$ mM-Hepes/Tris, pH 7.5, at a concentration of about 3 mg of protein/ml. In some experiments which required liposomes with a different intravesicular-medium composition, the buffer used for chromatography and for the final resuspension of the proteoliposomes was replaced by the appropriate solutions.

Transport measurements

Uptake of radiolabelled substrates by the proteoliposomes was measured by a rapid filtration method (Hopfer *et al.*, 1973). Unless specified otherwise, 5 μ l of the liposome suspension was diluted with 25 μ l of the appropriate uptake buffer and incubated at 25 $^{\circ}C$ for the indicated times. For the uptake of phosphate and amino acids, the reaction was stopped with 1 ml of an ice-cold stop solution containing 150 mM-KCl and 5 mM-Hepes/Tris, pH 7.5. The stop solution used for the uptake of glucose contained 150 mM-NaCl, 5 mM-Hepes/Tris, pH 7.5, and 1 mM-phlorizin. The suspension was filtered immediately under vacuum through a nitrocellulose filter (0.45 μ m pore size). The filter was rinsed with 7 ml of stop solution and processed for liquid-scintillation counting. All results are expressed as means $\pm$ s.d. of at least three independent experiments performed in triplicate. Statistical analyses were done by Student's *t* test.

SDS/PAGE

SDS/PAGE was performed with an LKB 2050 Midget electrophoresis apparatus and the Laemmli (1970) buffer system. The separating gel contained 10% (w/v) acrylamide and 0.3% (w/v) NN' -methylenebisacrylamide. Before the electrophoresis, samples were boiled for 2 min in sample buffer containing 1.0% (w/v) SDS and 2.5% (v/v) β -mercaptoethanol. Electrophoresis was carried out for 1 h at a constant current of 20 mA, and the gels were stained with Coomassie Brilliant Blue. Molecular-mass determinations were made by using a calibration curve prepared with the following proteins: phosphorylase *b* (97 kDa), BSA (66 kDa), ovalbumin (43 kDa) and carbonic anhydrase (31 kDa).

Other methods

Lipid phosphorus was measured by the ashing technique of Ames & Dubin (1960). Protein content was determined by the bicinchoninic acid assay with BSA as the standard protein (Smith *et al.*, 1985). Alkaline phosphatase was assayed as described by Kelly & Hamilton (1970).

Chemicals

[3H]Glucose and [3H]-labelled amino acids were purchased from Du Pont/New England Nuclear, and [^{32}P]P_i (carrier-free) was from ICN Biochemicals. CHAPS, monensin, phlorizin, phos-

phonoformic acid and phosphonoacetic acid were from Sigma, cholesterol and glycerol were from Anachemia, asolectin was from Associated Concentrates, the bicinchoninic acid assay reagents were from Pierce, and the reagents used for electrophoresis were from Bio-Rad. Other chemicals were of the highest purity available commercially.

RESULTS AND DISCUSSION

Reconstitution of Na^+ -dependent phosphate-transport activity

Proteins extracted from purified brush-border membrane vesicles, in the presence of glycerol and brush-border membrane lipids, were reconstituted into proteoliposomes prepared with brush-border membrane lipids enriched with cholesterol. The rate of phosphate uptake into these proteoliposomes was rapid at first and decreased progressively during the first 2 h (Fig. 1a). Thereafter, the amount of phosphate taken up increased more slowly for at least 4 h more. Phosphate uptake was strongly stimulated by the presence of a trans-membrane Na^+ gradient. The Na^+ -dependent transport activity, which was calculated by subtracting the uptake values measured in the absence of Na^+ from those measured in its presence, reached a maximum at about 4 h and decreased slightly after 6 h of incubation (Fig. 1b). The phosphate taken up in a Na^+ -dependent manner accumulated into an osmotically sensitive space, since it was decreased considerably when the proteoliposomes were lysed by dilution into a hypo-osmotic medium (Table 1). The Na^+ -independent uptake was, however, much less sensitive to osmotic shock.

