Identification of Functionally Relevant Residues of the Rat Ileal Apical Sodium-dependent Bile Acid Cotransporter

Received for publication, January 3, 2006, and in revised form, March 23, 2006 Published, JBC Papers in Press, April 11, 2006, DOI 10.1074/jbc.M600342020

An-Qiang Sun†, Natarajan Balasubramaniyan‡, Haijun Chen§, Mohammad Shahid‡, and Frederick J. Suchy‡
From the †Department of Pediatrics, Mount Sinai School of Medicine, New York, New York 10029 and ‡Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06520

The mechanisms underlying the transport of bile acids by apical sodium-dependent bile acid transporter (Asbt) are not well defined. To further identify the functionally relevant residues, thirteen conserved negatively (Asp and Glu) and positively (Lys and Arg) charged residues plus Cys-270 of rat Asbt were selected, and their roles in transport function and membrane localization were examined by a combination of molecular biological, biochemical, and electrophysiological methods.

EXPERIMENTAL PROCEDURES

Materials—[3H]Taurocholic acid (2.1–3.47 Ci/mmol) was purchased from PerkinElmer Life Sciences. Unlabeled taurocholate was purchased from Sigma. Sulfo-N-hydroxysuccinimide-SS-biotin was from Pierce. Cell culture supplies were obtained from Invitrogen. Subcloning reagents, restriction enzymes, and competent cells were obtained from Stratagene (La Jolla, CA), Invitrogen, and New England Biolabs (Beverly, MA).

Generation of GFP-fused Rat Ileal Bile Acid Transporter (rAsbt-GFP) cDNA and Site-directed Mutagenesis—Full-length wild-type rat Asbt cDNAs were inserted in-frame into the HindIII and BamHI sites of a green fluorescent protein (GFP) vector, pEGFPN2 (Clontech, Palo Alto, CA), to produce the GFP-fused plasmid constructs as described previously (4). Potential functional determinant residues were mutated by site-specific mutagenesis using rat Asbt-GFP as template. The QuikChange™ site-directed mutagenesis kit (Stratagene) was used to convert codons for potential active determinant residues to alanine or glutamine residues according to the manufacturer’s directions with minor modification as described previously (5). After subcloning into expression vectors, fidelity of all the constructs was verified by DNA cycle sequencing using a PerkinElmer GeneAmp 9600, ABI Prism 377 DNA sequencer at the DNA Core Facility, Mount Sinai School of Medicine.

Cell Culture and Transient Transfection—COS-7 (SV40-transformed monkey kidney fibroblast) cells were maintained in complete Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum, 50 units/ml of penicillin, 50 μg/ml of streptomycin, and 2 mM l-glutamine. Cells were transiently transfected with plasmids containing wild-type or mutant rat Asbt-GFP cDNA using Lipofectamine™ reagent (Invitrogen) as described previously (3). Transfected cells were harvested 24–48 h later for bile acid transport and confocal microscopy analysis.
Confocal microscopy was carried out as described previously (7). Briefly, confocal microscopy was performed on a confluent monolayer of transfected cells cultured on glass coverslips. The cells were fixed and permeabilized for 7 min in 100% methanol at -20 °C, followed by rehydration in phosphate-buffered saline. After being washed with phosphate-buffered saline, the cells on coverslips were inverted onto a drop of VectaShield (Vector Laboratories, Inc., Burlingame, CA). Fluorescence was examined with a Leica TCS-SF (UV) 4-channel confocal laser scanning microscope in the Imaging Core Facility Microscopy Center, Mount Sinai School of Medicine.

**Bile Acid Influx Transport Assay and Inhibition Study**—The Na+-dependent taurocholate (TC) influx assay was done as described previously (4, 7). Briefly, taurocholate uptake was performed at 37 °C for 10 min. The confluent cell monolayers grown on 12-well plates were washed twice with warm uptake buffer (116 mM NaCl (or choline), 5.3 mM KCl, 1.1 mM KH2PO4, 0.8 mM MgSO4, 1.8 mM CaCl2, 11 mM D-glucose, 10 mM Hepes, at pH 7.4), and each well was incubated with uptake buffer containing 10 μM [3H]taurocholate at the final concentrations. The sodium concentration was kept constant at 116 mM. The data were fitted to the Michaelis-Menten equation by nonlinear regression with the enzyme kinetic software Enzfitter for MS-DOS.

