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Characterization, cDNA Cloning, and Functional Expression of Mouse Ileal Sodium-Dependent Bile Acid Transporter[1]

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Mouse ileal sodium dependent bile acid transporter (ISBT) was characterized using isolated enterocytes. Only enterocytes from the most distal portion showed Na^+-dependent [^{3}H]taurocholate uptake. Northern blot analysis using a probe against mouse ISBT revealed the expression of mouse ISBT mRNA to be restricted to the distal ileum. The K_m and V_{max} for Na^+-dependent [^{3}H]taurocholate transport into isolated ileocytes were calculated as 27 μM and 360 pmol/mg protein/min, respectively. Uptake of [^{3}H]taurocholate was inhibited by *N*-ethylmaleimide. We have cloned ISBT cDNA from mouse ileum. The cDNA included the entire open reading frame coding 348 amino acid protein with seven hydrophobic segments and two *N*-glycosylation sites. COS-7 cells transfected with the expression vector containing this cDNA expressed Na^+-dependent [^{3}H]taurocholate uptake activity with a K_m of 34 μM.

Key words: bile acid, ileum, Na^+-dependence, taurocholate, transporter.

After secretion into the small intestine, bile acids are reabsorbed and returned to the liver. The absorption of bile acids is mediated by both passive and active mechanisms. While the passive uptake mechanism is distributed throughout the entire intestine, active uptake is observed only at the distal ileum (*1-4*). The first step in active absorption is mediated by the ileal sodium-dependent bile acid transporter (ISBT), which is located at the brush border of the ileocytes (*5*). ISBT-mediated uptake is dependent on the Na^+-gradient across the brush border membrane (*1, 2, 6*). In the liver, bile acids are extracted from the portal vein by another Na^+-dependent bile acid transporter (Na^+/taurocholate cotransporting polypeptide, NTCP) (*7-10*).

The cDNA encoding ISBT was first isolated from hamster by expression cloning (*11*). Based on the nucleotide sequence of the cDNA, hamster ISBT was predicted to be a 37.9-kDa protein composed of 348 amino acids with seven predicted transmembrane domains and three putative *N*-glycosylation sites. COS cells transfected with hamster ISBT cDNA show Na^+-dependent [^{3}H]taurocholate uptake with a K_m value of 33 μM. Because this K_m is comparable to the values obtained in transport experiments using brush-border membrane vesicles (*4, 12*), the isolated cDNA was considered to encode the main component of the active absorption machinery located at the distal ileum. Subsequently, cDNAs encoding ISBT were isolated from rats (*13*), humans (*14*), and rabbits (S. Stengelin *et al.*, direct submission to GenBank under accession number of Z54357). The cDNA encoding NTCP was isolated from rats (*15*) and humans (*16*). Nucleotide sequence analyses revealed that ISBT and NTCP share similar hydropathy profiles, indicating that the membrane topologies of both proteins are similar (*11*).

The structure-function relationship of ISBT has only been reported for human ISBT by investigation of naturally occurring mutations (*14, 17*). Substitutions of lysine 243 by proline, threonine 262 by methionine, and proline 290 by serine abolished the bile acid transport activity of human ISBT while replacing alanine 171 by serine had no effect. These mutations had no effect on ISBT expression or sorting to the plasma membrane. Thiol group-specific chemical modifiers have been reported to inactivate the uptake of [^{3}H]taurocholate into the brush-border membrane vesicles from rabbit ileum (*4*). Cholate uptake by hepatocytes has also been reported to be sensitive to a thiol modifier (*10*). These findings suggest that (a) cysteine residue(s) is/are located at or close to a critical region in ISBT and NTCP. However, no specific cysteine residue required for bile acid transport has been identified. To further clarify the structure-function relationships of ISBT, we here report the cloning of ISBT cDNA from mouse, the experimental animal neglected in the course of studies on the ileal Na^+-dependent bile acid transporter.

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Abbreviations: BBMV, brush border membrane vesicles; ISBT, ileal sodium-dependent bile acid transporter; NEM, *N*-ethylmaleimide; NTCP, Na^+/taurocholate cotransporting polypeptide.

