Biochimica et Biophysica Acta, 1145 (1993) 250–256

BBAMEM 75862

Solubilization and functional reconstitution of the human placental taurine transporter

Sammanda Ramamoorthy, Palaniappan Kulanthaivel, Frederick H. Leibach, Virendra B. Mahesh and Vadivel Ganapathy

Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA (USA)

(Received 28 April 1992)

Key words: Taurine transporter; Solubilization; Reconstitution; Brush-border membrane; (Human placenta)

The taurine transporter from purified human placental brush-border membranes was solubilized and reconstituted into proteoliposomes in a functional form. Solubilization was done with 2.5% cholate in the presence of 4 M urea. The proteins in the solubilizate were precipitated with 6% poly(ethylene glycol) and the precipitated proteins were reconstituted into proteoliposomes with an asolectin/protein ratio of 10:1. Under these experimental conditions, the taurine transport activity in the proteoliposomes was maximal. SDS-PAGE analysis of proteins, however, revealed that the proteoliposomes still contained a majority of the proteins originally present in the brush-border membranes. Uptake of taurine in the reconstituted proteoliposomes was obligatorily dependent on the presence of Na^+ as well as Cl^-. Substitution of Na^+ with other monovalent cations such as K^+ and Li^+ reduced the taurine transport activity drastically. Similarly, substitution of Cl^- with other monovalent anions such as SCN^-, F^-, I^- and NO_3^- could support the transport activity only to a maximum of 30% of the control activity. In the presence of Cl^-, the uptake rate was sigmoidally related to Na^+ concentration, resulting in a Na^+/taurine coupling ratio of 2:1. The apparent dissociation constant for Na^+ was about 195 mM. In the presence of Na^+, the uptake rate was hyperbolically related to Cl^- concentration, indicating a Cl^-/taurine coupling ratio of 1:1. The apparent dissociation constant for Cl^- was about 205 mM. The NaCl-dependent taurine uptake was stimulated by an inside-negative membrane potential, showing that the uptake process was electrogenic. The uptake system was specific for β-amino acids. The affinity of the system for taurine was high with an apparent dissociation constant of 2.7 ± 0.1 μM. It is concluded that the taurine transporter can be dislodged from the placental brush-border membranes and reconstituted in a catalytically active form in proteoliposomes with no significant change in its characteristics.

Introduction

Taurine (β-aminoethanesulfonic acid) uptake in animal cells occurs via a specific transport system which is obligatorily dependent not only on the presence of Na^+ but also on the presence of Cl^- for its transport function [1,2]. For many years we have been interested in the mechanisms of taurine transfer across the human placenta because of the essential role of this amino acid in the growth and development of the placenta and the fetus [3–5]. We have studied placental taurine transport with isolated brush-border membrane vesicles from human term placentas [6,7] as well as with cultured human placental choriocarcinoma cells [8,9]. These studies have demonstrated that the human placenta possesses a very active mechanism for the transport of taurine from mother to fetus. The characteristics of the placental taurine transporter are similar to those described for the taurine transporter in other tissues.

The progress made thus far in the understanding of the nature of the taurine transporter in the placenta deals only with the general characteristics such as substrate specificity, ionic dependence, energetics and regulation. Virtually nothing is known about the transporter at the molecular level. The goal of identification, purification and characterization of the protein(s) responsible for the transport function still remains elusive. An important contributing factor to this problem is the lack of specific assay methods for monitoring the transport protein during various purification steps. This problem is solved if the transport protein can be dislodged from the membrane by detergent solubilization and subsequently reconstituted in functional form

Correspondence to: V. Ganapathy, Department of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA 30912-2100, USA.

Abbreviations: PEG, poly(ethylene glycol); PMSF, phenylmethylsulfonyl fluoride; DTT, dithiothreitol.

in proteoliposomes. The transport function of the protein can be measured in these proteoliposomes and this procedure can be used to monitor the protein during purification. Success of this approach, however, relies upon the availability of a simple, effective and reproducible reconstitution procedure. We report in this paper on the solubilization and functional reconstitution of the human placental taurine transporter. We have also characterized the taurine transporter in this reconstituted system and our results show that the properties of the reconstituted transporter are very similar to those of the native transporter.