Membrane Biotinylation Analysis—rAsbt-GFP and mutant cell surface expression was detected essentially as described by Ho et al. (8). Briefly, COS-7 cells were grown on 6-well plates and transfected with rAsbt-GFP and mutants using a similar protocol for transport experiments. Sixteen hours post-transfection, cells were washed with ice-cold phosphate-buffered saline Ca2+/Mg2+ (138 mM NaCl2, 2.7 mM KCl, 1.5 mM KH2PO4, 9.6 mM Na2HPO4, 1 mM MgCl2, 0.1 mM CaCl2, pH 7.3) and then treated with a membrane-impermeable biotinylating agent (sulfo-N-hydroxysuccinimide-SS-biotin, 1.5 mg/ml; Pierce) at 4 °C for 1 h. Subsequently the cells were washed three times with ice-cold phosphate-buffered saline Ca2+/Mg2+ containing 100 mM glycine and then incubated for 20 min at 4 °C with the same buffer to remove the remaining labeling agent. After washing, cells were disrupted with 700 μl of lysis buffer (10 mM Tris base, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, pH 7.4) containing protease inhibitors (Complete; Roche Applied Science) at 4 °C for 1 h with constant agitation. Following centrifugation, 140 μl of streptavidin-agarose beads (Pierce) at 4 °C for 1 h was added to 600 μl of cell lysate and incubated overnight in a cold room. Beads were washed four times with ice-cold lysis buffer, and the biotylated proteins were released by incubation of the beads with 2 mM Laemmli buffer for 30 min at room temperature. Similar to total cell lysates, samples of the biotinylated fractions (25 μl) were subjected to Western analysis for detection of immunodetectable rAsbt-GFP and mutants with polyclonal anti-GFP antibody (1:2000 dilution). Protein concentration was normalized to the amount of actin protein detected by an anti-actin antibody (1:5000).

Oocyte Expression and Electrophysiological Experiments—Oocyte culture and current-voltage analysis were performed as described previously (9). Briefly, oocytes were isolated from Xenopus laevis frogs, defolliculated by collagenase treatment. On the following day, they were microinjected with 46 nl of sterile water containing 5 ng of rAsbt-GFP or mutant cRNA per oocyte in standard experiments. Whole-oocyte currents were measured by two-electrode voltage clamp after 4 days of cRNA injection (Oocyte Clamp, Warner Instruments, Hamden, CT) with constant perfusion (1 ml/min, solution exchange 3 s). Data were sampled at 1 kHz and filtered at 0.25 kHz. All experiments were per-

---

**FIGURE 1.** Protein sequence alignments of ileal Asbt from different species. An alignment of five members of the Asbt family from rat (Q62633), mouse (AB002693), rabbit (Z54357), hamster (A49607), and human (U10417) shows the conserved amino acids. The asterisks (*) indicate the selected amino acid residues for mutagensis.
formed at room temperature and repeated with at least two branches of oocytes. Standard bath solution was ND-96 (in mM): 96 NaCl, 2 KCl, 1 MgCl₂, 0.3 CaCl₂, and 5 HEPES/NaOH, pH 7.5.

Statistics Analysis—The results were expressed as mean value ± S.E. and analyzed by using unpaired t test and one-way analysis of variance to test for a difference in means between two groups that may have unequal sizes. The p values were adjusted by Bonferroni correction to deal with multiple comparison. p ≤ 0.05 was considered statistically significant.

RESULTS

Effects of Site-directed Mutagenesis on Initial Rate of Taurocholate Transport by rAsbt-GFP—Previous studies on bile acid–binding proteins and sodium-coupled cotransporters (10, 11) indicated that charged amino acids of these proteins may be critical as binding sites for sodium ions and the cotransported substrate. The negatively charged residues Asp-115 (located in the large intracellular loop 3) and Glu-257 (located in the large extracellular loop 6) of rat Ntcp have been identified as being important for bile acid transport activity (10). Recently, Zahner et al. (10) suggested that Cys-266 of rat Ntcp, which is located in the large extracellular loop 6, is involved in taurocholate transport. The importance of the conserved charged residues of Asbt is not fully understood, especially the conserved positively charged residues.

