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Molecular cloning, tissue distribution, and expression of a 14-kDa bile acid-binding protein from rat ileal cytosol

(cDNA cloning/Northern blot analysis/cDNA expression)

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ABSTRACT **A cDNA clone encoding the major intestinal cytosolic 14-kDa bile acid-binding protein (14-kDa I-BABP) was isolated from a rat ileal λgt22A library following immunoscreening using a monospecific antiserum raised against a 14-kDa polypeptide found in the rat ileal cytosol. One clone of 516 bp encoded a 128-amino acid protein with a predicted molecular mass of 14,544 Da. The deduced amino acid sequence of 14-kDa I-BABP showed 100% homology to rat intestinal 15-kDa protein (I-15P) and 72% homology to porcine 15-kDa gastrotropin, whereas comparison of I-BABP to rat 14-kDa fatty acid-binding proteins of liver, intestine, and heart revealed homologies of 44%, 25%, and 28%, respectively. Northern blot analysis revealed a single transcript of ≈0.5 kb in ileum and ovary; however, the abundance of I-BABP mRNA was much greater in ileum than in ovary. No transcript was seen in RNA extracted from stomach, jejunum, colon, liver, adrenal, brain, heart, kidney, or testis. Transfection of the I-BABP cDNA into COS-7 cells resulted in the expression of a 14-kDa protein that was identical to the ileal cytosolic I-BABP as determined by immunoblotting. Photoaffinity labeling of expressed 14-kDa protein was saturable with respect to increasing concentrations of 7,7-azo[^{3}H]taurocholate (K_m, 83.3 μM; V_{max}, 6.7 pmol/mg per 5 min). Taurocholate inhibited 7,7-azotaurocholate labeling by >96% with lesser inhibition by taurochenodeoxycholate (83.1%), chenodeoxycholate (74.6%), cholate (50.5%), and progesterone (38.5%), whereas oleic acid and estradiol did not inhibit binding.**

Bile acids (BAs) play a critical role in the micellar solubilization of phospholipid and cholesterol in bile and the lipolytic products of digestion in the intestinal lumen. To maintain effective concentrations for micellarization, BAs undergo an enterohepatic circulation. BAs are synthesized from cholesterol in the liver, secreted in bile into the proximal small intestine, absorbed throughout the small bowel and colon by passive transport and in the ileum by an active transport process, and returned to the liver via the portal blood (1).

A series of studies employing intestinal segments (2, 3), isolated enterocytes (4, 5), and plasma membrane vesicles (6–9) demonstrated that BA uptake across the apical membrane of the ileal cell occurs by a Na^+-dependent process, mediated by an intrinsic membrane protein with a molecular mass of 99 kDa. The identity of this transport protein was initially established by photoaffinity labeling with photolabile BA derivatives (10) and later confirmed using specific antiserum to the transport protein (11). Utilizing similar approaches, the exit of BA across the basolateral membrane was demonstrated to occur by an anion-exchange mechanism (12) involving a putative transport protein with a molecular mass of 54 kDa (13).

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The photoaffinity labeling of rat ileal enterocytes, followed by their fractionation, localized subcellular fractions and proteins that may be involved in intracellular BA transport (14). These studies suggested that intracellular BA-binding proteins (BABPs) can be separated into cytosolic proteins with molecular masses of 14, 35, and 59 kDa, the 43-kDa actin, and a 20-kDa microsomal protein. The 14-kDa protein was shown to be the major intestinal BABP (14-kDa I-BABP) (14). Partial amino acid sequence analysis of the purified 14-kDa I-BABP showed that it is highly homologous to porcine and canine 15-kDa gastrotropins, which are members of the cytosolic fatty acid-binding protein (FABP) family (15, 16). The purified 14-kDa I-BABP was found to be immunologically distinct from rat 14-kDa liver FABP (L-FABP) and I-FABP (17).

We herein report the full-length cDNA sequence for 14-kDa I-BABP isolated from a rat ileal λgt22A library using a 14-kDa I-BABP monospecific polyclonal antiserum.‡ The 14-kDa I-BABP cDNA and deduced amino acid sequence were compared to our previously reported partial amino acid sequence (15) and to other members of the FABP family that bind ligands with distinct and shared properties (18, 19). The tissue distribution of 14-kDa I-BABP transcripts and the functional expression of the 14-kDa I-BABP cDNA in COS-7 cells are also reported.

MATERIALS AND METHODS

Animals and Reagents. Sprague–Dawley rats, 180–220 g, were obtained from Charles River Breeding Laboratories. Goat anti-rabbit IgG horseradish peroxidase (HRP) conjugate was purchased from Sigma. Photoaffinity labeling was performed with the sodium salt of (7,7-azo-3α,12α-dihydroxy-5β-[3β-^{3}H]cholan-24-oyl)-2-aminoethanesulfonate (7,7-azo-[^{3}H]TC) having a specific radioactivity of 3.75 Ci/mmol (1 Ci = 37 GBq). The synthesis and properties of this photolabile derivative have been described (20).