Materials and Methods

Materials

Cholate (sodium salt), PEG (average molecular weight, 8000), PMSF, valinomycin and unlabelled amino acids were purchased from Sigma (St. Louis, MO, USA). Ultrapure urea was purchased from Pierce (Rockford, IL, USA). Asolectin was from Associated Concentrates (Woodside, NY, USA).

[2(n)-^{3}H]Taurine (specific radioactivity, 25.6 Ci/mmol) was obtained from DuPont-New England Nuclear (Boston, MA, USA).

Methods

Preparation of placental brush-border membranes. The procedure for the preparation of placental brush-border membranes was the same as previously described from this laboratory [10,11]. Membranes were suspended in 5 mM Tris-HCl buffer (pH 7.4), containing 150 mM NaCl. Protein concentration of the membrane suspension was adjusted to 10 mg/ml and stored in 1 ml aliquots in liquid N_2.

Solubilization and reconstitution. The procedure for solubilization of the placental brush-border membranes and for reconstitution of the solubilized proteins into proteoliposomes was essentially similar to those described by McCormick et al. [12] and by Tamarappoo and Kilberg [13]. The procedure involves solubilization of the membranes with cholate in the presence of urea, precipitation of the solubilized proteins with PEG and reconstitution using asolectin in the presence of K^+. We have recently used this procedure successfully for solubilization and functional reconstitution of the placental serotonin transporter [14].

2 ml of the brush-border membrane suspension (20 mg of protein) were thawed at 37°C and mixed with 8 ml of solubilization buffer (10 mM Hepes-Tris (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.3 mM DTT and 1 μM PMSF) containing 5 M urea and 3.125% cholate. The mixture was stirred constantly at 4°C for 30 min. The suspension was then diluted with an equal volume of the solubilization buffer and centrifuged at 220 000 $\times g$ for 1 h. The solubilized proteins were recovered in the clear supernatant. In some experiments, the insoluble material recovered in the pellet was also saved and suspended in the solubilization buffer containing 2 M urea and 1.25% cholate.

Proteins in the solubilizate and in the pellet suspension were precipitated with 6% PEG. The mixture was kept on ice for 15 min and then centrifuged at 140 000 $\times g$ for 30 min. The resulting pellet was rinsed four times with the reconstitution buffer (10 mM Hepes-Tris (pH7.5), 200 mM potassium gluconate and 1 mM magnesium gluconate) and then suspended in a small volume of the same buffer by passing through a 25 gauge needle. Protein concentration of the suspension was adjusted to 1 mg/ml and then immediately used for reconstitution.

The reconstitution step involved freezing and thawing of the protein-phospholipid mixture followed by sonication. Asolectin suspension was prepared in the reconstitution buffer at a concentration of 40 mg/ml by first vortexing the suspension for 6 min and then by sonicating the material for 15 min in a bath type sonicator. These steps were carried out under N_2. The final sample was translucent. The PEG-precipitated proteins were mixed with a desired volume of the asolectin suspension to give an asolectin/protein ratio of 10:1. The mixture was subjected to freezing and thawing two times in an ethanol/dry ice bath and then sonicated for 3 min. The suspension was diluted with 20 volumes of the reconstitution buffer and the proteoliposomes were harvested by centrifugation of the suspension at 85 000 $\times g$ for 30 min. The pellet containing the proteoliposomes was suspended in a small volume of the reconstitution buffer by passing through a 25 gauge needle. The suspension was once again sonicated for 3 min and then immediately used for uptake measurements.

The insoluble material obtained after cholate-urea solubilization represents detergent-disrupted membrane fragments. The same procedure described above for reconstitution of the solubilized proteins was employed in the case of the insoluble material to restore transport-competent vesicles by the addition of exogenous phospholipids.

Uptake measurements. This was done using a rapid filtration technique as described previously [15]. Proteoliposomes (40 μl) were rapidly mixed with uptake medium (160 μl) containing radiolabelled taurine. After incubation for a desired time, the mixture was filtered through a Millipore filter (DAWP type, 0.65 μm pore size). The filter was then washed and the radioactivity associated with the filter was counted. The uptake medium in most experiments was 10 mM Hepes-Tris (pH 7.5), containing 200 mM NaCl. The composition of the stop buffer was 5 mM Hepes-Tris (pH 7.5), containing 210 mM KCl.