MATERIALS AND METHODS

Materials—[^{3}H]Taurocholate (128 and 74 GBq/mmol for experiments described in Figs. 1 and 3–8 and in Fig. 8, respectively) was purchased from DuPont-NEN. A cDNA library was constructed using the Lambda ZAP II vector provided as a cloning kit from Stratagene. The mammalian expression vector pZeoSV2(+) was from Invitrogen. Sequence analysis was done with GeneWorks (Oxford Molecular Group) and the freely distributed program ClustalX. The construction of expression vector was supported by the GeneConstructionKit 2 (Textco).

Isolation of Enterocytes—Epithelial cells were isolated from mouse small intestine by hyaluronidase treatment (*18*). Briefly, the entire small intestine was removed from an 8-week-old ICR male mouse, washed with phosphate-buffered saline, and cut into four segments of approximately equal length. These segments were opened lengthwise and incubated with 1 mg/ml hyaluronidase in 20 mM Tris·HCl buffer (pH 7.4, containing 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 200 mM mannitol, and 1 mg/ml bovine serum albumin; the buffer was oxygenated for 30 min in advance) at 37°C for 30 min with gentle shaking. Cell suspensions were filtered through cotton gauze, and cells were collected by centrifugation at 2,000 rpm for 5 min. Cells were washed twice with ice-cold 20 mM Tris·HCl buffer (pH 7.4, containing 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 200 mM mannitol), and suspended in 500 μl of the same buffer. Cells in 50 μl of suspension were lysed by mixing with an equal volume of 0.2 M NaOH and homogenization through a 26-gauge needle. Protein was measured by the method of Bradford with BSA as the standard (*19*).

Transport of [^{3}H]Taurocholate into Isolated Enterocytes—Fifty microliters of cell suspension was mixed with an equal volume of uptake buffer (20 mM Tris·HCl, pH 7.4, 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 200 mM NaCl or KCl, and 0.72 μM [^{3}H]taurocholate; the buffer was oxygenated for 30 min in advance) and incubated at 37°C. At the indicated time, 4 ml of ice-cold Krebs-Ringer Tris buffer (20 mM Tris·HCl, pH 7.4, 3 mM K_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 200 mM mannitol) was added to stop the reaction, and the cell suspension was immediately filtered through a glass filter (GC50 from Advantec, Tokyo) presoaked in 1 mM taurocholate. The filter was washed twice with the same buffer and the uptake of [^{3}H]taurocholate into cells was measured.

RT-PCR—Single-stranded cDNAs were synthesized from mouse ileal total RNA by reverse transcriptase of Moloney murine leukemia virus using a random hexamer as a primer. A 1,043-bp segment was amplified by *ExTaq* (Takara Biochemicals, Kyoto) using a 5′-primer (5′-CGGAATTCCATGGATAACTCCTCCGTCTGTTCC-3′) and a 3′-primer (5′-CGGAATTCCTCGAGGTTTGTCTCCTGGAATGATGGC-3′) designed on the basis of the nucleotide sequence of rat ISBT cDNA by 31 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 30 s, and extension at 72°C for 2 min. The underlined portions of the 5′- and 3′-primers correspond to positions 116–139 and 1114–1135 of rat ISBT cDNA, respectively, and these are flanked at their 5′-ends by additional segments containing *Eco*RI, *Nco*I, *Eco*RI, and *Xho*I restriction sites, respectively. The PCR product was cloned into M13mp18, and the nucleotide sequences of the three clones were determined. A clone with no nucleotide sequence difference between the consensus sequences of the three clones was used as a probe for Northern blot analysis and the cDNA cloning of mouse ISBT.

Cloning of Mouse ISBT cDNA—Double-stranded cDNA was synthesized from mouse ileal poly(A)$^+$ RNA with a cDNA synthesis kit (TimeSaver cDNA Synthesis Kit from Amersham-Pharmacia) using a random hexamer as a primer for synthesis of the first strand. An *Eco*RI/*Not*I adopter was ligated to the end of the double-stranded cDNA. cDNA was then ligated to Lambda ZAP II vector at the *Eco*RI site, and packaged *in vitro*. The primary titer of this cDNA library was 1.0×10^6 pfu/ml.

The mouse ileal cDNA library was screened with a probe amplified from mouse ileal total RNA by RT-PCR, with a yield of four positive clones. Because restriction endonuclease-site mapping was similar among these clones, we chose the longest for further experiments. The plasmid portion of the vector was isolated by self excision according to the manufacturer's protocol, and the nucleotide sequence of the cDNA insert was determined by making deletion mutants.

