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Association of hepatic system A amino acid transporter with the membrane-cytoskeletal proteins ankyrin and fodrin

M.E. Handlogten, E.E. Dudenhausen, W. Yang, M.S. Kilberg *

Department of Biochemistry and Molecular Biology, The University of Florida, College of Medicine, Box 100245, JHMHC, Gainesville, FL 32610, USA

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Abstract

System A activity is a highly regulated mechanism for the active transport of zwitterionic amino acids into mammalian cells. Monoclonal antibodies generated against a previously unidentified rat liver plasma membrane-associated protein were shown to immunoprecipitate solubilized System A transport activity. The immunoreactive protein was later determined by immunoblotting and peptide microsequencing to be rat liver α-fodrin (non-erythroid spectrin). Antibody against ankyrin, a protein that often serves as a bridge between integral membrane proteins and fodrin, also immunoprecipitated System A transport activity. Fractionation of solubilized plasma membrane proteins on sucrose gradients revealed that the System A transporter co-migrated as a complex with fodrin and ankyrin, even in the presence of detergent and urea. In contrast, the System N amino acid transporter does not co-migrate with ankyrin and fodrin, nor does the anti-fodrin antibody immunoprecipitate System N activity. The present data are the first to demonstrate an association between an organic solute transporter and the membranocytoskeletal proteins ankyrin and fodrin.

Keywords: Polyethyleneglycol 8000; Hepatic system A; Arkyrin; Fodrin; (Madine Darby canine kidney cell)

1. Introduction

Transport of neutral amino acids across the mammalian plasma membrane is mediated by several independent activities with distinct but overlapping substrate specificity [1,2]. System A, a Na^+-dependent transporter that mediates the uptake of small neutral amino acids, has been detected in every nucleated cell or tissue type tested, and regulation is extensive [2–4]. A number of studies suggest that induction of System A is the result of increased synthesis of a plasma membrane glycoprotein, either the transporter itself or an associated protein [5,6]. In the hepatocyte, newly synthesized System A transport activity is delivered first to the basolateral membrane, and then transferred by transcytosis to the canalicular domain [7].

Previous observations suggest that there are important interactions between the cytoskeleton and amino acid transporters. We have recently shown by immunohistochemistry that the cationic amino acid transporter CAT1 (System y^+ activity) exists within the fibroblast plasma membrane in discrete islands or clusters that appear to occur randomly over the entire cell surface [8]. In contrast, antibody against the GLUT1 glucose transporter demonstrated that it is uniformly distributed over the entire cell surface. Treatment with nocodazole to disrupt the microtubules resulted in dispersion of the CAT1 transporter over the entire cell surface that generated a staining pattern similar to GLUT1. Interestingly, the CAT1-containing clusters reformed within 3 h after removal of the inhibitor and incubation of the cells in fresh medium. These changes in CAT1 clustering were paralleled by the appropriate changes in microtubule organization [8]. With regard to System A, in most cell types, disruption of the cytoskeleton only slightly affects basal transport activity, but blocks hormone or adaptive induction by 40–80% [9–11]. Furthermore, Karl et al. [12] reported that use of chaotropic agents to strip cytoskeletal proteins from isolated placental plasma membrane vesicles resulted in the loss of System A transport activity.

Studies investigating membrane–cytoskeleton interactions have demonstrated that ankyrin and fodrin (the latter sometimes referred to as 'non-erythroid spectrin') are im-

Abbreviations: PEG, polyethyleneglycol 8000; EDTA, ethylenediamine tetracetic acid; AIB, 2-aminoisobutyric acid; EGTA, (ethylenebis(oxyethylenenitrilo)tetracetic acid; MDCK, Madine Darby canine kidney cells.

* Corresponding author. Fax: +1 (352) 3926511.

