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Simple and effective purification of a Na^+-dependent amino acid transport system from Ehrlich ascites cell plasma membrane

(reconstitution/immunoprecipitation of transport activity/essential sulfhydryl groups/"A" system amino acid transporter)

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ABSTRACT **A reconstitution assay was used to measure transport activity during purification of a Na^+-dependent amino acid transporter from Ehrlich cell plasma membrane. Cholate/urea-solubilized membranes were fractionated on a Sepharose 6B column and transport activity was recovered in the column void volume. Centrifugation of the void volume fraction at 105,000 × *g* and reextraction of the pellet with 1% octyl glucoside led to recovery of an extract whose specific transport activity was nearly 30-fold higher than that of the original solubilized extract with a recovery of 38% of the original activity. The properties of amino acid uptake in the purified reconstituted transporter were identical to those in native plasma membrane vesicles. The major component present in the purified fraction had a molecular mass of 120–130 kDa. Strong evidence that this 120- to 130-kDa peptide contains a component of the amino acid transporter was obtained by immunoprecipitation of transport activity from solubilized membranes with an antibody against the 120- to 130-kDa peptide. This study tentatively identifies a component of the Na^+-dependent amino acid transporter as a peptide with an apparent molecular mass of 120–130 kDa.**

For many years, the Ehrlich ascites cell has been a valuable model for the study of Na^+-dependent and Na^+-independent amino acid transport systems (1). The three major routes by which the Ehrlich cell takes up amino acids are the Na^+-dependent A and ASC systems (2, 3), and the Na^+-independent L system (2). Although the transport characteristics (2–4) and the cellular regulation of transporter activity (1) of the three systems have been studied extensively, the protein components involved in the transport process have not been identified.

In the present study, the A transport system has been purified from Ehrlich cell plasma membrane and activity monitored by a sensitive and reproducible reconstitution assay. With the aid of an antibody against the major component present in the purified fraction, a peptide with a molecular mass of 120–130 kDa has been tentatively identified as a major component of the Na^+-dependent amino acid transporter in the Ehrlich ascites cell.

MATERIALS AND METHODS

Membrane Solubilization and Purification of Na^+-Dependent Amino Acid Transport Activity. Ehrlich ascites cell plasma membranes were solubilized by suspension (3–4 mg of membrane protein per ml) in 2.5% cholic acid (wt/vol)/4 M urea/100 mM NaCl/0.1 mM EDTA/5 mM Tris·HCl, pH 7.4/10 μM phenylmethylsulfonyl fluoride and incubation at 4°C for 30 min (5). After centrifugation (120,000 × *g*, 60 min), the supernatant (≈10 ml) was applied to a Sepharose 6B column, (2.5 × 50 cm) equilibrated in 100 mM KCl/5 mM Tris·HCl, pH 7.4/0.25% cholic acid (wt/vol)/50 μM dithiothreitol/0.05% asolectin (wt/vol). Fractions (4 ml) were collected and pooled in lots of three (12 ml) and a 1-ml aliquot from each pool was assayed for Na^+-dependent α-aminoisobutyric acid (AIB) transport activity. The active fractions from two Sepharose 6B columns were combined and centrifuged at 105,000 × *g* for 30 min. The supernatant was discarded and the pellet (≈450 μg of protein per ml) was resuspended in 1% octyl glucoside (wt/vol), in 10 mM Tris·HCl, pH 7.4/0.1% asolectin (wt/vol)/100 mM KCl/50 μM dithiothreitol (buffer A) and incubated at 4°C for 15 min. The suspension was then centrifuged at 105,000 × *g* for 30 min and the octyl glucoside supernatant was applied to a Sephacryl S-200 column (1.5 × 46 cm) equilibrated in 1% cholic acid (wt/vol) in buffer A. Fractions (2.6 ml) were collected, pooled in lots of three, and assayed for Na^+-dependent amino acid transport activity.