Construction of Expression Vector for Mouse ISBT—For an unknown reason, the *Eco*RI site at the junction of the 3′-end of the mouse ISBT cDNA insert and vector was disrupted. Thus the plasmid-cloned mouse ISBT cDNA was digested with *Eco*RI and *Eco*RV to isolate a 1,649-bp fragment. This fragment was ligated to pZeoSV2(+) at *Eco*RI and *Eco*RV sites to construct a mouse ISBT expression vector, pZmISBT.

Transient Expression of Mouse ISBT in COS-7 Cells—COS-7 cells at 80% confluency on a 35-mm dish were transfected with 0.8 μg of pZmISBT or pZeoSV2(+) by a cationic liposome-mediated procedure (Lipofectamine Plus from Lifetechnologies, according to the manufacturer's protocol). Two days after transfection, the medium was changed to fresh medium and the cells were incubated for 3 h. The medium was removed and the cells were washed twice with wash buffer (20 mM Tris·HCl, pH 7.5, 3 mM K_2HPO_4, 200 mM mannitol). Cells were covered with 0.45 ml of uptake buffer (20 mM Tris·HCl, pH 7.5, 3 mM K_2HPO_4, 100 mM NaCl or choline chloride, and 0.63 μM [^{3}H]taurocholate) and incubated at 37°C. Ten seconds before the indicated time, buffer was removed, and just at the indicated time 1 ml of ice-cold wash buffer containing 1 mM unlabeled taurocholate was added. The cells were then washed with wash buffer two more times and lysed in 1 ml of 0.2 M NaOH, and protein and [^{3}H]taurocholate uptake were measured.

RESULTS AND DISCUSSION

Characterization of the Mouse Na$^+$-Dependent Bile Acid Transporter Using Isolated Enterocytes—Although the mouse is an experimental animal for which the most advanced techniques of genetic engineering have been developed, detailed investigations on the Na$^+$-dependent bile acid transporter have not been done in this animal. We characterized the ileal Na$^+$-dependent bile acid transporter. The small intestine removed from an 8-week-old ICR mouse was divided into quarters and epithelial cells were isolated by hyaluronidase treatment. The time course of

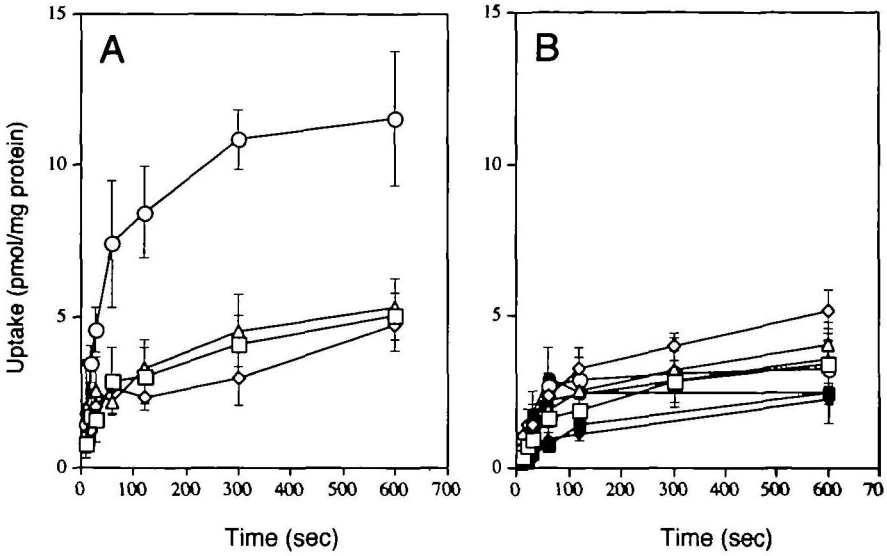

Fig. 1. **Time course of [^{3}H]taurocholate uptake into isolated enterocytes.** The small intestine from an 8-week-old ICR mouse was cut into four segments and the epithelial cells were isolated. The cells were incubated with 0.72 μM [^{3}H]taurocholate for 10, 20, 30, 60, 120, 300, or 600 s and [^{3}H]taurocholate uptake was measured. A, in the presence of 100 mM Na^+; B, in the presence of 100 mM K^+ (open symbols) or choline (closed symbols) instead of Na^+. □, ◇, △, and ○ correspond to proximal, mid-upper, mid-lower, and distal segments from the pylorus to the ileocecal valve, respectively. Points and bars represent the averages and standard errors of three independent experiments.