Fig. 1. Time course for the uptake of phosphate into reconstituted membrane vesicles

(a) Proteoliposomes were prepared as described in the text and suspended in 5 mM-Hepes/Tris, pH 7.5, 200 μ M- $[^{32}P]KH_2PO_4/K_2HPO_4$ (1 μ Ci/assay) and 150 mM- $NaNO_3$ ($\square$) or - KNO_3 ($\blacksquare$). (b) The Na^+ -dependent transport activity was calculated by subtracting the uptake values measured in the presence of KNO_3 from those measured in the presence of $NaNO_3$. Values are means $\pm$ s.d. of seven independent experiments performed in triplicate.

Table 1. Effect of osmotic shock on the retention of phosphate taken up by reconstituted membrane vesicles

Proteoliposomes (5 μ l) were incubated in 25 μ l of 5 mM-Hepes/Tris, pH 7.5, 200 μ M-[32 P]KH $_2$ PO $_4$ /K $_2$ HPO $_4$ (1 μ Ci/assay) and 150 mM-NaNO $_3$ or -KNO $_3$. After a 60 min incubation period, control liposomes were diluted into stop solution and filtered immediately, and the test liposomes were diluted into 270 μ l of 5 mM-Hepes/Tris, pH 7.5, for 30 s before addition of the stop solution. Values are means $\pm$ S.D. of three independent experiments performed in triplicate.

	Phosphate uptake (nmol/mg of protein)	
	NaNO $_3$	KNO $_3$
Control	6.71 $\pm$ 0.31	3.60 $\pm$ 0.25
Osmotic shock	4.32 $\pm$ 0.28	2.96 $\pm$ 0.25

Table 2. Phosphate-transport activity of proteoliposomes prepared with different lipid mixtures

Proteoliposomes were prepared as described in the Materials and methods section with the indicated lipid mixtures and incubated in 5 mM-Hepes/Tris, pH 7.5, 200 μ M-[32 P]KH $_2$ PO $_4$ /K $_2$ HPO $_4$ (1 μ Ci/assay) and 150 mM-NaNO $_3$ or -KNO $_3$. Na $^+$ -dependent transport was calculated by subtracting the uptake values measured in the presence of KNO $_3$ from those measured in the presence of NaNO $_3$. For each experiment, the lipids used for the preparation of the liposomes were also included in the buffer used for solubilizing the proteins. Values are means $\pm$ S.D. of three independent experiments performed in triplicate.

Source of lipid mixture	Na $^+$ -dependent phosphate uptake (nmol/mg of protein)	
	15 min	2 h
Brush-border membrane	2.33 $\pm$ 0.36	5.72 $\pm$ 1.42
Kidney cortex homogenate	2.52 $\pm$ 1.40	6.84 $\pm$ 0.72
Asolectin + cholesterol*	0.71 $\pm$ 0.36	0.63 $\pm$ 0.40
Egg-yolk phospholipids + cholesterol*	0.43 $\pm$ 0.21	0.29 $\pm$ 0.27

* 30 mol %.

Fig. 2. Effect of monensin on the uptake of phosphate into reconstituted membrane vesicles

Proteoliposomes were incubated in 5 mM-Hepes/Tris, pH 7.5, 200 μ M-[32 P]KH $_2$ PO $_4$ /K $_2$ HPO $_4$ (1 μ Ci/assay), 0.5 % ethanol and 150 mM-NaNO $_3$ ($\square$, $\blacksquare$) or -KNO $_3$ ($\circ$, $\bullet$) with ($\blacksquare$, $\bullet$) or without ($\square$, $\circ$) 21.6 μ M-monensin. Values are means $\pm$ S.D. of three independent experiments performed in triplicate.

When monensin was added to the incubation medium to make the proteoliposomes permeable to Na $^+$, phosphate uptake was decreased to a level which approached that measured in the absence of Na $^+$ (Fig. 2). Monensin, however, had no effect on the uptake of phosphate in the absence of Na $^+$. The incomplete inhibition of the Na $^+$ -dependent transport activity by monensin is probably due to the ability of the carrier to transport significantly more phosphate in the presence of Na $^+$ than in its absence, even when Na $^+$ is equilibrated on both sides of the membrane, as was demonstrated with brush-border membrane vesicles (Strvey *et al.*, 1990). This Na $^+$ -gradient-independent activity was also unaffected by monensin. In an effort to measure the uptake of phosphate under Na $^+$ -equilibrium conditions, proteoliposomes were prepared in NaNO $_3$ instead of KNO $_3$. The total amount of phosphate taken up by these proteoliposomes was greatly decreased, and no Na $^+$ -dependent transport activity could be detected (Fig. 3). A similar loss of Na $^+$ -dependent transport activity was previously described by McCormick *et al.* (1985) for the reconstituted amino acid co-transporter from Ehrlich ascites cells. These authors demonstrated that replacing K $^+$ by Na $^+$, or any of several other cations, in the buffer used for the preparation of the liposomes resulted in substantially decreased intravesicular volumes and poor transport activities. The importance of K $^+$ ions for the reconstitution of transport activities was also shown to be related to its effect on the degree of vesicle fusion during the freeze-thaw cycle, rather than to a direct effect on the carrier molecule (McCormick *et al.*, 1985).