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portant links between integral plasma membrane proteins and the cytoskeleton [13,14]. Ankyrin-fodrin complexes exhibit high-affinity binding sites for membrane proteins and may be involved in the maintenance of cell polarity for polarized epithelial cells [15–18]. Several ion transport proteins are complexed with ankyrin-fodrin including Na^+ channel [19], Na^+-Ca^{2+} exchanger [20], H^+K^+-ATPase [21], and Na^+K^+-ATPase [17,18,22,23]. It is proposed that selective localization of the Na^+K^+-ATPase to the basolateral membrane of polarized MDCK cells may be related to its interaction with the ankyrin-fodrin complex which is specifically associated with this membrane domain in confluent cells [17]. In support of this hypothesis, in retinal pigment epithelial cells where the localization of the Na^+K^+-ATPase is 'reversed' such that the activity resides on the apical surface, the presence of ankyrin and fodrin is also predominantly apical in their distribution [18]. In the present study, we show that monoclonal antibodies against either α-fodrin or ankyrin co-precipitate the System A transporter and that, during sucrose density fractionation, the transport activity migrates in a complex with ankyrin and fodrin.

2. Materials and methods

2.1. Solubilization of System A activity

Rat liver plasma membrane vesicles were isolated following the protocol described by Prpic et al. [24]. System A transport activity and associated cytoskeletal components were solubilized by incubating the membranes in a buffer containing 2.5% cholate and 4 M urea [25]. After centrifugation for 1 h at $100\,000 \times g$, the proteins in the supernatant were precipitated with a final concentration of 20% polyethylene glycol 8000 (PEG) by the addition of 2 volumes of 30% PEG and incubation on ice for 15 min. The mixture was centrifuged at $100\,000 \times g$ for 30 min, and the resulting pellet was washed three times in KMB buffer (200 mM KCl, 1 mM $MgCl_2$, and 10 mM Hepes, pH 7.5). The precipitated proteins were resuspended at a concentration of 1 mg protein/ml in STAB buffer (20% glycerol, 2 mM EDTA, 0.05% Triton X-100, 0.25% asolectin and 10 mM Tris, pH 7.4), as described previously [26]. After sonication for 10 s, the solution was centrifuged at $15\,000 \times g$ for 2 min to remove particulate matter and the proteins remaining in the supernatant were subjected to selective precipitation using 3% PEG. The final protein fraction is enriched for System A activity by 70-fold over that in plasma membrane vesicles (Table 1) and approx. 2000-fold over that measured in total cell homogenates [25,27]. This System A-enriched protein fraction was used as antigen to immunize mice for the generation of monoclonal antibodies. Protein content was determined by a modification of the Lowry procedure as previously described [25].

2.2. Preparation of monoclonal antibodies

Monoclonal antibodies were prepared by the Hybridoma Core Facility of the Interdisciplinary Center for Biotechnology at the University of Florida using a modified method of Kohler and Milstein [28]. Details of the preparation and screening of these hybridoma supernatants have been described [26].

2.3. Immunodepletion of System A transport activity

To test for precipitation of transporter activity by antibody, an immunodepletion assay was performed as described previously [26]. Briefly, a 1-ml aliquot of the appropriate secondary antibody, covalently linked to agarose beads (Sigma Chemical Co., St. Louis, MO), was incubated for 1 h with hybridoma supernatant or 5 μg of primary antibody IgG at 4°C and then centrifuged for 1 min at 50 g, after which the supernatant was discarded. After incubation of the agarose-bound primary antibody for 1 h at 4°C with an aliquot of the solubilized membrane protein fraction enriched for System A and its associated proteins, the beads were pelleted at 50 g for 1 min and discarded. The supernatant was saved, dithiothreitol was added to a final concentration of 2 mM, and then the proteins were reconstituted into proteoliposomes. Negative controls included incubations employing agarose beads lacking a bound antibody or a bound antibody that was shown previously to produce no immunodepletion of System A. System A transport activity was measured as the Na^+-dependent uptake of 200 μM [^{3}H]2-aminoisobutyric acid (AIB) as described by Fafournoux et al. [25].

Table 1
Enrichment of System A activity solubilized from rat liver plasma membrane

Protein fraction	AIB transport	Fold-enrichment
Plasma membrane vesicles	228 ± 4	1
SOLUB protein fraction	3185 ± 132	14
STAB protein fraction	16291 ± 1474	72

Rat liver plasma membrane vesicles were isolated from glucagon-treated animals. System A activity in vesicles or proteoliposomes was measured for one min at 37°C as the Na^+-dependent uptake of 200 μM [^{3}H]AIB. The membrane vesicle transport activity was solubilized with 2.5% cholate/4 M urea, and then precipitated with 30% PEG. A portion of the pellet (SOLUB) was reconstituted to measure enrichment (SOLUB), and the remainder was resuspended in STAB buffer, sonicated for 10 s, centrifuged to remove non-solubilized material, and subjected to a selective precipitation procedure using 3% PEG to obtain the maximum enrichment of System A activity [25]. The resulting pellet (STAB) was the material used to immunize mice for the production of antibody as indicated in Section 2. The transport velocities are expressed as pmol · mg^{-1} protein · min^{-1} and are the averages ± S.D. of assays in quadruplicate. Similar results were obtained in each of many independent experiments.