TABLE I

Distribution of taurine transport activity in the solubilizate and in the pellet after treatment of the placental brush-border membranes with cholate in the presence of urea

Brush-border membranes (20 mg protein) were solubilized for 30 min in the presence of 2.5% cholate and 4 M urea and the supernatant (solubilizate) and the pellet were separated by centrifugation for 1 h at 220000 ×g. Precipitation of the proteins by PEG and reconstitution of the proteins into proteoliposomes were done as described in Materials and Methods. Uptake of taurine (0.3 μM) into proteoliposomes was measured with a 1-min incubation. Total protein recovered in the final proteoliposome preparations from the solubilizate and the pellet was 1.70 and 1.24 mg, respectively.

Uptake conditions	Solubilizate	Pellet
Specific activity (pmol/mg of protein per min)		
NaCl	5.10 ± 0.01	1.82 ± 0.15
KCl	0.24 ± 0.00	0.26 ± 0.01
Na^+-dependent	4.85 ± 0.01	1.56 ± 0.09
Total activity (pmol/min)		
Na^+-dependent	8.10 ± 0.01	1.94 ± 0.11
Percent distribution	81	19

Data analysis. Uptake measurements were done in duplicate or triplicate and the variation among the replicate values was always less than ±5% of the mean value. The experiments were repeated two or three times and the results from these experiments are given as means ± S.E. When not given, the error is small and lies within the symbol. Kinetic and statistical analyses were done using a commercially available computer statistics package Stagraphics (STSC, Rockville, MD, USA).

Results

Placental brush-border membranes were solubilized with cholate in the presence of urea and the taurine transport activity in the solubilizate and in the pellet was determined after reconstitution into proteoliposomes (Table I). It was found that about 80% of the taurine transport activity was associated with the proteins in the solubilizate and only 20% with the proteins in the insoluble material. The specific activity of the Na^+-dependent taurine transport in the proteoliposomes prepared with the solubilized proteins was approximately three times higher than the specific activity in the proteoliposomes prepared with the insoluble proteins (4.85 ± 0.01 versus 1.56 ± 0.09 pmol/mg of protein per min at a taurine concentration of 0.3 μM).

The characteristics of the solubilized taurine transporter were then studied in reconstituted proteoliposomes. Fig. 1 describes the time course of taurine uptake in these proteoliposomes in the presence of either NaCl or KCl in the uptake medium. In the absence of NaCl, uptake of taurine was negligible at all time periods studied. The presence of NaCl in the uptake medium increased the uptake 50- to 100-fold. With a 1-min incubation and at a taurine concentration of 0.2 μM, uptake in the presence of NaCl was 5.3 ± 0.2 pmol/mg of protein whereas the corresponding value in the absence of NaCl was only 0.10 ± 0.02 pmol/mg of protein. Uptake from the NaCl medium initially increased with time, but at longer periods of incubation (> 60 min) taurine concentration inside the proteoliposomes decreased considerably because the rate of efflux became increasingly greater with time than the rate of influx due to the gradual dissipation of the NaCl gradient. However, equilibrium was not reached even when the incubation was continued for 5 h.

In order to determine the cation and anion specificity of the reconstituted taurine transporter, we measured taurine uptake from different uptake media with varying cationic and anionic composition (Table II). It was found that the uptake of taurine from the media containing either KCl or LiCl was negligible, representing only 3 to 5% of the uptake measured from the NaCl-containing medium. Comparison of the uptake rates measured in the presence of sodium salts of Cl^-, F^-, I^-, NO_3^- or SCN^- revealed that the uptake was highest in the presence of Cl^- and lowest in the presence of F^-. The anions I^-, NO_3^- and SCN^- could substitute for Cl^- to a small but significant extent. These data show that the reconstituted placental taurine transporter exhibits an absolute requirement for Na^+ and Cl^- for its catalytic activity.