Fig. 3. Effect of medium composition on the reconstitution of Na $^+$ -dependent phosphate-transport activity

Proteoliposomes were prepared as described in the Materials and methods section with 5 mM-Hepes/Tris, pH 7.5, and 150 mM-KNO $_3$ ($\circ$, $\bullet$) or -NaNO $_3$ ($\square$, $\blacksquare$) for gel-filtration chromatography and final resuspension of the liposomes. These were then incubated in 5 mM-Hepes/Tris, pH 7.5, 200 μ M-[32 P]KH $_2$ PO $_4$ /K $_2$ HPO $_4$ (1 μ Ci/assay) and 150 mM-NaNO $_3$ ($\circ$, $\square$) or -KNO $_3$ ($\bullet$, $\blacksquare$). Values are means $\pm$ S.D. of three independent experiments performed in triplicate.

Addition of brush-border membrane lipids and glycerol to the extraction buffer appears to be crucial for the efficient reconstitution of the renal phosphate co-transporter. A strong Na $^+$ -dependent phosphate-transport activity could be reconstituted, however, when brush-border membrane lipids were replaced by lipids extracted from kidney cortex homogenate (Table 2). In contrast, proteoliposomes prepared with asolectin or egg-yolk phospholipids and 30 % (w/v) cholesterol were unable to transport phosphate in a Na $^+$ -dependent manner (Table 2).

A Na $^+$ -dependent phosphate-transport activity was only observed when protein was reconstituted into the liposomes. The proteoliposomes nevertheless accumulated substantial amounts

Fig. 4. Effect of phosphate concentration on the uptake of phosphate by reconstituted membrane vesicles

(a) Proteoliposomes were incubated for 3 h in 5 mM-Hepes/Tris, pH 7.5, 150 mM-NaNO₃ (□) or -KNO₃ (■), and 1–500 μM-[³²P]KH₂PO₄/K₂HPO₄ (1 μCi/assay), pH 7.5. (b) The Na⁺-dependent transport activity was calculated by subtracting the uptake values measured in the presence of KNO₃ from those measured in the presence of NaNO₃. Values are means ± s.d. of three independent experiments performed in triplicate.

Table 3. Effect of *trans* phosphate on the uptake of phosphate in reconstituted membrane vesicles

Proteoliposomes were prepared as described in the Materials and methods section, except that the buffer used for gel-filtration chromatography and final resuspension of the liposomes was replaced by 135 mM-KNO₃, 5 mM-Hepes/Tris and 10 mM-K₂SO₄ or -KH₂PO₄/K₂HPO₄, pH 7.5. The proteoliposomes (5 μl) were diluted into 245 μl of 5 mM-Hepes/Tris, pH 7.5, containing a trace of [³²P]KH₂PO₄/K₂HPO₄ (1 μCi/assay) and 150 mM-NaNO₃ or -KNO₃ and incubated for 3 h. Values are means ± s.d. of three independent experiments performed in triplicate.

Trans phosphate (mM)	Phosphate uptake (nmol/3 h per mg of protein)	
	NaNO ₃	KNO ₃
0	10.99 ± 0.94	7.19 ± 0.58
10	10.57 ± 0.66	4.07 ± 0.37

of phosphate even when Na⁺ was replaced by K⁺ in the incubation medium. This Na⁺-independent uptake is probably due, in large part, to binding of phosphate to the liposomes. Brush-border membrane lipid extracts are known to contain phosphate-binding proteolipids (Kessler *et al.*, 1982; Kessler & Vaughn, 1984; Debic & Lorenc, 1988). Liposomes prepared with such lipid extracts, without added protein, took up 0.26 ± 0.02 and

0.27 ± 0.04 nmol of phosphate/μmol of phospholipid after 2 h, in the presence and absence of Na⁺, respectively. Since relatively long incubation times had to be used to demonstrate maximal Na⁺-dependent transport activity, diffusion of phosphate across the lipid bilayer probably also contributes to the Na⁺-independent uptake.