2.4. Fractionation of protein complexes on sucrose gradients

Rat liver plasma membrane proteins were solubilized for 30 min at 4°C in 1 ml of gradient buffer (10 mM Tris-HCl, pH 7.5, 145 mM KCl, 2 mM EGTA, 2 mM EDTA, 2 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 μg/ml each of aprotinin, leupeptin, pepstatin, *N*-tosyl-L-phenylalanine chloromethyl ketone, and N^{α}-*p*-tosyl-L-lysine chloromethyl ketone) and 2.5% cholate or 2.5% cholate plus 2 M urea (see figure legends). Following centrifugation for 5 min at 48 000 × *g*, approx. 2 mg of protein (in 1 ml) was layered onto a 4.2 ml linear 5–20% (w/w) sucrose gradient prepared in gradient buffer. The gradient was centrifuged at 300 000 × *g* for 5 h at 4°C in a Beckman SW50.1 rotor and 18 fractions containing approx. 0.25 ml each were collected. A portion of each fraction was used to measure the total protein content, determine the refractive index, and separate proteins for immunoblot analysis. Proteins in the remainder of each fraction were precipitated with a final concentration of 20% PEG, and then System A activity was reconstituted into proteoliposomes and transport activity assayed as described above.

2.5. Immunoblot analysis

Protein samples were dissolved in sample dilution buffer and subjected to one-dimensional polyacrylamide gel electrophoresis and immunoblot analysis as described previously [26]. Binding of primary antibody was detected by incubating the blots for 1 h in blocking buffer containing secondary antibody conjugated to horseradish peroxidase. Secondary antibody bound was assayed by use of an enhanced chemiluminescence kit according to the manufacturer's instructions (Amersham Corp., Arlington Heights, IL).

3. Results

3.1. Partial purification of hepatic System A transporter activity

System A activity present in rat liver plasma membrane proteins is not readily solubilized by detergents alone [25]. However, as first reported by McCormick et al. [29] for the Ehrlich cell, greater than 80% of the activity is released when 4 M urea is included during the solubilization procedure [25]. Enhanced solubilization in the presence of urea has been described for fodrin as well [15]. Following solubilization of System A activity in 2.5% cholate/4 M urea and then precipitation by the addition of 30% PEG, extraction of a subset of proteins was achieved by resuspension of the pellet in STAB buffer, sonication and centrifugation to remove non-soluble particulates, and then selective protein precipitation with 3% PEG. The latter procedure causes precipitation of only 10% of the total protein, but nearly all of the System A transport activity [25]. Subsequent reconstitution into proteoliposomes resulted in an enrichment of System A activity of more than 70-fold over the value in plasma membrane vesicles (Table 1). Analysis of this membrane protein fraction by 1D-PAGE revealed about 8–10 primary polypeptides and a number of minor proteins. After we discovered that α-fodrin is a component in this final fraction, we analyzed several of the intermediate steps along this purification scheme to establish that there was immunoblotting parallel enrichment between α-fodrin and the System A transport activity (data not shown). Immunization of mice for the production of monoclonal antibodies was performed using this partially purified transporter fraction as antigen, as described previously [26]. The resulting hybridoma supernatants were screened for the ability to immunodeplete the System A activity prior to reconstitution and, as described below, a number of them were capable of precipitating System A activity.