In this study, we selected eleven positively charged residues, two negatively charged residues, Asp-122 (Asp-115 in liver Ntcp) and Glu-261 (Glu-257 in liver Ntcp), and Cys-270 (Cys-266 in liver Ntcp) of rat Asbt to examine their potential functional importance. An alignment (Fig. 1) of the amino acid sequences of five Asbt proteins from rat, mouse, hamster, rabbit, and human reveals that these fourteen selected residues are conserved in all five species. Because GEP is easily detected and has been shown not to interrupt transport activity, cellular distribution, and protein stability of bile acid transporters (4, 12, 13), we first constructed a rAsbt-GFP (see “Experimental Procedures”). The QuikChange site-directed mutagenesis kit was used to convert codons for the charged amino acids of interest and Cys (C) to non-charged alanine (A) or glutamine (Q). The wild type and mutants of rAsbt-GFP were then expressed in COS-7 cells, and the initial rate of taurocholate uptake was examined.

Fig. 2 shows that the initial rate of TC transport was significantly reduced in cells transfected with nine of the rAsbt-GFP thirteen mutants. Taurocholate transport activity decreased by 85–90% in the cells transfected with D122A, R256Q, E261A, and double mutated K312Q,K313Q (KK313QQ) mutants. K191Q- and K225Q-transfected cells showed an ~80% decrease in transport activity and K57Q, R64Q, and R254A showed ~35–55% decrease in transport activity compared with wild-type rat Asbt-GFP. Mutations of Lys-150, Lys-185, and Lys-189 of rAsbt-GFP caused no significant change in transport activity compared with wild-type rat Asbt-GFP. In contrast, replacement of Cys-270 with Ala enhanced the TC transport activity >20% compared with wild-type rAsbt-GFP-transfected cells.

Kinetic Analysis of rAsbt-GFP and Mutants—A decrease in transport activity after a single amino acid mutation may be caused by a change in substrate binding affinity and/or in membrane distribution of the transport protein (4, 5, 8). Therefore, we next evaluated the kinetic parameters (apparent $K_m$) of Na⁺-dependent taurocholate uptake of wild-type and mutant rAsbt-GFP in transfected COS-7 cells. Initial rates of [³⁵S]taurocholic acid accumulation in COS-7 cells were measured at varying concentrations of taurocholic acid as described under “Experimental Procedures.” The data were fitted by nonlinear regression to the Michaelis-Menten equation. As shown in Table 1 and Fig. 3, the wild-type rAsbt-GFP had a $K_m$ value for taurocholate transport of 26.3 ± 8.3 pmol/mg/min, which is comparable with the previously reported $K_m$ values of 12–37 μM in studies of rat ileal brush-border membrane vesicles and Chinese hamster ovary cells transfected with human or hamster Asbt (14–17). These studies showed that fusion with GFP did not significantly affect taurocholate binding affinity of rAsbt. As shown in Table 1, the $K_m$ values of D122A, K191Q, R256Q, E261A, and K312Q,K313Q (KK313QQ) mutants were increased 3–6-fold compared with wild-type Asbt-GFP. The $K_m$ values of R64Q and K225Q mutants were increased ~2.5-fold compared with wild-type rAsbt-GFP. The apparent $K_m$ values for K150Q, K185Q, and K189Q mutants were similar to that of wild-type rAsbt-GFP. Furthermore, corresponding experiments comparing the wild-type and C270A mutant of rAsbt-GFP revealed no significant change in $K_m$ value for taurocholate transport. However, the increased initial rate of taurocholate uptake by the C270A mutant appears to be due to an increased $V_{max}$ (C270A = 164.9 ± 4.8 pmol/...
mg/min versus WT = 129.0 ± 6.3 pmol/mg/min) for the transport process. These data suggest that the decrease of initial TC transport activity of the mutants is due to altered substrate binding affinity.

Cellular Distribution of Rat Asbt Mutants in Transfected Cells—To define the potential effects of point mutations on the cellular distribution of rAsbt, mutated rAsbt-GFPs were transiently transfected into COS-7 cells. The plasma membrane distributions of these mutants were visualized by confocal microscopy. The confocal images demonstrated that plasma membrane expression of these mutated proteins in transfected COS-7 cells was similar to wild-type rAsbt-GFP. Quantitative plasma membrane biotinylation analysis further confirmed these results. Fig. 5 shows no significant differences in cell surface protein expression between the mutants and wild-type rAsbt-GFP-transfected COS-7 cells. Therefore, we conclude that the altered transport activities of these mutants were not due to synthesis and trafficking defects but to mutation-induced changes in the affinity of Asbt for taurocholate.