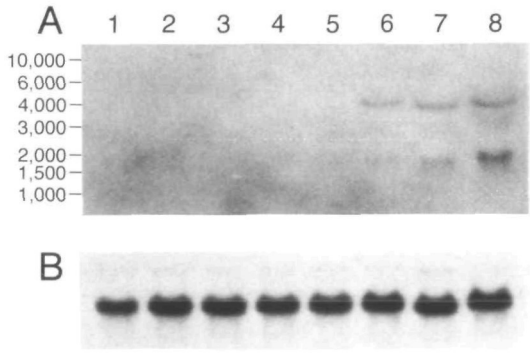

Fig. 2. **Northern blot analysis of the ileum-specific expression of ISBT mRNA.** The small intestine from an 8-week-old ICR mouse was cut into eight segments of equal length and numbered 1-8, proximal to distal. 20 μg of total RNA from each segment was hybridized with (A) a probe amplified by RT-PCR from mouse ileal total RNA or (B) a probe for β-actin.

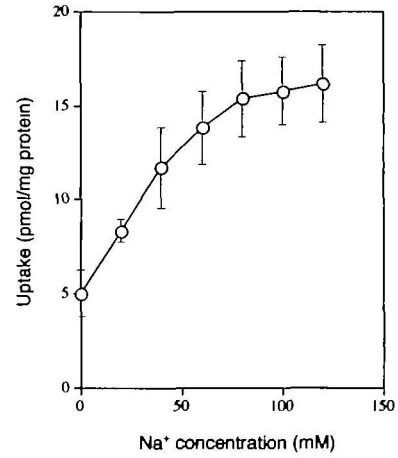

Fig. 3. **Na^+-dependence of [^{3}H]taurocholate uptake by isolated ileocytes.** Uptake of [^{3}H]taurocholate after a 5-min incubation into the epithelial cells prepared from the terminal ileal segment was measured at various Na^+ concentrations. The osmotic pressure was adjusted by replacing Na^+ with K^+. Points and bars represent the averages and standard errors of three independent experiments.

[^{3}H]taurocholate uptake into isolated enterocytes was measured (Fig. 1). Uptake by the epithelial cells isolated from the most distal segment was markedly higher than uptake by epithelial cells from other segments. When Na^+ was replaced by K^+ or choline, uptake by the ileocytes was reduced to the level of epithelial cells from other segments. This indicates that the expression of the Na^+-dependent bile acid transporter is restricted to the ileum, consistent with the results of Northern blot analysis showing that mouse ISBT mRNA is expressed only in the distal ileum (Fig. 2). In Fig. 2, two bands can be observed in lanes 6-8. For human ISBT, it has been reported that the length of the 3′-untranslated region of mRNA is 2,137 nucleotides, and the longest mRNA is 3,779 nucleotides plus a poly(A) tail (*20*). If this is the case for mouse ISBT, the longer band can be considered to be a major transcript of the mouse ISBT gene, and the shorter band might be degraded molecules.

To examine the Na^+-dependence of [^{3}H]taurocholate uptake into ileocytes in detail, uptake was measured at various Na^+ concentrations (Fig. 3). Uptake increased with the increase in the concentration of Na^+, and the maximum uptake was observed at a Na^+ concentration of 100-120 mM.

To characterize the kinetics of [^{3}H]taurocholate uptake by mouse ileocytes, uptake was measured in the presence of various concentrations of taurocholate (Fig. 4). Na^+-dependent uptake was a saturable process with an apparent Michaelis constant (K_m) and maximum uptake (V_{max}) of 27 μM and 360 pmol/mg protein/min, respectively.

Primary or secondary bile acids and glycine or taurine conjugates were examined for their inhibitory effects on [^{3}H]taurocholate uptake into mouse ileocytes (Fig. 5). These free and conjugated forms of bile acids were inhibitory in the order of taurocholate > chenodeoxycholate/deoxycholate > glycocholate/cholate > lithocholate.