3.2. Fodrin is the antigen corresponding to the System A-precipitating monoclonal antibodies

The System A-enriched membrane protein fraction used as antigen for the preparation of the monoclonal antibodies was subjected to immunoblotting after separation of the proteins by two-dimensional polyacrylamide gel electrophoresis [30]. After subcloning positive hybridomas by serial dilution, a large number of monoclonal antibodies recognized a polypeptide of approx. 240 kDa (data not shown). To positively identify the immunoreactive protein, after separation by 2D-PAGE the polypeptide was subjected to cyanogen bromide cleavage and subsequent microsequencing by the Protein Core Facility of the Interdisciplinary Center for Biotechnology at the University of Florida. Three distinct peptide sequences were obtained and used to search the SWISS-PROT database for homologous sequences using the IntelliGenetics Inc. software PC-GENE (Mountain View, CA). The only significant similarities that were identified resulted from a comparison of the peptide sequences to previously reported human and chicken α-fodrin sequences. The 240 kDa polypeptide detected by our monoclonal antibodies is similar to the native size of α-fodrin [31,32]. As shown in Fig. 1, the first 25 residue peptide sequence from the rat was identical to residues 278-302 of both human [33,34] and chicken [35] α-fodrin. The second rat peptide, also 25 residues in length, differed from the human sequence (residues 380-404) by only one amino acid (Fig. 1) and was identical to the chicken sequence [35]. The third peptide, 31 residues in length, was analogous to residues 653-683 of either the chicken or human α-fodrin sequence and contained one conservative substitution (isoleucine to valine) and one unknown residue. These data represent an overall amino

acid identity of 98% between the rat, chicken, and human sequences, consistent with published reports demonstrating a high degree of conservation of fodrin across species [34,36,37]. The published portions of rat kidney [38] and rat brain [36] α-fodrin sequences begin at residues 1292 and 1776 of the chicken sequence [35] and therefore, do not contain our peptide sequences.

3.3. Immunoprecipitation of System A transport activity by anti-fodrin antibody

Following subcloning of positive hybridoma cell lines, a number of the α-fodrin monoclonal antibodies, including 2A12-1/1B1 and several independent subclones of hybridoma 4H10-3 (1G1 and 4C9 are shown as examples), all produced about 30–35% immunodepletion of System A transport activity (Table 2). Experiments using serial dilutions of hybridoma supernatants, previously concentrated 5- to 10-fold in a protein microdialysis apparatus (Bio-Molecular Dynamics, Beaverton, OR), demonstrated that immunoprecipitation of transport activity was dependent on antibody concentration and inhibition of up to 79% could be achieved (e.g., 2736 ± 191 and 564 ± 49 pmol · mg^{-1} protein · min^{-1} for control and anti-fodrin treatment, respectively). The α-fodrin antibodies that immunodepleted System A activity did not immunoprecipitate solubilized hepatic System N transport activity when tested under the same conditions (Table 2, see also Ref. [26]. System N is a Na^+-dependent amino acid carrier with a markedly different substrate specificity than System A, but it is also present in the membrane protein fraction used for the immunodepletion assays and antibody production. We have previously reported that two monoclonal antibodies (1E7-3/4E3 and 3E1-2/4H1) that recognize a polypeptide of approximately 100 kDa and that are effective in immunodepleting solubilized System N activity, do not immunoprecipitate System A activity [26].

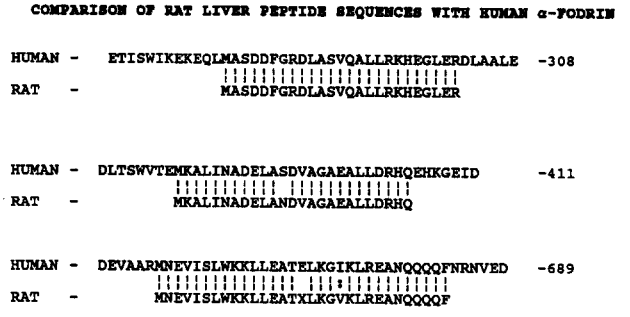

Fig. 1. Comparison of peptide sequences from the rat liver 2A12-1/1B1 antigen with human α-fodrin. The immunoreactive polypeptide was separated by 2D-PAGE, subjected to cyanogen bromide cleavage, and microsequenced as outlined in the text. The resulting peptide sequences were compared to the protein database SWISS-PROT for possible homologous sequences. The most significant sequence matches were against chicken brain and human lung α-fodrin. Of the 80 identified (1 unknown) residues contained within the three rat peptides, all but two were identical to the published human and chicken α-fodrin sequences, as discussed in the text.