We then studied the kinetics of the Na^+- and Cl^--dependence of taurine uptake. In these experiments,

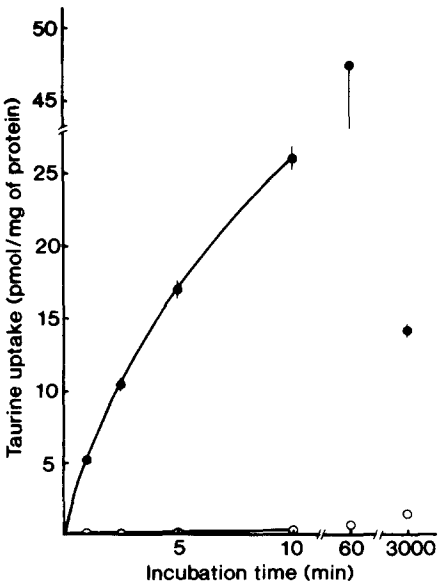

Fig. 1. Time course of taurine uptake (0.2 μM) in reconstituted proteoliposomes measured in the presence of either NaCl (●) or KCl (○) in the uptake medium.

TABLE II

Influence of anions and cations on the activity of the reconstituted placental taurine transporter

Uptake of taurine (0.3 μM) into proteoliposomes was measured with a 1-min incubation. Uptake buffer was 10 mM Hepes-Tris (pH 7.5) containing 187.5 mM of the indicated inorganic salts.

Salt	Taurine uptake	
	pmol/mg of protein per min	%
NaCl	4.31 ± 0.10	100
LiCl	0.13 ± 0.02	3
KCl	0.20 ± 0.03	5
NaF	0.19 ± 0.01	5
NaI	0.75 ± 0.01	18
$NaNO_3$	0.64 ± 0.01	15
NaSCN	1.35 ± 0.01	31

proteoliposomes were reconstituted in 10 mM Hepes-Tris buffer (pH 7.5), containing 400 mM potassium gluconate and 1 mM magnesium gluconate. First, we measured the initial rates of taurine uptake at varying concentrations (0–300 mM) of the external Na^+ while keeping the external Cl^- concentration constant (300 mM) (Fig. 2). The dependence of the uptake rate on the concentration of Na^+ was sigmoidal indicating that the coupling ratio of Na^+/taurine is greater than one. The number of Na^+ ions involved per transport of one

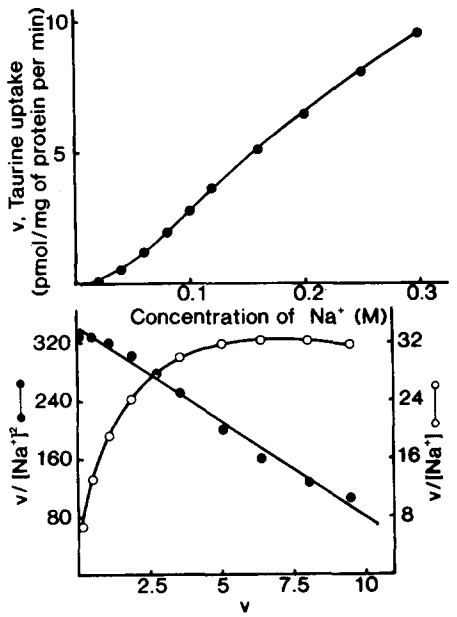

Fig. 2. Dependence of the activity of the taurine transporter in proteoliposomes on the concentration of Na^+. Proteoliposomes were prepared with a reconstitution buffer containing 400 mM K gluconate instead of 200 mM. Uptake of taurine (0.2 μM) was measured with a 1-min incubation in the presence of varying concentrations of Na^+ (0–300 mM) and fixed concentration of Cl^- (300 mM). Uptake buffers were prepared by appropriately mixing two solutions containing either 400 mM NaCl or 400 mM KCl. Both solutions were buffered with 10 mM Hepes-Tris (pH 7.5).