Antibody Production. The void volume fraction from the Sephacryl S-200 column was pooled and lyophilized after extensive dialysis against H_2O. The protein was dissolved in gel running buffer (6), boiled for 5 min, and applied to a 5–15% gradient polyacrylamide slab gel (≈30 μg of protein per lane). After localization by Coomassie blue staining, and destaining overnight, the 120- to 130-kDa band was excised, washed twice, and suspended in 0.9% NaCl by Teflon/glass homogenization. Aliquots of the gel suspension (≈0.5 ml) were emulsified with 0.6 ml of complete Freund's adjuvant and injected intradermally in a New Zealand rabbit. Approximately 150 μg of the peptide was injected. Two additional injections of antigen (≈150 μg of protein) in complete and incomplete Freund's adjuvant, respectively, were given 10 and 36 days after the initial injection. Fourteen days after the final injection, the animal was bled from the ear, the serum was heat-inactivated, and the IgG fraction (fraction I) was purified on a protein A-Sepharose column. Two other IgG fractions (IgG fractions 2 and 3) were purified from serum removed from the same rabbit after further injections of antigen 5 months and 8 months after the first injection. Nonimmune IgG was purified from serum removed from the same animal before antigen injection or from other nonimmunized rabbits.

Western Blot Analysis. For western blotting, proteins were electrophoretically transferred to nitrocellulose paper after $NaDodSO_4$/gel electrophoresis of Ehrlich cell plasma membranes (7). Goat anti-rabbit IgG conjugated to horseradish peroxidase and HRP Color Development Reagent (Bio-Rad) were used to detect antibody binding.

Immunoprecipitation of Na^+-Dependent Amino Acid Transport Activity. Plasma membranes were solubilized and dialyzed overnight against 100 mM KCl/5 mM Tris·HCl, pH 7.4/0.1 mM $MgCl_2$/0.1 mM $CaCl_2$ (K^+ buffer) containing 1% cholic acid (wt/vol) and 1 μM phenylmethylsulfonyl fluoride.

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Abbreviations: AIB, α-aminoisobutyric acid; MalNEt, *N*-ethylmaleimide.

The solubilized membrane preparation (800 μl) was incubated with 1.5 mg or 3 mg of nonimmune or immune IgG for 60 min at 22°C. Antibody–antigen complexes were removed by addition of a suspension of *Staphylococcus aureus* in K^+ buffer/0.7% cholic acid or protein A-Sepharose followed by incubation for 30 min at 22°C and centrifugation at 3000 × *g* for 5 min. The supernatants were carefully removed and reconstituted to measure Na^+-dependent amino acid transport activity.

Reconstitution Assay. Samples from the different purification steps were removed, diluted to 2 ml with K^+ buffer, and reconstituted as described (5, 8) after addition of 10 mg of asolectin.

Measurement of Na^+-Dependent Amino Acid Uptake. Amino acid transport activity was measured as described (5, 8) except that the assay volume was reduced from 300 to 150 μl and the proteoliposome volume used was 25 μl. Activity in native plasma membrane vesicles was measured by the procedures described by Colombini and Johnstone (9). All transport activity measurements are reported as the Na^+-dependent component of uptake.

Protein Determination. Protein concentration was usually determined by a modified Lowry procedure (10, 11). When amino acid transport was expressed in terms of the protein incorporated into proteoliposomes, protein concentration was determined by the Kaplan–Pedersen assay (12). However, accurate measurement of small quantities of protein in the presence of phospholipid using this assay was difficult, so in some cases transport activity was expressed in terms of the protein content of the protein/phospholipid mixture before reconstitution. The specific activity of transport was underestimated in this procedure, because generally 30% of the protein in the mixture was reincorporated into the proteoliposomes. The particular procedure used to express transport activity in each experiment is identified in the figure and table legends.

RESULTS

Sepharose 6B Chromatography. Preliminary fractionation of solubilized membranes was achieved by Sepharose 6B column chromatography (Fig. 1). The Na^+-dependent transport activity eluted in the void volume fractions. The pooled fractions from the Sepharose 6B columns, containing the peak level of Na^+-dependent AIB transport activity, contained ≈10% of the protein applied to the column. From five experiments, the average degree of enrichment of transport activity was 8.2 ± 4.7-fold and the average percentage recovery of activity was 67% ± 32% (n = 5). Purification was also apparent upon $NaDodSO_4$ gel electrophoresis when the protein profiles of unfractionated solubilized membranes were compared to those in the active Sepharose 6B column fractions (Fig. 2).