Table 2
Selective immunodepletion of System A activity by α-fodrin monoclonal antibodies

Antibody/systems	Transport (pmol · mg^{-1} protein · min^{-1})	Percent inhibition
System A activity		
Control	2655 ± 330	–
2A12-1/1B1	1794 ± 252	32 *
4H10-3/1G1	1720 ± 276	35 *
4H10-3/4C9	1936 ± 170	27 *
System N activity		
Control	2529 ± 53	–
2A12-1/1B1	2482 ± 90	2

Solubilized membrane proteins enriched for System A activity were subjected to immunodepletion prior to reconstitution as described in the text. System A and System N activities in the resulting proteoliposomes were measured as the Na^+-dependent uptake of 200 μM [^{3}H]AIB or 50 μM [^{3}H]glutamine, respectively, at 37°C for one min and are reported as the averages ± S.D. for assay in quadruplicate. Immunodepletion was tested using antibody-containing culture medium from the hybridomas listed. Non-inhibitory monoclonal antibodies were used to obtain the negative 'control' velocities. A hybridoma medium was considered to be a negative control if it contained an antibody against a protein in the fraction, but did not produce inhibition of transport when compared to conditioned medium obtained from myeloma cell cultures. Two independent experiments are shown as representative from a large number of reproducible tests for immunodepletion of both Systems A and N. Statistical differences were determined by the Student's *t*-test and the * indicates a *P*-value of < 0.025.

3.4. Immunoprecipitation of System A transport activity by anti-ankyrin antibody

Fodrin binding to plasma membrane proteins is often bridged by an ankyrin molecule in between the two [13,16,22]. To determine if ankyrin was also associated with the hepatic System A transporter, anti-ankyrin antibody, kindly provided by Dr. W. James Nelson (Stanford University) or obtained commercially (Zymed Laboratories, San Francisco), were used to test for immunoprecipitation of enriched System A activity by the immunodepletion assay described above. As demonstrated for the fodrin monoclonal antibodies, the ankyrin antibody also caused a reproducible immunodepletion of System A activity. The reconstituted velocities were 2736 ± 191 pmol · mg^{-1} protein · min^{-1} in the presence of a non-immune IgG and 1100 ± 103 pmol · mg^{-1} protein · min^{-1} ($P < 0.005$) in the presence of anti-ankyrin IgG. We have not optimized this inhibition with regard to concentration of antibody required for maximal immunoprecipitation of transporter, but tests with three different antibody concentrations indicated that the inhibition was dependent on the concentration of the anti-ankyrin IgG. These results, suggesting that both ankyrin and fodrin are associated with the System A transporter, are supported by the co-migration of these three proteins on sucrose gradients as described below.

3.5. Co-migration of ankyrin, fodrin, and System A on sucrose gradients

Nelson and Hammerton [23] have solubilized plasma membrane protein complexes and subjected them to fractionation on linear sucrose gradients to document the specific interaction between fodrin and the Na^+K^+-ATPase. A similar approach also was used by that research group to identify fodrin/E-cadherin complexes [16]. We have taken advantage of this same procedure to determine if the hepatic System A amino acid transport activity co-migrates with α-fodrin and ankyrin. The data in Fig. 2 illustrate that the System A transport activity migrates at a higher density than the System N transporter protein when solubilized membrane proteins are fractionated on a 5–20% sucrose gradient. When each of the collected fractions were tested for the presence of α-fodrin and ankyrin by immunoblot analysis, those fractions exhibiting the highest System A transport activity, also contained the greatest amount of the two membranocytoskeletal proteins (Fig. 2). In contrast, the System N transporter migrated in fractions that contained a relatively small amount of ankyrin and no α-fodrin.

As outlined in Section 2, the membrane protein fraction from which System A transport activity and fodrin were co-enriched was originally solubilized with 2.5% cholate containing 4 M urea. To further demonstrate that the association of ankyrin, α-fodrin, and the System A transport activity was reasonably strong, membrane proteins were solubilized in 2.5% cholate/2 M urea and then subjected to sucrose gradient fractionation in the presence of 1 M urea. The addition of urea to the fractionation buffer caused a shift in the migration of transport activity on the sucrose gradient, the peak transport occurring in fractions 4-5 instead of fractions 7-8 (compare Figs. 2 and 3). However, when each of the gradient fractions were