We tested the effect of *N*-ethylmaleimide (NEM) on [^{3}H]taurocholate transport by mouse ileocytes (Fig. 6). While Na^+-independent uptake was not affected by NEM,

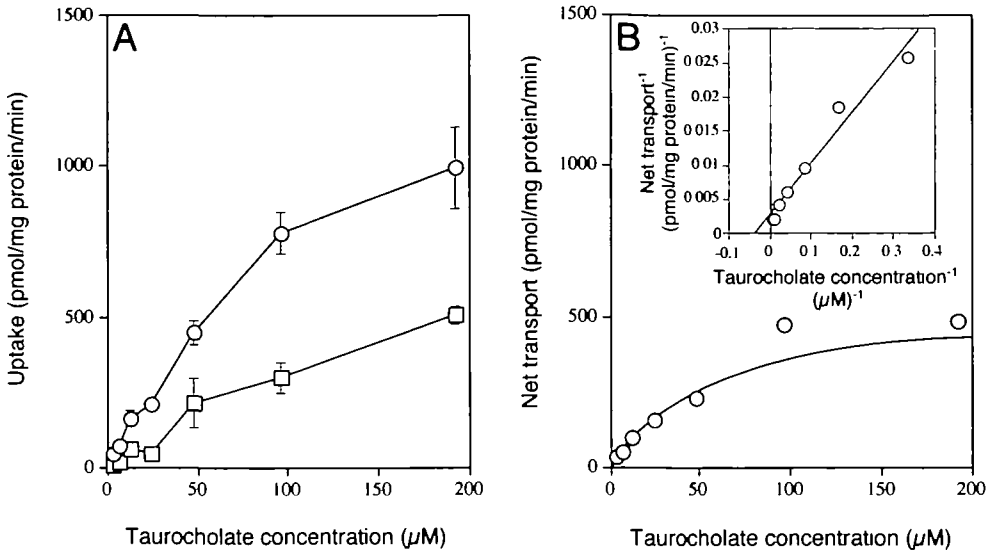

Fig. 4. **Kinetic analysis of Na^+-dependent [^{3}H]taurocholate uptake by isolated ileocytes.** A: Uptake after 20 s of incubation was measured at various concentrations of taurocholate in the presence of 100 mM Na^+ (○) or 100 mM K^+ (□). Because the uptake of [^{3}H]taurocholate increased linearly with time within 20 s, we used the rate of uptake at 20 s as the initial rate. Points and bars represent the averages and standard errors of three independent experiments. B: Net Na^+-dependent transport was calculated by subtracting the uptake in the absence of Na^+ from that in the presence of Na^+. The apparent Michaelis constant (K_m) and the maximum uptake (V_{max}) were calculated by linear regression of the double reciprocal plot (inset).

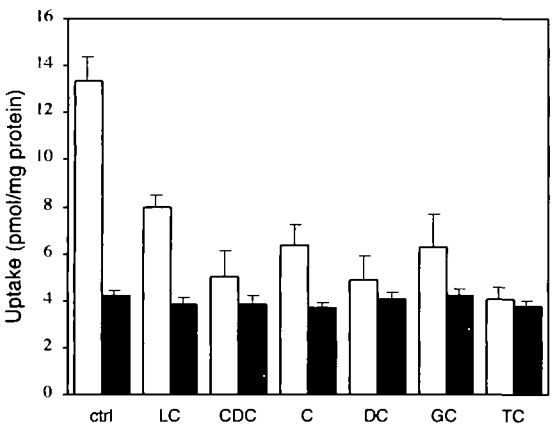

Fig. 5. **Effect of various bile acids on Na^+-dependent [^{3}H]taurocholate uptake into isolated ileocytes.** The uptake of [^{3}H]taurocholate was measured after a 5-min incubation in the presence of a 100-fold excess of unlabeled bile acids. The open and stippled columns show the uptake in the presence and absence of 100 mM Na^+, respectively. Ctrl, control; LC, lithocholic acid; CDC, chenodeoxycholic acid; C, cholic acid; DC, deoxycholic acid; GC, glycocholic acid; TC, taurocholic acid. Data are the averages and standard errors of three independent experiments.