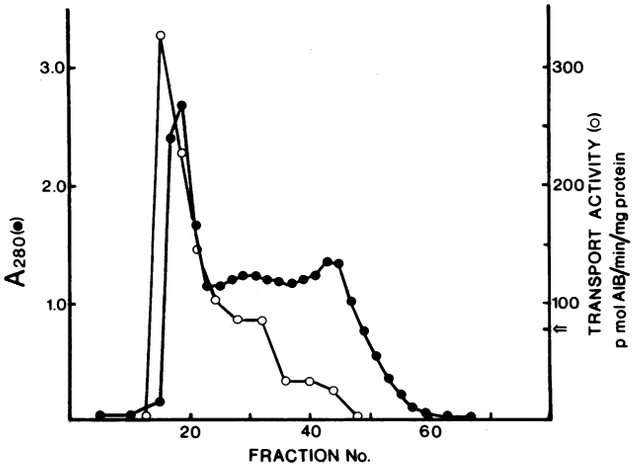

FIG. 1. Sepharose 6B chromatography of solubilized plasma membranes. Solubilized plasma membranes were loaded on a Sepharose 6B column. Fractions were assayed for Na^+-dependent AIB uptake (○) by reconstitution. The reconstituted transport activity of the original solubilized membranes is shown by the arrow. Transport activity is expressed in terms of the protein present in the proteoliposomes.

When the active fractions from the Sepharose 6B column were centrifuged at 105,000 × *g* for 30 min, 90% of the protein present was pelleted, indicating extensive protein aggregation during gel chromatography. Resuspension of the 105,000 × *g* pellet in 10 mM Tris·HCl buffer (pH 7.4) followed by reconstitution led to recovery of Na^+-dependent AIB transport activity, which was ≈25% higher than that of the uncentrifuged Sepharose 6B fraction. Therefore, the aggregated protein/phospholipid suspension, even in the absence of detergent, provided an effective protein source for reconstitution of amino acid transport.

Octyl Glucoside Extraction. Further purification and enrichment of activity was achieved after reextraction of the 105,000 × *g* pellet with 1% octyl glucoside containing 0.1% asolectin. Although only 19% of the protein in this pellet was extracted by octyl glucoside, the solubilized fraction had very high levels of amino acid transport. In three experiments, the average Na^+-dependent transport activity found in the 105,000 × *g* pellet was 368 ± 17 pmol of AIB per min per mg of protein. The corresponding activity in the octyl glucoside extract was 1620 ± 330 pmol of AIB per min per mg of protein, 4.4-fold higher. (Values are based on the protein content of the reconstitution mixture.) This dramatic increase in transport activity is shown graphically in Fig. 3 where the initial velocity of Na^+-dependent AIB uptake in proteoliposomes derived from the 105,000 × *g* pellet and the corresponding octyl glucoside extract are compared. In the absence of asolectin (0.1%) ≈50% less transport activity was extracted from the 105,000 × *g* pellet. Transport activity in the octyl glucoside extract was 27-fold higher than in solu-

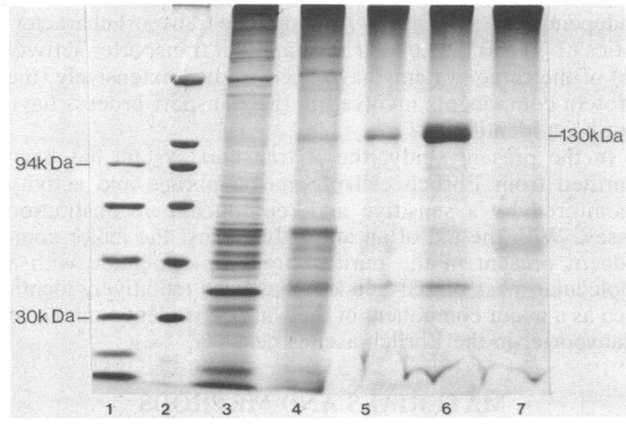

FIG. 2. $NaDodSO_4$ gel electrophoresis of samples generated during purification of the Na^+-dependent amino acid transport system. Lanes: 1 and 2, small and large molecular mass standards, respectively; 3, solubilized Ehrlich plasma membranes (load, ≈50 μg of protein); 4, active fractions from a Sepharose 6B column (Fig. 1) (load, ≈100 μg of protein); 5, protein extracted from the 105,000 × *g* pellet with octyl glucoside (load, ≈50 μg of protein); 6, protein from a Sephacryl S-200 column void volume fraction, which contained 41% of the transport activity loaded on the column (load, ≈50 μg of protein); 7, protein in a second peak of the Sephacryl S-200 column, which contained only 7% of the loaded transport activity (load, ≈50 μg of protein). Samples from the different fractions were dialyzed against H_2O and lyophilized before electrophoresis (6, 8).