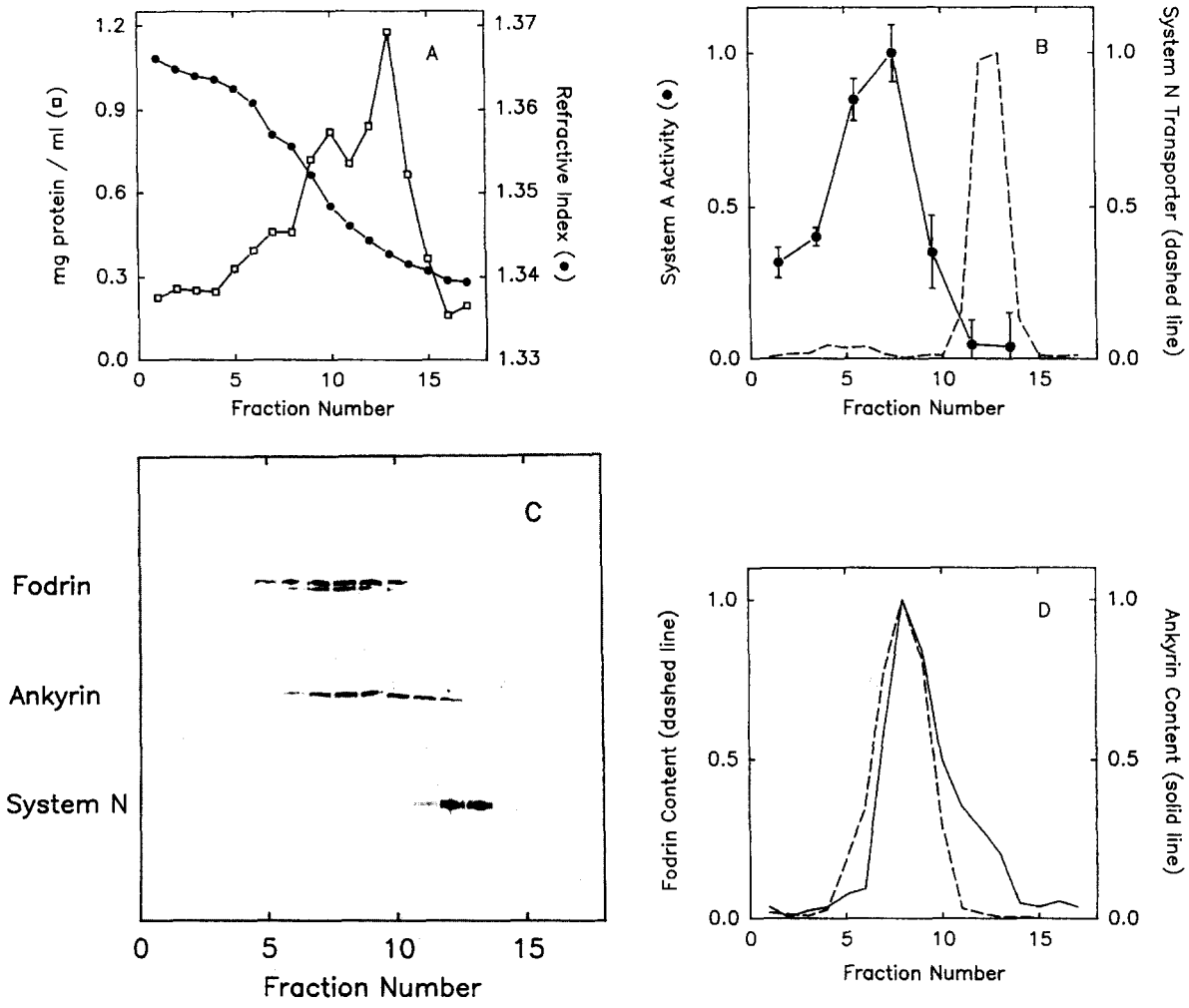

Fig. 2. Sucrose gradient fractionation of rat liver plasma membrane proteins solubilized with 2.5% cholate. A system A-enriched membrane protein fraction was prepared and then subjected to fractionation on a 5–20% sucrose gradient as described in Section 2. (A) Protein concentration (□) and refractive index (●) was determined for each fraction, the bottom of the gradient was assigned fraction 1. (B) Proteins from pooled fractions were reconstituted into proteoliposomes and the system A activity measured as the Na^+-dependent uptake of 200 μM [^{3}H]AIB (●). The system N transporter protein content was determined by immunoblot analysis and then quantitated by densitometry (dashed line). The protein content of α-fodrin (dashed line) and ankyrin (solid line) in each fraction was assayed by immunoblot analysis (C) and then quantitated by densitometry (D).