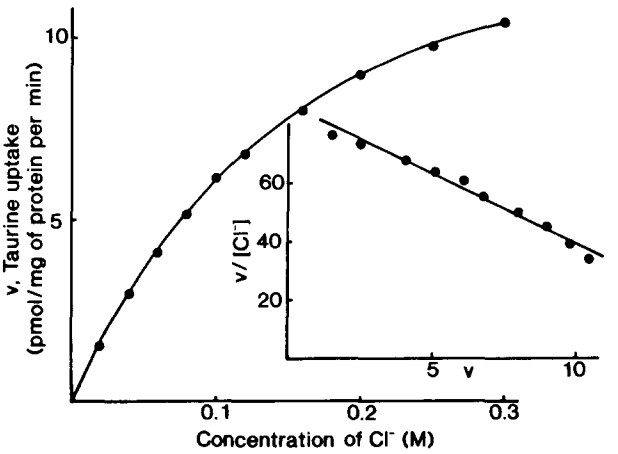

Fig. 3. Dependence of the activity of the taurine transporter in proteoliposomes on the concentration of Cl^-. Proteoliposomes were prepared with a reconstitution buffer containing 400 mM potassium gluconate instead of 200 mM. Uptake of taurine (0.2 μM) was measured with a 1-min incubation in the presence of varying concentrations of Cl^- (0–300 mM) and a fixed concentration of Na^+ (300 mM). Uptake buffers were prepared by appropriately mixing two solutions containing either 400 mM NaCl or 400 mM sodium gluconate. Both solutions were buffered with 10 mM Hepes-Tris (pH 7.5).

taurine molecule could be calculated from this experiment by a procedure described by Turner [16]. In this method, the experimental data are fitted to a Hill-type equation

$$v = \frac{V_{max}[Na^+]^n}{K^n_{t(Na^+)} + [Na^+]^n}$$

where v is the taurine uptake rate, $K_{t(Na^+)}$ is the Michaelis-Menten constant (apparent dissociation constant) for Na^+ for interaction with the transporter and n is the Na^+/taurine coupling ratio (i.e., the number of Na^+ ions involved per transport of one taurine molecule). The plots of v versus $v/[Na^+]^n$ were constructed, assigning different numerical values for n. The plot was clearly curvilinear when $n = 1$ whereas it was close to a straight line ($r^2 > 0.99$) when $n = 2$. Thus, two Na^+ ions are involved per transport of one taurine molecule in these reconstituted proteoliposomes. With the value of $n = 2$, the apparent dissociation constant for Na^+ was calculated to be approx. 195 mM.

We also studied the Cl^- kinetics of the reconstituted taurine transporter (Fig. 3). The dependence of taurine uptake on $[Cl^-]$ was hyperbolic, suggesting a Cl^-/taurine ratio of 1:1. This was supported by the observation that the plot of v versus $v/[Cl^-]$ was linear ($r^2 > 0.99$). The approximate value for $K_{t(Cl^-)}$ was 205 mM.

Taurine exists as a zwitterion at pH 7.5. If the stoichiometry of Na^+/Cl^-/taurine for the reconsti-

tuted taurine transporter is 2:1:1 as suggested by the above experiments, the uptake process would be electrogenic, resulting in the transfer of net positive charge across the membrane. Therefore, we determined the electrogenicity of the taurine uptake process in the proteoliposomes by studying the influence of an inside-negative, K^+-diffusion potential on the initial rate of taurine uptake. The NaCl-dependent taurine uptake was measured with an outwardly directed K^+ gradient ($[K^+]_i = 200$ mM; $[K^+]_o = 40$ mM) in the presence or absence of valinomycin, a K^+ ionophore. The ionophore is expected to generate an inside-negative K^+-diffusion potential under these experimental conditions. When the uptake rates (taurine concentration, 0.3 μM) measured in the presence and absence of valinomycin were compared, it was found that the uptake in the presence of the ionophore was significantly greater than the uptake in its absence (control, 2.23 ± 0.03 pmol/mg of protein per 15 s; membrane potential, 3.23 ± 0.03 pmol/mg of protein per 15 s; $P < 0.001$). These data provide evidence for the electrogenic nature of the reconstituted taurine transporter.

Human placental brush-border membranes possess a number of amino acid transport systems with characteristic substrate specificities [17,18]. In order to show that the taurine transport activity measured in the reconstituted proteoliposomes actually represents the activity of the β-amino acid transport system which accepts only the β-amino acids such as taurine as substrates, we studied the effects of the representative substrates for the various amino acid transport systems of the placental brush-border membrane on the uptake of radiolabelled taurine in the proteoliposomes. The results given in Table III show that the uptake of radiolabelled taurine was markedly inhibited by taurine, hypotaurine and β-alanine. All of these are β-amino acids. On the contrary, the substrates of the other amino acid transport systems, e.g., alanine (system ASC), proline (system A), aspartic acid (system X_{AG}^-), lysine (system y^+) and leucine (system L), had no significant effect. Thus, the amino acid transport system responsible for the uptake of taurine in the reconstituted proteoliposomes accepts only β-amino acids as substrates.