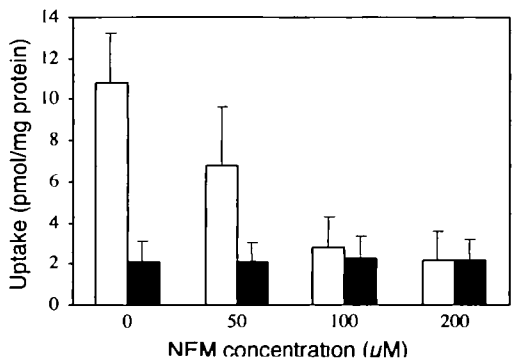

Fig. 6. **Effect of *N*-ethylmaleimide on Na^+-dependent [^{3}H]-taurocholate uptake by isolated ileocytes.** Isolated ileocytes were treated with the indicated concentrations of *N*-ethylmaleimide for 5 min. Then the cells were incubated for 5 min with [^{3}H]taurocholate in the presence of 100 mM Na^+ (open columns) or 100 mM K^+ (stippled columns). Data are the averages and standard errors of four independent experiments.

Na^+-dependent uptake was inhibited by NEM in a concentration-dependent manner.

cDNA Cloning and Functional Expression of Mouse ISBT—A cDNA segment was amplified from mouse ileal total RNA by RT-PCR with primers designed on the basis of the nucleotide sequence of rat ISBT cDNA. Using this segment as a probe, the mouse ileum cDNA library was screened and a positive clone was isolated. The isolated 1,629-bp cDNA included the entire open reading frame encoding a 348-amino acid protein with a calculated molecular weight of 38,089, and 49- and 536-bp 5′- and 3′-untranslated regions, respectively. No polyadenylation signal was found in the 3′-untranslated region. Comparison of the amino acid sequence of mouse ISBT with those of rat, hamster, rabbit, and human ISBT revealed 94, 86, 82, and 76% identity (Fig. 7A). Two potential *N*-glycosylation sites were found at positions 3 and 10. A Kyte-Doolittle hydrophobicity plot with a window of 11 amino acids showed seven hydrophobic peaks (Fig. 7B). For NTCP, a membrane topology with seven transmembrane segments, an extracellular N-terminus, and an intracellular C-terminus has been proposed based on the results of immunostaining and glycosylation analysis (*15, 16*). The hydrophobicity plot of mouse ISBT is consistent with this membrane topology since ISBT and NTCP share similar hydrophobicity profiles. However, more complex analyses that take the optimal length for transmembrane segments into consideration suggest that ISBT may have eight transmembrane segments (data not shown), because the second hydrophobic peak is too long for a single transmembrane segment. Therefore, further study of the actual structure of ISBT in cellular membranes is needed.

COS-7 cells transfected with the expression vector for

A

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mouse    MDNSSV-CPPNATVCEGDSCVVPESNFNAILNTVMSTVLTILLAMVMFSMGCNVEVHKFL   59
rat      MDNSSV-CSPNATFCEGDSCLVTESNFNAILSTVMSTVLTILLAMVMFSMGCNVEINKFL   59
hamster  MDNSSI-CNPNATICEGDSCIAPESNFNAILSVVMSTVLTILLALVMFSMGCNVELHKFL   59
rabbit   MSNLTVGCLANATVCEGASCVAPESNFNAILSVVLSTVLTILLALVMFSMGCNVEIKKFL   60
human    MNDPNS-CVDNATVCSGASCVVPESNFNNILSVVLSTVLTILLALVMFSMGCNVEIKKFL   59
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mouse    GHIKRPWGIFVGFLCQFGIMPLTGFILSVASGILPVQAVVVLIMGCCPGGTGSNILAYWI   119
rat      GHIKRPWGIFVGFLCQFGIMPLTGFILSVASGILPVQAVVVLIMGCCPGGTGSNILAYWI   119
hamster  GHLRRPWGIVVGFLCQFGIMPLTGFVLSVAFGILPVQAVVVLIQGCCPGGTASNILAYWV   119
rabbit   GHIRRPWGIFIGFLCQFGIMPLTGFVLAVAFGIMPIQAVVVLIMGCCPGGTASNILAYWV   120
human    GHIKRPWGICVGFLCQFGIMPLTGFILSVAFDILPLQAVVVLIIGCCPGGTASNILAYWV   119
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mouse    DGDMDLSVSMTTCSTLLALGMMPLCLFVYTKMWVDSGTIVIPYDSIGISLVALVIPVSFG   179
rat      DGDMDLSVSMTTCSTLLALGMMPLCLFIYTKMWVDSGTIVIPYDSIGISLVALVIPVSIG   179
hamster  DGDMDLSVSMTTCSTLLALGMMPLCLFIYTKMWVDSGTIVIPYDSIGTSLVALVIPVSIG   179
rabbit   DGDMDLSVSMTTCSTLLALGMMPLCLYVYTKMWVDSGTIVIPYDNIGTSLVALVVPVSIG   180
human    DGDMDLSVSMTTCSTLLALGMMPLCLLIYTKMWVDSGSIVIPYDNIGTSLVALVVPVSIG   179
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mouse    MFVNHKWPQKAKIILKIGSITGVILIVLIAVIGGILYQSAWIIEPKLWIIGTIFPIAGYS   239
rat      MFVNHKWPQKAKIILKIGSIAGAILIVLIAVVGGILYQSAWIIEPKLWIIGTIFPIAGYS   239
hamster  MYVNHKWPQKAKIILKIGSIAGAILIVLIAVVGGILYQSAWTIEPKLWIIGTIYPIAGYG   239
rabbit   MFVNHKWPQKAKIILKVGSIAGAVLIVLIAVVGGILYQSAWIIEPKLWIIGTIFPMAGYS   240
human    MFVNHKWPQKAKIILKIGSIAGAILIVLIAVVGGILYQSAWIIAPKLWIIGTIFPVAGYS   239
         *:***************:***:*.:********:********* * *********:*:***.