Effects of Various Bile Acids on Taurocholate Uptake in COS-7 Cells Transfected with rAsbt-GFP Mutants—The charged side chain at the binding cavity of carrier proteins may be critical for ligand binding affinity and/or substrate specificity and may interact with polarized groups of ligands through H-bonding and/or electrostatic interactions. To understand whether these selected charged residues determine substrate binding specificity, cholate, taurodeoxycholate (TDC), taurochenodeoxycholate (TCDC), taurodehydrocholate (TDHC), and lithocholic acid (LCA) were used as inhibitors in these experiments. These bile acids, differing in the number and position of hydroxyl groups in the steroid nucleus and conjugation with taurine group, were used to map potential ligand-carrier protein-interacting sites in wild-type and mutant transporters (see “Discussion”). As shown in Fig. 6, taurocholate uptake by wild-type rAsbt-GFP was significantly inhibited by 10 min of exposure to cholate, TCDC, TDC, and lithocholic acid, but not by TDHC. These results agree with previous studies by Kramer et al. (18). As seen in Fig. 6, the inhibition by TCDC (absence of 12α-OH group) on the taurocholate transport was almost abolished by mutation of D122A, R256Q, E261A, and K312Q,K313Q (KK313QQ) and was also significantly decreased by K191Q, K225Q, and R254A mutants compared with wild-type rAsbt-GFP. Similar to the effects of TCDC, the inhibition of taurocholate transport by TDC (absence of 7α-OH group) was also significantly decreased in the K225Q, R256Q, E261A, and K312Q,K313Q mutants. In contrast, taurocholate transport by all of the mutants was inhibited to the same degree as wild-type rAsbt-GFP by cholate, lithocholic acid, and TDHC. These results suggest that residues Asp-122, Lys-191, Lys-225, Arg-254, Arg-256, Glu-261, and Lys-312,Lys-313 may be important for substrate binding specificity. Asp-122, Lys-191, and Arg-254 may interact with the 7α-hydroxyl group of bile acids (see “Discussion”).
Effects of Non-bile Acid Organic Anions on Taurocholate Uptake by COS-7 Cells Transfected with rAsbt-GFP Mutants—The ileal Na\(^+\)-dependent bile acid carrier exhibits wide substrate specificity and may be inhibited by many cholephilic organic substances of high structural diversity through competitive or non-competitive interactions (19–22). To further identify functionally relevant residues that may determine substrate specificity, we investigated the effects of five structurally different non-bile acid organic anions on taurocholate transport in COS-7 cells transfected with the cDNAs of rAsbt-GFP and mutated transporters. Fig. 7 shows that bromosulfolathaline (BSP), bumetanide (BUM), and diisothiocyanostilbene disulfonate (DIDS) significantly inhibited \(^{3}H\)taurocholate uptake by wild-type rAsbt-GFP-transfected cells, whereas estron-3-sulfate (E3S) and probenecid had no effect. These results are in agreement with the previous studies except for DIDS, which has been reported to inhibit rabbit Ntcp but not rabbit Asbt (18). Fig. 7 shows the BSP inhibition of taurocholate uptake is enhanced by >50% in D122A mutant transfected cells. In cells transfected with R64A, K191Q, K225Q, R256Q, E261A, and K312Q,K313Q mutants, the inhibition of taurocholate influx by BSP was also increased by 20–30%. The inhibition of \(^{3}H\)taurocholate transport by the other non-bile acid organic anions tested (BUM, DIDS, E3S, and probenecid) was not significantly changed in cells transfected with mutants compared with wild-type rAsbt-GFP-transfected cells.