TABLE III

Influence of unlabelled amino acids on the uptake of radiolabelled taurine in reconstituted proteoliposomes

Uptake of radiolabelled taurine (0.2 μM) into proteoliposomes was measured with a 1-min incubation. Final concentration of unlabelled amino acids during uptake was 250 μM.

Unlabelled amino acid	Taurine uptake	
	pmol/mg of protein per min	%
None	5.51 ± 0.03	100
Taurine	0.04 ± 0.00	1
Hypotaurine	0.08 ± 0.05	2
β-Alanine	0.17 ± 0.01	3
Alanine	4.78 ± 0.05	87
Leucine	5.44 ± 0.01	99
Proline	5.06 ± 0.08	92
Aspartic acid	5.09 ± 0.14	92
Lysine	5.20 ± 0.06	94

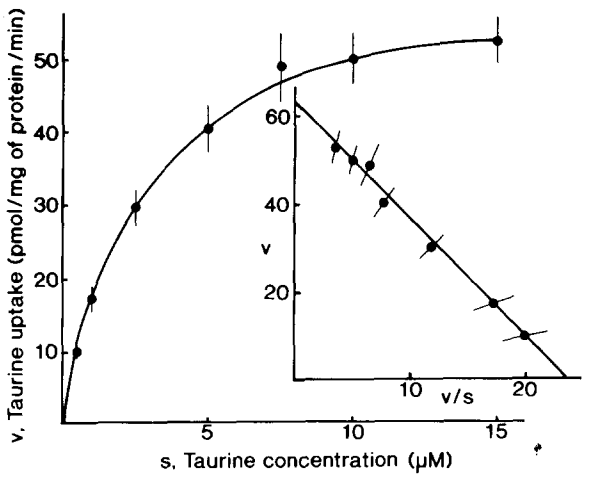

Fig. 4. Dependence of the uptake rate on the concentration of taurine in proteoliposomes. Uptake of taurine was measured with a 1-min incubation over the taurine concentration range of 0.5–15 μM. At each concentration, the amount of radiolabelled taurine was kept constant (0.3 μM) and the amount of unlabelled taurine was varied to give the desired taurine concentration. Uptake of radiolabel measured in the presence of 1 mM unlabelled taurine was used to calculate the non-carrier-mediated component of the uptake. This value was subtracted from the total uptake at each taurine concentration to determine the carrier-mediated component. Only the latter component was used in the kinetic analyses. Inset: uptake rate/taurine concentration versus uptake rate (Eadie-Hofstee plot).

The dependence of the uptake rate on substrate concentration was investigated for taurine uptake in proteoliposomes. In this experiment, uptake was measured with a 1-min incubation over a taurine concentration range of 0.5–15 μM in the presence of an inwardly directed NaCl gradient. When the uptake rate was plotted against taurine concentration, the plot was hyperbolic (Fig. 4), suggesting that a single transport system participates in the uptake of taurine in these reconstituted proteoliposomes. The data were then analyzed by the Eadie-Hofstee plot (uptake rate/taurine concentration versus uptake rate). The plot was linear ($r^2 = 0.99$) (Fig. 4, inset). The kinetic parameters (K_t, apparent dissociation constant and V_{max}, maximal velocity) for the transport system were calculated from the plot. The K_t was 2.7 ± 0.1 μM and the V_{max} was 63.2 ± 1.4 pmol/mg of protein per min.

Discussion

Human placental brush-border membranes possess several amino acid transport systems, including the

taurine transporter, which participate in the transfer of amino acids from mother to fetus. To our knowledge, there has been no report on the reconstitution of any of the placental amino acid transport systems. Ours is the first demonstration of successful solubilization and reconstitution of an amino acid transport system in a catalytically active form from the human placenta. However, functional reconstitution of the taurine transporter from non-placental tissues such as heart has been reported by other investigators [19].