mouse    LGFFLARLAGQPWYRCRTVALETGMQNTQLCSTIVQLSFSPEDLNLVFTFPLIYTVFQLV   299
rat      LGFFLARLAGQPWYRCRTVALETGMQNTQLCSTIVQLSFSPEDLNLVFTFPLIYTVFQLV   299
hamster  LGFFLARIAGQPWYRCRTVALETGLQNTQLCSTIVQLSFSPEDLNLVFTFPLIYSIFQIA   299
rabbit   LGFFLARIAGQPWYRCRTVALETGMQNTQLCSTIVQLSFSPEDLTYVFTFPLIYSIFQIA   300
human    LGFLLARIAGLPWYRCRTVAFETGMQNTQLCSTIVQLSFTPEELNVVFTFPLIYSIFQLA   299
         ***:***:** ********:***:***************:**:*. ********::**:.

mouse    FAAVILGIYVTYRKCYGKNDAEFLEKTDNEMDSRPSFDETNKGFQPDEK              348
rat      FAAIILGMYVTYKKCHGKNDAEFLEKTDNDMDPMPSFQETNKGFQPDEK              348
hamster  FAAILLGAYVAYKKCHGKNNTELQEKTDNEMEPRSSFQETNKGFQPDEK              348
rabbit   FAAIFLGIYVAYRKCHGKNDAEFPDIKDTKTEPESSFHQMNGGFQPGA-              348
human    FAAIFLGFYVAYKKCHGKNKAEIPESKENGTEPESSFYKANGGFQPDEK              348
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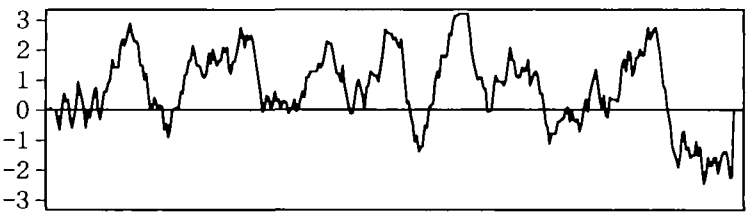

Fig. 7. **A: Alignment of the amino acid sequences of mammalian ISBTs.** The amino acid sequence predicted from the nucleotide sequence of mouse ISBT was aligned with those of ISBTs isolated from other sources. Asterisks, colons, and dots indicate identical amino acids, and strongly and weakly conservative substitutions, respectively. Seven hydrophobic segments predicted for mouse ISBT by the Kyte-Doolittle hydrophobicity plot with a window of 11 amino acids (panel B) are underlined. Two potential *N*-glycosylation sites are boxed.

mouse ISBT cDNA (pZmISBT) showed Na^+-dependent [^{3}H]taurocholate uptake (Fig. 8A). The apparent K_m value was 37 μM, almost equal to the K_m obtained with mouse ileocytes. These K_m values are comparable to the values of 36, 37, 40, 48, and 33 μM for taurocholate uptake by rabbit ileal BBMV (*4*), human ileal BBMV (*12*), porcine ileal BBMV (*21*), porcine ileal-poly(A)$^+$ RNA-injected *Xenopus* oocytes (*21*), and COS cells transfected with isolated hamster ISBT cDNA (*11*), respectively. This, as well as the high amino acid sequence homology among mammalian ISBTs, indicates that the structural and functional characteristics of ISBT are conserved among mammals.