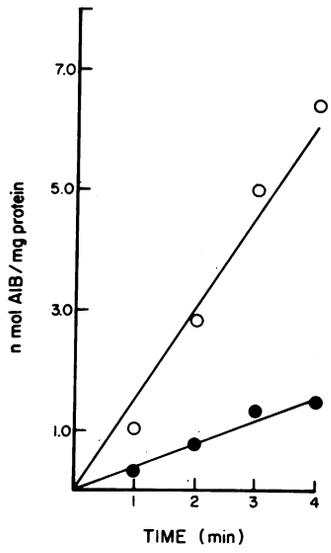

FIG. 3. Octyl glucoside extraction of Na^+-dependent AIB transport activity. Plasma membranes were solubilized in cholate/urea containing 10 μM phenylmethylsulfonyl fluoride and loaded on a Sepharose 6B column (Fig. 1). The fractions containing transport activity were pooled, divided into two equal portions, and centrifuged (105,000 × g; 30 min). One pellet was suspended in 10 mM Tris·HCl, pH 7.4/100 mM KCl, and a 200-μl aliquot (74 μg of protein) was reconstituted and assayed for Na^+-dependent AIB transport (●). The second pellet was extracted with 1% octyl glucoside/0.1% asolectin, and a 200-μl aliquot (14 μg of protein) was reconstituted and assayed for Na^+-dependent amino acid transport (○). Transport activity is based on the protein content of the reconstitution mixture.

bilized membranes with a 38% recovery of total transporter activity (Table 1).

The properties of [^{14}C]AIB uptake in proteoliposomes prepared from the octyl glucoside extract were identical to those in intact cells or plasma membrane vesicles. Table 2 shows that 5 mM Me-AIB, a specific A system substrate (1, 3) inhibited [^{14}C]AIB uptake by 96% in proteoliposomes derived from the octyl glucoside extract. In contrast leucine, a poor A system substrate (2), inhibited AIB uptake by only 10%. The effects of Me-AIB and leucine in the reconstituted extract were identical to those measured in plasma membrane vesicles (Table 2). Furthermore, in intact cells and native plasma membrane vesicles AIB uptake was strongly inhibited by pretreatment with *p*-chloromercuribenzenesulfonic acid as shown in Table 3. In contrast, treatment with an identical concentration of *N*-ethylmaleimide (MalNEt) had little effect on uptake. This pattern of inhibition was maintained after solubilization and purification of the amino acid transporter.

Comparisons of the proteins present at each purification step by $NaDodSO_4$ gel electrophoresis (Fig. 2) showed clearly that a peptide with a broad molecular mass range of

Table 2. Inhibition of AIB transport by Me-AIB and leucine in native plasma membrane vesicles and in proteoliposomes reconstituted from an octyl glucoside extract

Conditions	Na^+-dependent AIB uptake, pmol of AIB per min per mg of protein	
	Plasma membrane vesicles	Reconstituted octyl glucoside extract
Control	427 (100%)	2654 (100%)
Me-AIB	82 (19%)	105 (4%)
Leucine	371 (87%)	2389 (90%)

Plasma membrane vesicles were prepared as described (9). For uptake measurements, 25-μl aliquots of the membrane suspension in K^+ buffer (62 μg of protein) were added to 125 μl of K^+ or Na^+ buffer containing 0.15 mM [^{14}C]AIB with or without 5 mM Me-AIB or leucine. After incubation for 30 s at 22°C, a 130-μl aliquot was removed (9) and processed as described. Five hundred-microliter aliquots (30 μg of protein) of the purified octyl glucoside extract were reconstituted and the proteoliposomes were resuspended in K^+ buffer. [^{14}C]AIB uptake in 25-μl aliquots of the proteoliposome suspension (0.9 μg of protein) was measured as described above except that incubation was for 5 min at 22°C and [^{14}C]AIB concentration was 0.1 mM. Uptake was calculated on the basis of the protein content of the proteoliposomes.

120–130 kDa became enriched during purification, suggesting that this peptide had a role in Na^+-dependent AIB transport.