The salient features of the solubilization and reconstitution procedure described in the present paper include (a) solubilization of the membrane proteins by cholate in the presence of urea, (b) removal of cholate/urea and simultaneous precipitation of proteins from the solubilizate by PEG, (c) reconstitution of the PEG-precipitated proteins into proteoliposomes by a freeze-thaw procedure in the presence of K^+, and (d) addition of a sonication step to the reconstitution procedure. The inclusion of urea during solubilization was introduced by McCormick et al. [12]. Preliminary experiments in our laboratory showed that solubilization of the human placental brush-border membranes by 2.5% cholate in the absence of urea led to a significant inactivation of the taurine transport activity. Use of PEG to remove cholate/urea was adopted from the procedure described by Fafournoux et al. [20]. Inclusion of this step improved the efficiency of the procedure in many ways. PEG precipitation provided a fast and efficient method to remove the detergent and urea, rendering the time-consuming dialysis or column chromatography steps described for this purpose in various reconstitution procedures unnecessary. In addition, Lin et al. [21] have shown that the presence of PEG during reconstitution reduces the nonspecific permeability of the reconstituted proteoliposomes. This allows the maintenance of the experimentally imposed transmembrane ion gradients in the proteoliposomes for a longer period of time than is expected in the absence of PEG. As a consequence, the activity of ion gradient-dependent transport systems is expected to be higher in the proteoliposomes prepared in the presence of PEG than in its absence. In our procedure, PEG was not added during the reconstitution step. However, trace amounts of PEG are expected to be present in the PEG-precipitated proteins which might significantly enhance the NaCl gradient-dependent taurine transport activity in the final preparations of proteoliposomes. The advantage of the presence of K^+ during reconstitution was realized from the work of McCormick et al. [22]. These investigators have shown that reconstitution in the presence of K^+ allows the formation of functionally active proteoliposomes whereas reconstitution in the presence of Na^+, Li^+, or choline does not.

Whether sonication of the proteoliposomes has any significant beneficial effect on the incorporation of proteins into liposomes and consequently on the transport activity of the final preparation is equivocal. Many investigators have successfully used sonication to improve the transport function of the proteoliposomes [13,20,23] whereas some others have not [12]. We compared in the present study the taurine transport activity in proteoliposomes prepared with or without the sonication step in the reconstitution procedure (data not shown). We found that the proteoliposomes without sonication showed very little taurine transport activity. Moreover, the activity varied significantly from preparation to preparation. On the other hand, if the sonication step was included, the proteoliposomes showed consistently much higher taurine transport activity and, in addition, there was little variability in the activity among preparations.

The present paper also describes the general characteristics of the reconstituted human placental taurine transporter. This enables us to compare these characteristics with those of the native transporter. The characteristics studied are the dependence on cations and anions, substrate specificity, electrogenicity, kinetics and the Na^+- and Cl^--stoichiometry. The reconstituted transporter is obligatorily dependent upon Na^+ and Cl^-. The system interacts specifically with β-amino acids. The uptake process is electrogenic, with a Na^+/Cl^-/taurine stoichiometry of 2:1:1. The taurine transporter in native placental brush-border membrane vesicles also exhibits similar characteristics [6,7]. The Michaelis-Menten constant for the native transporter has been reported to be in the range of 4.0–6.5 μM [6,7,24] which is close to the value (2.7 μM) obtained in the present study for the reconstituted transporter. In contrast to our studies with the native transporter [6,7] and the reconstituted transporter [present study] which demonstrate the Cl^--dependence of the system, Karl and Fisher [24] have reported that Cl^- failed to stimulate the native taurine transporter in the presence of Na^+. However, these investigators determined the Cl^--dependence of the system in the presence of SCN^-, an anion which could substitute for Cl^- to a significant extent [6,7]. In fact, the study by Karl and Fisher itself [24] has shown that SCN^- was as good as Cl^- in supporting taurine uptake in the presence of Na^+. Therefore, the failure by these investigators to observe the Cl^- dependence of the native transporter was most likely due to the presence of SCN^-.