Effects of *cis* phosphate on the uptake of phosphate

Both in the presence and in the absence of Na⁺, the rate of phosphate uptake increased rapidly with phosphate concentration at low phosphate concentrations and more slowly at higher concentrations (Fig. 4a). The Na⁺-dependent transport activity became saturated at about 200 μM-phosphate, with an apparent half-saturation constant of about 40 μM (Fig. 4b). This value is in reasonably good agreement with those of 80 μM (Hoffmann *et al.*, 1976) and 85 μM (Cheng & Sacktor, 1981) obtained with intact brush-border membrane vesicles, although the measurements of phosphate uptake into reconstituted vesicles were done after a fairly long incubation period.

Effect of *trans* phosphate on the uptake of phosphate

Phosphate transport into brush-border membrane vesicles is known to be strongly stimulated by the presence of high concentrations of phosphate on the *trans* side of the membrane (Béliveau & Strévey, 1988). The uptake of phosphate into proteoliposomes containing 0 and 10 mM-phosphate is compared in Table 3. Although the total uptake of phosphate was unaffected by *trans* phosphate in the presence of Na⁺, it was decreased by 43 % when Na⁺ was absent. This decrease is interpreted as being due to the competition of intravesicular phosphate for phosphate-binding sites. Since a similar decrease in phosphate binding should also occur in the presence of Na⁺, we conclude that *trans* phosphate stimulated the Na⁺-dependent transport activity, which increased from 3.80 ± 0.76 nmol/3 h per mg of protein in the absence of *trans* phosphate to 6.50 ± 0.51 nmol/3 h per mg of protein in the presence of 10 mM-*trans* phosphate.

Effect of inhibitors of phosphate transport

Phosphonoformic acid and phosphonoacetic acid, two phosphate analogues which are considered as specific inhibitors of the brush-border membrane phosphate-transport system (Szczepanska-Konkel *et al.*, 1986), had very little effect on the uptake of phosphate by the proteoliposomes (Table 4). The ability of phosphonoformate and phosphonoacetate to inhibit phosphate uptake into intact isolated bovine brush-border membranes and, as a control, those from rat was therefore tested (Table 4). Although the inhibition of phosphate transport into rat brush-border membrane vesicles by both compounds was similar to previously published results, the bovine phosphate carrier was much less sensitive than those of the rat and several other species (Szczepanska-Konkel *et al.*, 1986). Arsenate, which is also known to be a potent inhibitor of phosphate transport, decreased phosphate uptake into proteoliposomes as well as in brush-border membrane vesicles from both species tested (Table 4).

Other substrates

Proteoliposomes were tested for their ability to take up other substrates which are normally transported across the brush-border membrane by Na⁺-dependent carriers. The uptake of D-glucose, L-alanine, L-proline, L-leucine, L-glycine, L-glutamate and β-alanine was only slightly stimulated by the presence of a Na⁺ gradient, in contrast with the uptake of phosphate by the same proteoliposomes. The presence of Na⁺ and phosphate in the extraction medium may have contributed to protect specifi-

Table 4. Effect of phosphate-transport inhibitors on the uptake of phosphate by proteoliposomes and by brush-border membrane vesicles isolated from bovine and rat kidney cortex

Proteoliposomes were incubated for 3 h and membrane vesicles were incubated for 20 s in a solution containing 5 mM-Hepes/Tris, pH 7.5, 200 μ M- $[^{32}\text{P}]\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ (1 μ Ci/assay), 150 mM-NaNO₃ or -KNO₃ and the indicated inhibitors. Values are means $\pm$ S.D. of three independent experiments performed in triplicate.