When [^{3}H]taurocholate uptake into COS-7 cells transfected with pZmISBT was measured in the presence of unlabeled bile acids, the inhibitory effect differed from the effect on [^{3}H]taurocholate uptake into isolated ileocytes in that lithocholate was most effective and taurocholate least effective (Fig. 8B).

We tested NEM for its effect on [^{3}H]taurocholate uptake into COS-7 cells transfected with pZmISBT. No inhibitory effect was observed even at NEM concentrations up to 2 mM (data not shown), while uptake by mouse ileocytes and by rabbit ileal BBMV was inhibited (Fig. 6) (*4*). From these studies of inhibition by unlabeled bile acids and NEM, it can be speculated that an additional component other than ISBT, which with ISBT comprises the bile acid uptake machinery in ileocytes, is sensitive to NEM. We have obtained preliminary data, however, that replacement of cysteine 51 of mouse ISBT by alanine markedly decreases the transport activity. Because twelve cysteine residues in mouse ISBT are all conserved among mammalian ISBTs and some are conserved in NTCPs, it is likely that cysteine residues would play an important role. We have constructed mutated mouse ISBTs in which the twelve cysteine

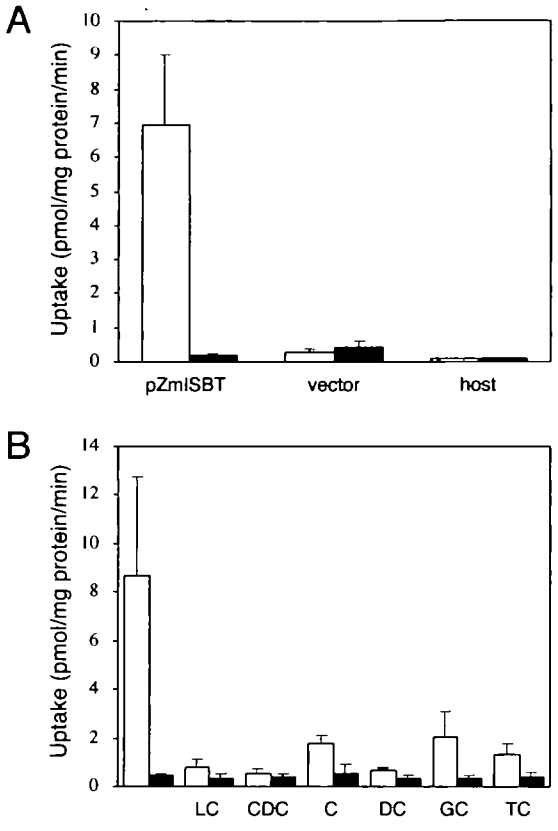

Fig. 8. **Functional expression of mouse ISBT in COS-7 cells.** A: COS-7 cells were transfected with pZmISBT, pZoeSV2(+), or no plasmid by a cationic liposome-mediated procedure. Two days after transfection, [^{3}H]taurocholate uptake after 1 min incubation was measured in the presence of 100 mM NaCl (open columns) or 100 mM choline chloride (stippled columns). Data are the averages and standard errors of triplicated experiments. B: [^{3}H]Taurocholate uptake into pZmISBT-transfected COS-7 cells after a 1-min incubation was measured in the presence of a 115-fold excess of unlabeled bile acids. The open and stippled columns show uptake in the presence of 100 mM NaCl and choline chloride, respectively. −, control; LC, lithocholic acid; CDC, chenodeoxycholic acid; C, cholic acid; DC, deoxycholic acid; GC, glycocholic acid; TC, taurocholic acid. Data are the averages and standard errors of three independent experiments.

residues are individually changed to alanine. Using these mutant ISBTs, we are examining the involvement of each cysteine residue in the function of ISBT.

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