The SDS-PAGE analysis of proteins revealed that the proteoliposomes contained most of the proteins which were originally present in the native brush-border membranes (data not shown). There was no significant enrichment of the taurine transporter activity in the proteoliposomes compared to the native placental brush-border membrane vesicles. The specific activity of the taurine transporter in proteoliposomes was in

the range of 2.9–5.5 pmol/mg of protein per min at 0.2 μM taurine. Under comparable conditions, the specific activity in native placental brush-border membrane vesicles was in the range of 1.8–4.8 pmol/mg of protein per min [6,7].

Acknowledgements

This work was supported by National Institutes of Health Grant HD 24451. The authors thank Ida O. Thomas and Marcia D. Lewis for expert secretarial assistance.

References

1 Chesney, R.W. (1985) Adv. Pediatr. 32, 1–42.
2 Turner, R.J. (1986) J. Biol. Chem. 261, 16060–16066.
3 Sturman, J.A., Rassin, D.K. and Gaull, G.E. (1977) J. Neurochem. 28, 31–39.
4 Ejiri, K., Akahori, S., Kudo, K., Sekiba, K. and Ubuka, T. (1987) Biol. Neonate 51, 234–240.
5 Ghisolfi, J., Berrebi, A., Nguyen, V.B., Thouvenot, J.P., Rolland, M., Putet, G., Dabadie, A. and Pontonnier, G. (1989) Biol. Neonate 56, 181–185.
6 Miyamoto, Y., Balkovetz, D.F., Leibach, F.H., Mahesh, V.B. and Ganapathy, V. (1988) FEBS Lett. 231, 263–267.
7 Kulanthaivel, P., Leibach, F.H., Mahesh, V.B. and Ganapathy, V. (1989) Biochim. Biophys. Acta 985, 139–146.
8 Kulanthaivel, P., Cool, D.R., Ramamoorthy, S., Mahesh, V.B., Leibach, F.H. and Ganapathy, V. (1991) Biochem. J. 277, 53–58.
9 Ramamoorthy, S., Leibach, F.H., Mahesh, V.B. and Ganapathy, V. (1992) Pediatr. Res. 32, 125–127.
10 Balkovetz, D.F., Leibach, F.H., Mahesh, V.B., Devoe, L.D., Cragoe, Jr., E.J. and Ganapathy, V. (1986) Am. J. Physiol. 251, C852–C860.
11 Kulanthaivel, P., Simon, B.J., Burckhardt, G., Mahesh, V.B., Leibach, F.H. and Ganapathy, V. (1990) Biochemistry 29, 10807–10813.
12 McCormick, J.I., Tsang, D. and Johnstone, R.M. (1984) Arch. Biochem. Biophys. 231, 355–365.
13 Tamarappoo, B.K. and Kilberg, M.S. (1991) Biochem. J. 274, 97–101.
14 Ramamoorthy, S., Cool, D.R., Leibach, F.H., Mahesh, V. and Ganapathy, V. (1992) Biochem. J. 286, 89–95.
15 Ganapathy, V., Mendicino, J.F. and Leibach, F.H. (1981) J. Biol. Chem. 256, 118–124.
16 Turner, R.J. (1983) J. Membr. Biol. 76, 1–15.
17 Yudilevich, D.L. and Sweiry, J.H. (1985) Biochim. Biophys. Acta 822, 169–201.
18 Smith, C.H., Moe, A.J. and Ganapathy, V. (1992) Annu. Rev. Nutr. 12, 183–206.
19 Schaffer, S.W., Kulakowski, E.C. and Kramer, J.H. (1982) In Taurine in Nutrition and Neurology (Huxtable, R.J. and Pasantes-Morales, H., eds.), pp. 143–160, Plenum Press, New York.
20 Fafournoux, P., Dudenhausen, E.E. and Kilberg, M.S. (1989) J. Biol. Chem. 264, 4805–4811.
21 Lin, G., McCormick, J.I., Dhe-Paganon, S., Silvius, J.R. and Johnstone, R.M. (1990) Biochemistry 29, 4575–4581.
22 McCormick, J.I., Silvius, J.R. and Johnstone, R.M. (1985) J. Biol. Chem. 260, 5706–5714.
23 Kasahara, M. and Hinkle, P.C. (1977) J. Biol. Chem. 252, 7384–7390.
24 Karl, P.I. and Fisher, S.E. (1990) Am. J. Physiol. 258, C443–C451.