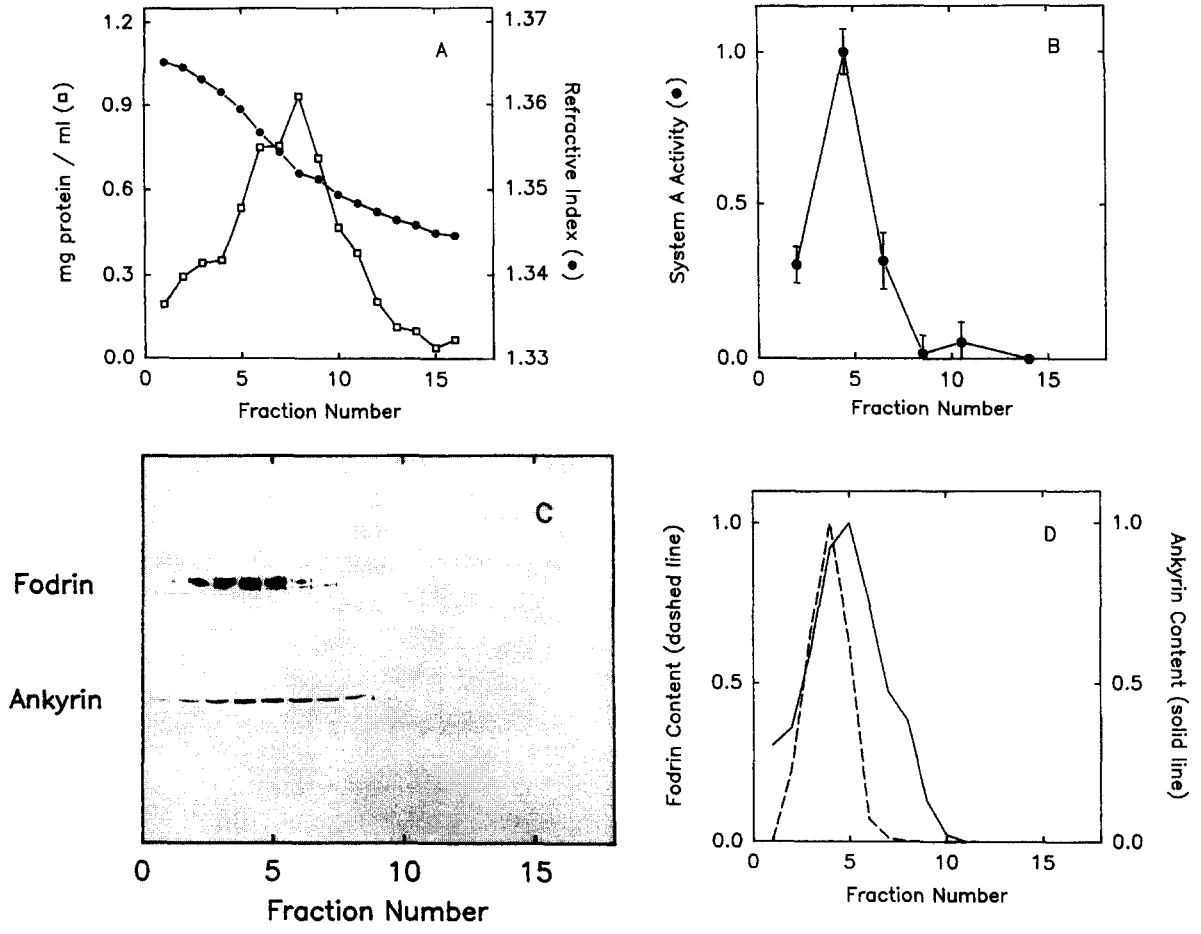

Fig. 3. Sucrose gradient fractionation of rat liver plasma membrane proteins in the presence of urea. Rat liver membrane proteins were solubilized in 2.5% cholate/2 M urea and then fractionated on a 5–20% sucrose gradient in the gradient buffer, but with the addition of 1 M urea. (A) Each fraction was analyzed for protein content (□) and refractive index (●). (B) The Na^+-dependent System A transport of 200 μM [^{3}H]AIB in pooled fractions was assayed following reconstitution into proteoliposomes and expressed as pmolmg^{-1} proteinmin^{-1} (●). Immunoblot analysis was performed to measure the content of α-fodrin (dashed line) and ankyrin (solid line) in each fraction (C) and then quantitated by densitometry (D).