Electrophysiological Analysis of Substrate-dependent Currents in the Xenopus Oocytes Expressing Wild-type and Mutant rAsbt-GFP—Previous studies indicated that the Na\(^+\)-dependent bile acid cotransport process is electrogenic with a substrate binding site composed of a closely positioned negatively charged group that interacts with a single sodium ion (10, 16). However, because of technical difficulties, so far there has been no direct evidence of electrical currents generated from bile acid and Na\(^+\) movement by Asbt. To further understand the mechanism of Na\(^+\)/bile acid cotransport, we examined the relationship between electrical and chemical driving forces of rAsbt in the Xenopus oocyte expression system, which is widely used to study structure and function of ion channels, receptors, and transporters. First, the surface expression of rAsbt-GFP and mutants in the Xenopus oocytes was examined by confocal microscopy. The results show that the wild-type and mutant rAsbt-GFP proteins were expressed on the cell membrane of oocytes and no GFP fluorescence was observed in non-injected oocytes (data not shown). The whole-oocyte currents were then obtained before and after application of 1 mM taurocholate with a family of test voltage pulses to the oocyte. The taurocholate-induced currents were obtained from the current difference in the absence and presence of taurocholate at each test voltage. In Fig. 8A, whole-oocyte family currents in non-injected oocytes showed no measurable voltage-dependent current changes before and after application of 1 mM taurocholate. In contrast, application of 1 mM taurocholate significantly increased whole-cell currents in oocytes expressing wild-type rAsbt-GFP (Fig. 8B). The taurocholate-induced currents were measured in the same way for the rAsbt-GFP mutants. Interestingly, a small fraction of whole-oocyte current was detected from oocytes expressing rAsbt-GFP even without application of taurocholate (Fig. 8B, left panel), suggesting that Na\(^+\) ions are able to move and bind to Asbt under the test voltages in the absence of taurocholate.

Fig. 8C shows the I–V curves of the taurocholate-induced currents in oocytes expressing rAsbt-GFP wild type or one of its mutants. These results demonstrate that a significant increase of whole-oocyte current only occurred when taurocholate was applied to oocytes injected with rAsbt-GFP or one of its mutant cRNAs. These inward negative currents were induced by taurocholate and were voltage dependent (Fig. 8C). As shown in Fig. 8, C and D, the taurocholate-induced currents from oocytes expressing D122A or E261A mutant were significantly decreased by >50% compared with those express-
ing wild-type rAsbt-GFP. In contrast, the taurocholate-induced currents in oocytes with C270A mutant were increased by >30% compared with those expressing wild-type rAsbt-GFP. These findings are consistent with our taurocholate transport results described above (see Fig. 2) and suggest that taurocholate uptake is closely accompanied by movement of Na" ions. These data further indicate that the residues of Asp-122 and Glu-261 are critical for both taurocholate and Na" movement and Cys-270 may be located in a sub-
strate binding-sensitive domain to optimize both taurocholate and Na\(^{+}\) uptake.

**DISCUSSION**

Early studies using mutagenesis by Hallén and colleagues (27) suggested that the maintenance of a negative charge by a D282E substitution in the mouse Asbt did not affect TC uptake activity. Zhang et al. (2) have recently shown that replacement of Glu-282 with alanine significantly decreased taurocholate transport activity of human ASBT. These data indicated that a negative charge at 282 of Asbt may be critical for interaction with the 12\(\alpha\)-OH group of the bile acids. Previous studies also predicted that positively charged residues of bile acid transporters...
were important for interaction with substrates (2, 24–26). So far, there is little experimental evidence reported for the functional relevance of these conserved positively charged residues of Asbt. Studies from topology scanning, putative three-dimensional structure, and computation prediction suggested that Arg-254 and Arg-256 of human ASBT may interact with the 12α-OH group (2). Our results showed that replacement of the charged Arg-64, Asp-122, Lys-191, Lys-225, Lys-256, Glu-261, and Lys-312, Lys-313 residues of rat Asbt with non-charged Ala (A) or Gln (Q) significantly decreased the initial taurocholate transport activity and binding affinity but had no effect on their plasma membrane expression. This indicates that these charged residues of rat Asbt are critical for ligand-carrier protein interaction but not for membrane trafficking.

The 7α- and 12 α-hydroxyl groups of natural bile acids are of importance for molecular recognition of a bile acid molecule by Asbt (24). If one of these two hydroxyl groups is removed to yield TCDC (12α-OH removed) or TDC (7α-OH removed), the affinity of the bile acid for Asbt is increased (24). The 3α-hydroxy group of bile acid is also required for optimal transport (24). If all of the three hydroxyl groups are removed, the resulting TDHC had no affinity for Asbt (24).

The data from the inhibition studies using bile acids reveal that mutations at Lys-225, Lys-256, Glu-261, and Lys-312, Lys-313 residues significantly reduced the inhibition of taurocholate uptake by TDC and TCDC but did not have any effect on the inhibition of taurocholate uptake by LCA, TDHC, and cholate. In contrast with the effects of bile acids, mutations at Arg-64, Asp-122, Lys-191, Lys-225, Lys-256, Glu-261, and Lys-312, Lys-313 residues significantly enhanced the inhibition of taurocholate uptake by BSP but did not affect the inhibitions by other non-bile acid organic anions (E3S, BUM, DIDS, and probenecid). These data indicate that these residues have different affinities for bile acids and non-bile acid organic anions and suggest that these residues are important for determination of substrate binding specificity.