Sephacryl S-200 Column Chromatography. Evidence supporting a role for the 120- to 130-kDa peptide in amino acid transport was obtained by chromatography of the octyl glucoside extract (Table 1) on a Sephacryl S-200 column. Three major protein peaks were eluted from the column but only the void volume fraction had appreciable levels of amino acid transport. However, enrichment of transport activity after passage through this column was not observed consistently. Of four experiments comparing transport activity per mg of protein in the octyl glucoside extract and in the void volume fraction from the Sephacryl S-200 column, two showed an enhanced activity of 2-fold and 1.3-fold, while two showed decreases in activity of 26% and 50%. In all experiments, however, peak 1 (void volume) was the only fraction that contained high transport activity and no activity was found in other peaks from the column. The major peptide in the void volume fraction migrates with a mass of 120–130 kDa (Fig. 2), again implicating this protein in amino acid transport.

Immunoprecipitation of Na^+-Dependent AIB Transport Activity. To obtain direct evidence that the 120- to 130-kDa peptide is involved in Na^+-dependent AIB transport, an antibody was raised against the 120- to 130-kDa peptide and the effect of this antibody on Na^+-dependent AIB transport was studied. The results in Fig. 4 show that preabsorption of solubilized plasma membrane with immune IgG resulted in loss of reconstitutable transport activity, whereas nonimmune IgG did not alter the transport activity. These results

Table 1. Summary of purification procedure

Fraction	Reconstituted transport activity, pmol of AIB per min per mg of protein	Total protein, mg	Transport activity units, pmol of AIB per min
Solubilized membranes	136	60 (100%)	8160 (100%)
Active fractions from Sepharose 6B column	1436	5.3 (9%)	7611 (93%)
Octyl glucoside extract	3717	0.84 (1.4%)	3122 (38%)

Transport activity units were obtained by the following calculation: transport activity (pmol of AIB per min per mg of protein) × total protein in the fraction (mg). All calculations were based on the protein content of the proteoliposomes.

Table 3. Effect of *p*-chloromercuribenzenesulfonic acid and MalNEt on Na^+-dependent AIB uptake

Treatment	Na^+-dependent AIB uptake, pmol of AIB per min per mg of protein			
	Intact cells*	Native plasma membranes*	Solubilized membranes†	Octyl glucoside extract†
None	1003 (100%)	389 (100%)	127 (100%)	1100 (100%)
PCMBS	178 (18%)	33 (8%)	0	0
MalNEt	936 (93%)	285 (73%)	113 (89%)	1183 (108%)

*Ehrlich ascites cells (in Na^+ Ringer's) and plasma membrane vesicles (in K^+ buffer) were incubated for 20 min and 15 min, respectively, with 500 μM (cells) or 100 μM (membranes) *p*-chloromercuribenzenesulfonic acid (PCMBS) or MalNEt. The amino acid transport capacity of the cells was then determined (5). The treated plasma membrane vesicles were washed in K^+ buffer before measurement of Na^+-dependent AIB uptake (Table 2).

†Solubilized plasma membranes (1500 μg of protein) and the purified octyl glucoside extract (30 μg of protein) were incubated with 500 μM (solubilized membranes) and 200 μM (octyl glucoside extract) PCMBS or MalNEt, respectively, for 15 min at 22°C. The material was then reconstituted and Na^+-dependent [^{14}C]AIB uptake was measured (Table 2). Uptake is expressed in terms of the protein content of the proteoliposomes. However, for the octyl glucoside extract, the protein content of the liposomes was calculated by assuming that an average of 31.3% ± 7.9% (seven experiments) of the protein in the octyl glucoside extract was reincorporated into proteoliposomes during reconstitution.

support the conclusion that the 120- to 130-kDa peptide is a component of the Na^+-dependent transport system. IgG fraction 2 (Fig. 4*B*) was considerably less active than either IgG fraction 1 or 3 in removing reconstitutable activity. Examination of the immunoblots (Fig. 5) with the three IgG fractions show that IgG fractions 2 and 3 (lanes 5 and 6) generated two immunoreactive species against Ehrlich cell plasma membrane proteins. Therefore, the 120- to 130-kDa peptide used as antigen contained at least two components. However, IgG fraction 1 (Fig. 5, lane 4) showed only a single reactive species that migrated with the less intense larger molecular mass band in lane 5 and the more intense larger molecular mass band in lane 6. Since excised bands from gels were used as immunogens, the lack of consistent separation of the bands probably led to variation in the quantities of the two components used for immunization. If care was taken to include the larger component from the 120- to 130-kDa area of the gel for immunization, the resulting IgG fraction (Fig. 5, IgG fraction 3) showed a large increase in the efficacy of the reactivity of the antibody against the larger component and also removed appreciable activity from the dissolved membranes. Thus, these data suggest that the presence of antibody against the larger antigen is necessary for removal of the active transporter from solubilized membranes (Fig. 4). The lower quantity of this antibody in IgG fraction 2 could then be the reason for the inability of this fraction to efficiently remove transport activity.