tested by immunoblotting for the presence of ankyrin and α-fodrin, the location of these proteins also shifted such that there continued to be co-migration with System A activity. It is unlikely that migration of the three proteins would be shifted identically if they were migrating as individual proteins. Therefore, the data argue against random chance as an explanation of the co-migration of the three proteins in the absence of urea. These results document that the transporter-ankyrin-fodrin complex survives in the presence of 1 M urea.

4. Discussion

The data presented here demonstrate that monoclonal antibodies against rat liver α-fodrin cause co-precipitation of System A transport activity. Along with ankyrin [13], fodrin is thought to play a role similar to that of red blood cell spectrin in maintaining membrane-cytoskeletal interactions [32]. Current models propose that ankyrin binds directly to integral plasma membrane proteins and fodrin serves as a bridge between the bound ankyrin and the cytoskeleton [22]. Our results show that anti-ankyrin antibodies also immunoprecipitate System A transport activity.

Fodrin is associated with the plasma membrane as an 'insoluble complex' as defined by insolubility in an extraction buffer containing 150 mM salt and 0.5% Triton X-100 [15,39]. Interestingly, attempts to solubilize System A transport activity in the presence of detergents alone, such as Triton X-100, have also proven unsuccessful [25]. For example, 1% cholate alone released only 14% of the total transport activity with the remainder still associated with the insoluble membrane pellet; Triton X-100, CHAPS, octyl glucoside, and Lubrol ($C_{12}E_9$) gave similar results. However, including 4 M urea with any of the detergents increased the solubilization of the transport activity to 80% or more [25]. Effective extraction by urea is also characteristic of fodrin release from detergent extracted plasma membrane remnants [15]. Co-enrichment of fodrin throughout the partial purification of System A transport activity provides further support that either the complex survives solubilization in the presence of 4 M urea or, less

likely, that reassociation occurs after removal of the urea by PEG precipitation.

As shown previously for both the Na^+K^+-ATPase and E-cadherin [16,23], the association of the System A transport activity with ankyrin and α-fodrin was sufficiently stable to permit separation of the complex on sucrose gradients. That the solubilized complex survived gradient fractionation in the presence of 1 M urea underscores the strength of interaction between these proteins. Furthermore, the observation that the System N transporter migrates independently of fodrin or ankyrin, and is not immunoprecipitated by anti-fodrin antibodies, demonstrates the specificity of the interaction between the System A transporter and these cytoskeletal proteins. These data are the first to document an association of a Na^+-dependent organic solute transporter with these membrane-cytoskeletal complexes.

Fodrin/ankyrin binding to domain-specific integral membrane proteins may play a role in the maintenance of the polarized state of epithelial cells [15,17,18,23]. In MDCK cells, fodrin/ankyrin complexes are preferentially associated with the basolateral membrane domain [17]. In contrast to these epithelial cells, the polarization of the hepatocyte is associated with maintenance of a canalicular specific domain that is involved in bile formation [40]. Performing immunohistochemistry on frozen liver thin sections with the 2A12-1/1B1 antibody, we have confirmed previous reports that α-fodrin is present on each of the hepatocyte plasma membrane domains [41,42]. Basal System A transport activity in liver is relatively low on both the basolateral and canalicular domains, but the activity is increased up to 20-fold by a wide variety of stimuli [2,43]. Following glucagon treatment of rats, the initial increase in hepatic System A-mediated transport is detected on the basolateral surface of the hepatocyte, but with time the enhanced activity also appears on the canalicular membrane, transferred to the latter domain by transcytosis [7]. When the stimulation of transport has reached a maximum, the specific activity in the two membrane regions is nearly equal. Thus, detection of α-fodrin within both domains is consistent with this distribution of System A activity. Whether or not α-fodrin plays a role in the sorting process prior to transcytosis or in the maintenance of hepatocyte polarity represents an interesting topic for future research. Furthermore, the role of these cytoskeletal proteins in the extensive regulation of System A transport remains to be investigated.

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