Mutations at Asp-122, Lys-191, and Lys-254 residues of rAsbt reduced the inhibition of taurocholate uptake by TCDC, but not by TDC. The D122A mutation almost completely abolished the inhibition of taurocholate uptake, suggesting that this residue acts as a hydrogen bond acceptor. Therefore, Asp-122 may interact with the 7α-OH of taurocholate. Based on the work of Zhang et al. (2) and our data, it is suggested that the 12α-OH and 7α-OH groups of taurocholate may form H-bonds with Glu-282 and Asp-122 of Asbt, respectively. When the Asp-122 group of Asbt is replaced with Ala (D122A), the 12α-OH group of TDC still can compete with taurocholate to form an H-bond with the carboxyl group of Glu-282 and reduce the taurocholate uptake activity. The 7α-OH group of TCDC (which has no 12α-OH group) cannot form an H-bond with the non-charged Ala group of D122A mutant. Therefore, the inhibition of taurocholate uptake by TCDC was abolished by D122A mutation.

The electrogenericity of Na⁺-dependent bile acid transporters (human ASBT and rat Ntcp) and other transporters (Na⁺/glucose and Na⁺/amino acid transporters) has been reported from several laboratories and suggests that the stoichiometry of transport is two sodium ions and one ligand molecule (10, 16, 23). Zahner et al. (10) reported that the negatively charged Glu-257 and Asp-115 of rat Ntcp are important for the Na⁺ ion movement and extracellular Glu-257 (as a sodium ion sensor) and cytoplasmic Asp-115 may constitute an appropriate pair of binding sites for sodium ions allowing ion translocation across the cell membrane. Our results for the first time present experimental evidence to show that taurocholate movement by Asbt induces voltage-dependent current change. Consistent with the taurocholate movement, mutation at Asp-122 and Glu-261 of rat Asbt significantly decreased the voltage-dependent current. These findings suggest that taurocholate transport is closely accompanied by movement of a Na⁺ ion and indicate that the residues of Asp-122 and Glu-261 of rat Asbt are critical for both ligand and Na⁺ transport.

Hällén et al. (17) demonstrated that an alanine substitution of cysteine 270 (human ASBT)/266 (human NTCP) significantly decreases the inactivation by thiol reagents but kinetic parameters for sodium activation and taurocholate transport were largely unaffected by the cysteine to alanine substitutions, showing that this residue is nonessential in a functionally important region of the transporter. Our results are consistent with Hällén’s findings. The data show that replacement of Cys-270 of rat Asbt with Ala results in enhancement of taurocholate uptake and voltage-dependent currents but there is no change in membrane distribution or $K_m$ value compared with wild-type Asbt. Cys-270 may be located in a region near the substrate binding site so that replacement of the Cys with Ala results in a change of the steric environment of the binding site, leading to an increase in $V_{max}$ for the transport process.

Topology studies of Na⁺/bile acid cotransporter proteins from several laboratories suggested a 7- or 9-transmembrane (TM) configuration.
Functionally Relevant Residues of Asbt

In summary, our data suggest the following. 1) Asp-122, Lys-191, Lys-225, Lys-254, Lys-312, Lys-313 of Asbt located at extracellular loop 6 and TM domain VI may function as substrate recognition sensors and interact with ligands. In contrast with Asp-115 of Ntcp, which is located at a ligand-sensitive region but is not movement-sensitive region but is not essential for transport. Our results provide further insight into understanding the molecular mechanisms underlying the structure and function of Na÷-dependent transport proteins and may further suggest strategies for drug design to inhibit intestinal bile acid reabsorption.

Acknowledgments—Confocal laser scanning microscopy was performed at the Sinai School of Medicine confocal laser scanning microscopy core facility, supported with funding from NCI, National Institutes of Health Shared Resources Grant 5R24 CA095823-04, National Science Foundation Major Research Instrumentation Grant DBI-9724504, and National Institutes of Health Shared Instrumentation Grant 1 S10 RR0 9145-01.