	Phosphate uptake			
	Control	Phosphonoformate (2 mM)	Phosphonoacetate (2 mM)	Arsenate (5 mM)
Proteoliposomes (nmol/3 h per mg of protein)				
NaNO ₃	11.14 $\pm$ 0.82	10.46 $\pm$ 0.56	10.72 $\pm$ 0.61	5.46 $\pm$ 0.41*
KNO ₃	4.60 $\pm$ 0.45	4.80 $\pm$ 0.31	4.40 $\pm$ 0.31	3.42 $\pm$ 0.27*
Bovine brush-border membrane vesicles (nmol/20 s per mg of protein)				
NaNO ₃	1.18 $\pm$ 0.06	0.92 $\pm$ 0.04*	1.06 $\pm$ 0.22	0.57 $\pm$ 0.07*
KNO ₃	0.016 $\pm$ 0.001	0.023 $\pm$ 0.002	0.016 $\pm$ 0.003	0.012 $\pm$ 0.002*
Rat brush-border membrane vesicles (nmol/20 s per mg of protein)				
NaNO ₃	2.66 $\pm$ 0.26	1.17 $\pm$ 0.03*	2.03 $\pm$ 0.42	0.82 $\pm$ 0.02*
KNO ₃	0.037 $\pm$ 0.006	0.063 $\pm$ 0.010	0.044 $\pm$ 0.005	0.051 $\pm$ 0.007

* Significantly lower than the corresponding control value ($P < 0.025$; unpaired t test).

Fig. 5. SDS/PAGE of brush-border membrane proteins

Lanes: 1, molecular-mass standards; 2, total bovine brush-border membrane proteins; 3, proteins that were not solubilized and were removed by ultracentrifugation; 4, CHAPS-solubilized proteins; 5, proteins reconstituted into proteoliposomes. Lanes 1–4 contained 20 μ g of protein and lane 5 contained 15 μ g.

strongly enriched for proteins of 96, 120 and 152 kDa. A similar selective incorporation of brush-border membrane proteins into reconstituted membrane vesicles has been observed previously (Kinne & Faust, 1977; Koepsell *et al.*, 1983; Lynch & McGivan, 1987).

Conclusions

The reconstitution protocol described here is both simple and reliable. It allows the rapid reconstitution of a strongly Na⁺-dependent phosphate-transport activity with characteristics similar to those described for the phosphate carrier in isolated brush-border membrane vesicles. The results described in the present paper compare favourably with those obtained with previously published protocols for the reconstitution of the renal Na⁺/phosphate co-transporter. Na⁺-dependent phosphate-transport activities were reported previously (Kinne & Faust, 1977; Lin *et al.*, 1984), but the characteristics of these reconstituted activities were not described in detail, and those studies were apparently not pursued further. The more detailed studies reported by Schäli & Fanestil (1985) and by Schäli *et al.* (1986) showed a Na⁺-dependence for phosphate uptake which was small in comparison with the total uptake. Our procedure should therefore prove useful for the purification and identification of the phosphate carrier, a step which is essential for the characterization of this transport activity at the molecular level and for our understanding of the mechanisms involved in the renal re-absorption of phosphate.

The skilful technical assistance of Ms. Sylvie Giroux is gratefully acknowledged. This work was supported by grants from the Medical Research Council (MRC) of Canada to R.B. and from the Natural Sciences and Engineering Research Council (NSERC) of Canada to R.L. V. V. was the recipient of a Post-Doctoral Fellowship from the Fonds de la recherche en santé du Québec (FRSQ), and M.-C.D. was the recipient of a Scholarship for graduate studies from the Fonds pour la formation de chercheurs et l'aide à la recherche (FCAR).

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cally the phosphate carrier from becoming inactivated during the solubilization of the membranes. Alternatively, the phosphate carrier may have been solubilized, and thus reconstituted, more efficiently than the other transporters. This interpretation is consistent with the fact that only about 80 % of the proteins originally present in the brush-border membrane were solubilized by the detergent. Furthermore, SDS/PAGE revealed strikingly different protein compositions in intact brush-border membranes, solubilized proteins and proteoliposomes (Fig. 5). When compared with intact brush-border membranes, the pellet containing the unsolubilized proteins was most strongly enriched with a protein of 45 kDa, probably actin. The proteoliposomes were

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Received 1 November 1990/9 April 1991; accepted 8 May 1991