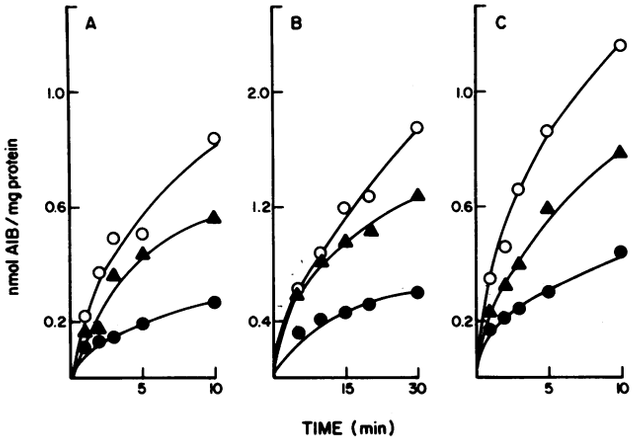

FIG. 4. Immunoprecipitation of Na^+-dependent amino acid transport activity. Plasma membranes were solubilized and dialyzed overnight against K^+ buffer/1% cholic acid/1 μM phenylmethylsulfonyl fluoride. Aliquots of the solubilized membrane suspension containing 1400, 1200, and 2200 μg of protein in *A*, *B*, and *C*, respectively, were incubated with 3 mg of IgG fraction 1 (*A*), IgG fraction 2 (*B*), and IgG fraction 3 (*C*). After incubation at 22°C (60 min in *A* and *B*; 45 min in *C*), a suspension of *Staphylococcus aureus* was added to *A* and *B* and protein A-Sepharose was added to *C*. The three incubates were centrifuged briefly, and the supernatants were removed and reconstituted (5, 8). Amino acid transport in the proteoliposomes was measured in K^+ (●) and Na^+ buffer (▲). In control incubations, solubilized membranes were treated in an identical manner with 3 mg of nonimmune IgG and the transport activity in the proteoliposomes formed was measured in K^+ (●) and Na^+ (○). The initial velocities of Na^+-dependent AIB transport in *A* were 125 and 56 pmol of AIB per min per mg of protein (55% decrease) for proteoliposomes derived from nonimmune and immune IgG-treated solubilized membranes, respectively. The equivalent values for *C* were 113 and 60 pmol of AIB per min per mg of protein (47% decrease). In *B* there was no apparent decrease in the initial velocity of Na^+-dependent AIB transport in the immune IgG-treated membranes. Lower transport activity was apparent only when uptake was measured for longer times (*B*). The uptakes with nonimmune IgG were indistinguishable from those without IgG treatment.

DISCUSSION

The reconstitution procedure (5, 8) used to measure Na^+-dependent AIB uptake is ideally suited for transporter puri-

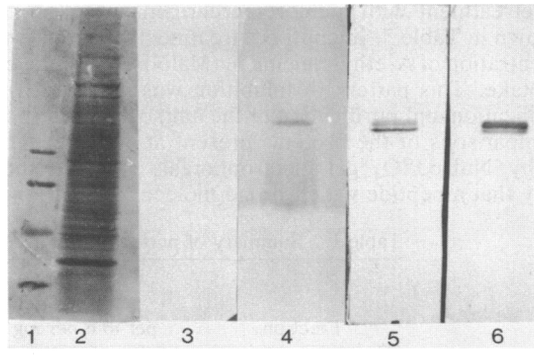

FIG. 5. Immunoblots of native Ehrlich cell plasma membranes with IgG fractions against the 120- to 130-kDa peptide. Ehrlich cell plasma membranes were resolved on $NaDodSO_4$ gels and transferred electrophoretically to nitrocellulose paper. One strip containing standards and plasma membrane proteins was cut off and stained with amido black (lanes 1 and 2). The remaining strips were incubated overnight at 4°C with 20 μg of nonimmune IgG (lane 3), IgG fraction 1 (lane 4), IgG fraction 2 (lane 5), and IgG fraction 3 (lane 6). Bound antibody was located by incubation with goat anti-rabbit IgG conjugated to horseradish peroxidase.

fication in that it is rapid, has a high degree of sensitivity, and is very reproducible. The most important step during purification was the aggregation induced by Sepharose 6B chromatography in 0.25% cholic acid and the subsequent selective extraction by octyl glucoside of the amino acid carrier from the aggregated protein. The physical form of the protein aggregate recovered from the Sepharose 6B fractions was not investigated but probably consists of a mixed micelle of protein, phospholipid, and detergent. This was the type of structure identified by Koepsell *et al.* (13) when the Na^+-dependent glucose transporter was enriched in a protein fraction precipitated by dialysis of solubilized renal brush-border membranes.