REFERENCES

1. Hallén, S., Brande’n, M., Dawson, P. A., and Sachs, G. (1999) Biochemistry 38, 11379–11388
2. Zhang, E. Y., Phelps, M. A., Banerjee, A., Khantwal, C. M., Chang, C., Helsper, F., and Swaan, P. W. (2004) Biochemistry 43, 11380–11392
3. Oelkers, P., Kirby, L. C., Heubi, J. E., and Dawson, P. A. (1997) J. Clin. Investig. 99, 1880–1887
4. Sun, A. Q., Arrese, M. A., Zeng, L., Swaby, I., Zhou, M. M., and Suchy, F. J. (2001) J. Biol. Chem. 276, 6825–6833
5. Sun, A. Q., Säkärä, R., Sachchidanand, Xu, S., Zeng, L., Zhou, M. M., and Suchy, F. J. (2003) J. Biol. Chem. 278, 4000–4009
6. Chen, H. J., Kim, L. A., Rajan, S., Xu, S., and Goldstein, S. A. (2003) Neuron 40, 15–23
7. Sun, A. Q., Ananthanarayanan, M., Soroka, C. J., Thervanant, S., Shneider, B. L., and Suchy, F. J. (1998) Am. J. Physiol. 275, G1045–G1055
8. Ho, R. H., Leake, B. F., Roberts, R. L., Lee, W., and Kim, R. B. (2004) J. Biol. Chem. 279, 7213–7222
9. Chen, H. J., Sesti, F., and Goldstein, S. A. N. (2003) Biochemical J. 384, 3679–3689
10. Zahnert, D., Eckhardt, U., and Petzinger, E. (2003) Eur. J. Biochem. 270, 1117–1127
11. Quick, M., and Jung, H. (1997) Biochemistry 36, 4631–4636
12. Xia, X., Roundtree, M., Meriki, A., Xu, X., Shentu, S., and LeSage, G. (2004) J. Biol. Chem. 279, 44931–44937
13. Sun, A. Q., Balasubramaniam, N., Liu, C. J., Shahid, M., and Suchy, F. J. (2004) J. Biol. Chem. 279, 16295–16300
14. Arefawi, W. A., Sarwar, Z., Tyagi, S., Saksena, S., Dudeja, P. K., and Gill, R. K. (2005) Am. J. Physiol. 288, G578–G585
15. Barnard, J. A., and Ghishan, F. K. (1987) Gastroenterology 93, 925–933
16. Weinmann, S. A., Carruth, M. W., and Dawson, P. A. (1998) J. Biol. Chem. 273, 34691–34695
17. Hallén, S., Frykland, J., and Sachs, G. (2000) Biochemistry 39, 6743–6750
18. Kramer, W., Stengelin, S., Baringhaus, K. H., Enhsen, A., Heuer, H., Becker, W., Corsiero, D., Gribig, F., Noll, R., and Weyland, C. (1999) J. Lipid Res. 40, 1604–1617
19. Swaan, P. W., Stokza, F. C., Jr., and Ote, S. (1997) J. Computer-Aided Mol. Design 11, 581–588
20. Kramer, W., Wess, G., Neckermann, G., Schubert, G., Fink, J. Gribig, F., Gutfahr, U., Kowalewski, S., Baringhaus, K. H., and Roger, G., (1994) J. Biol. Chem. 269, 10621–10627
21. Wess, G., Kramer, W., Enhsen, A., Glombik, H., Baringhaus, K. H., Boger, G., Urmann, M., Bock, K., Kleine, H., Neckermann, G., Hoffmann, A., Pittius, C., Falk, E., Fehlhabe, H. W., Kogler, H., and Friedrich, M. (1994) J. Med. Chem. 37, 873–875
22. Petzinger, E. (1994) Rev. Physiol. Biochem. Pharmacol. 123, 47–211
23. Meinild, A-K., Loo, D. D. F., Hirayama, B. A., Gallardo, E., and Wright, E. M. (2001) Biochemistry 40, 11897–11904
24. Baringhaus, K. H., Matter, H., Stengelin, S., and Kramer, W. (1999) J. Lipid Res. 40, 2158–2168
25. Luche, C., Zhang, F., Hamilton, J. A., James, C. Saccchettini, J. C., and Ruterjans, H. (2000) Eur. J. Biochem. 267, 2929–2938
26. Nichesola, D., Perduca, M., Capaldi, S., Carrizzo, M. E., Righetti, P. G., and Monaco, H. L. (2004) Biochemistry 43, 14072–14079
27. Hallén, S., Bjorquist, A., Ostlund-Lindqvist, A. M., and Sachs, G. (2002) Biochemistry 41, 14916–14924