Amino acid transport activity was readily extracted from the aggregated 105,000 × *g* pellet by octyl glucoside. The extracted material had high levels of Na^+-dependent AIB transport and the activity was ≈27-fold higher than that of the original solubilized membranes (Table 1). The transport characteristics of the purified transporter were identical to those of the intact cell and plasma membrane vesicle with respect to response to inhibition by competing amino acids and sulfhydryl agents. These data not only provide evidence that the transport system is functionally normal during solubilization and purification but also indicate that the transporter contains an essential sulfhydryl group. As is the case with the A system transporter in transformed liver cells (14), the essential sulfhydryl group in the Ehrlich cell transporter is more susceptible to modification with *p*-chloromercuribenzenesulfonic acid than MalNEt.

$NaDodSO_4$ gel electrophoresis of the octyl glucoside extract showed that only a small number of peptides were present in this extract and that the most enriched and major component presents a broad molecular mass range of 120–130 kDa (Fig. 2, lane 5). Three lines of evidence suggest that this peptide contains a component that is a part of the Na^+-dependent amino acid transporter: (*i*) At each purification step, increases in transport activity were accompanied by an enrichment of the 120- to 130-kDa peptide detected on $NaDodSO_4$ gel electrophoresis. (*ii*) Passage of the octyl glucoside extract on a Sephacryl S-200 column showed that transport activity was present only in the fractions containing the 120- to 130-kDa peptide. (*iii*) A polyclonal antibody against the 120- to 130-kDa peptide immunoprecipitated amino acid transport activity from solubilized membranes. Evidence for the role of this peptide in amino acid transport was also obtained in a previous study from this laboratory (8) in which a proteoliposome population with high transport capacity and specific enrichment of a 125-kDa peptide was isolated.

Although the purification data implicate the 120- to 130-kDa peptide with a significant role in Na^+-dependent AIB uptake, the uptake could also be due to minor components present in the purified fractions. For this reason, the loss of transport activity with an antibody directed against the 120- to 130-kDa peptide is particularly significant, since it confirms a role for this peptide in amino acid transport.

Although the immune IgG caused as much as a 60% removal of the transport activity, the inability to immunoprecipitate more activity may be due to a relatively low affinity of antibody for the antigen. In addition, the presence of cholic acid in the membrane extract during immunoprecipitation may inhibit antibody binding. In a similar study of the Na^+–Ca^{2+} exchanger from synaptic plasma membranes Barzilai *et al.* (15) raised an antibody against a 70-kDa peptide after its removal from $NaDodSO_4$ gels. They also observed that large quantities of the antibody were required to immunoprecipitate Na^+ gradient-dependent Ca^{2+} transport activity from cholate solubilized membranes. Alternatively, the inability to immunoprecipitate all the amino acid transport activity may be due to the presence of more than one transporter in the octyl glucoside extract. The antibody used in the present study may recognize and immunoprecipitate only one class of amino acid transporter.

This study associates A system transport activity with a membrane protein of 120–130 kDa. Im and Spector (16) fractionated solubilized Ehrlich membranes on a Bio-Gel A-15m column and reported high levels of L-alanine transport after reconstitution of a column fraction in which the most prominent component had a molecular mass of 40 kDa. However, that uptake was neither Na^+ dependent nor valinomycin sensitive and hence is unrelated to the activity described here. Identification of a Na^+-dependent proline carrier from rabbit intestinal brush-border membranes has been reported by Wright and Peerce (17) on the basis of inhibition of this system by fluorescein isothiocyanate. By substrate protection techniques, a peptide with a molecular mass of 100 kDa was identified as the imino carrier (17). Substrate protection against MalNEt labeling was also used to identify a 20-kDa peptide in normal rat liver membranes proposed by Hayes and McGivan (18) to be an essential component of the alanine transport system. However, no data are available as to the transport capacity of either of the above proteins after isolation from solubilized membranes.

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