Western Blot Analysis. Purification of 14-kDa I-BABP and production of a polyclonal antiserum to 14-kDa I-BABP were reported (17). Five milligrams of total ileal cytosolic proteins, 2 mg of ileal cytosolic proteins purified by lysylglycocholate affinity chromatography, and 20 mg of COS-7 cell lysates were subjected to SDS/PAGE. Separated proteins were transblotted onto a nitrocellulose membrane (Bio-Rad), and

Abbreviations: BA, bile acid; BABP, BA-binding protein; FABP, fatty acid-binding protein; I-, intestinal; L-, liver; 7,7-azo-TC, 7,7-azotaurocholate.

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‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. L22788).

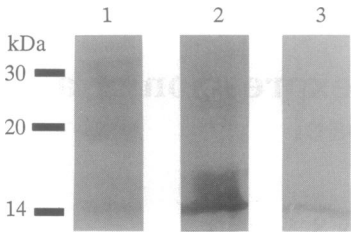

FIG. 1. Western blot analysis of 14-kDa I-BABP. Cytosolic proteins from rat ileal cells were analyzed by SDS/PAGE. Lane 1, total cytosolic proteins stained with Coomassie blue; lane 2, proteins purified by lysylglycocholate affinity chromatography and stained with Coomassie blue; lane 3, purified proteins blotted onto nitrocellulose and assayed by immunostaining with rabbit anti-rat 14-kDa I-BABP serum.

immunodetection was performed using a 1:200 dilution of rabbit anti-rat 14-kDa I-BABP serum and a 1:8000 dilution of HRP-conjugated goat anti-rabbit IgG serum (Sigma).

RNA Preparation and Analysis. Total cellular RNA was harvested from rat ileal enterocytes and various rat tissues and organs within and outside the digestive system by lysis in guanidine isothiocyanate (21). Denatured RNA was size-fractionated by electrophoresis (1% agarose in 1× Mops/2.2 M formaldehyde) and transferred to a Duralon-UV filter (Stratagene). Filters were probed with a [^{32}P]UTP-labeled full-length antisense 14-kDa I-BABP cRNA. Filters were washed to a final stringency of 0.1× SSC (1× SSC = 150 mM sodium chloride/10 mM sodium citrate, pH 7.4/20 mM EDTA) and 0.1% SDS at 65°C and autoradiographed at −70°C for 16 hr. Stripped filters were reprobed with a [^{32}P]UTP-labeled antisense mouse β-actin riboprobe.

cDNA Library Construction, Screening, and Sequencing. Rat ileal poly(A)$^+$ RNA isolated by oligo(dT)-cellulose chromatography was used for the construction of a λgt22A cDNA library (GIBCO/BRL). Primary library titer was 5.5×10^6 plaque-forming units (pfu)/ml. Following one round of library amplification (1×10^{10} pfu/ml), 5×10^5 plaques were immunoscreened using rabbit anti-rat 14-kDa I-BABP polyclonal antiserum (22). Six positive clones were isolated, two of which were chosen for additional analysis. Each was found to contain identical inserts of ≈500 bp. These were subcloned into the *Sal* I/*Not* I sites of pSPORT1 (GIBCO/BRL) for nucleic acid sequencing by the method of Sanger *et al.* (23) using Sequenase Version 2.0 (United States Biochemical).

Sequence Analysis. The translated amino acid sequence was screened against a nonredundant SwissProt + Protein Identification Resource + SPUpdate + GenPept + GPUpdate data base using the National Center for Biotechnology Information BLAST search service (24). Matching amino acid sequences were aligned using the CLUSTAL program/PC Gene Software (25). The deduced amino acid sequence of 14-kDa I-BABP was subjected to Prosite (26) analysis.

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-48  CGC GTC CGT CCT CAG TTG CCT CTC TGC TGC CAG CTC TCT CCC AGC

 -3  ACT ATG GCC TTC ACC GGC AAA TAC GAA TTT GAA AGT GAG AAG AAT
         MET Ala Phe Thr Gly Lys Tyr Glu Phe Glu Ser Glu Lys Asn   14

 43  TAC GAC GAG TTC ATG AAG CGC CTG GGT CTT CCA GAA GAT GTG ATT
     Tyr Asp Glu Phe Met Lys Arg Leu Gly Leu Pro Glu Asp Val Ile   29

 88  GAA AGG GGA CGT AAC TTC AAG ATC ATC ACA GAG GTC CAG CAG GAT
     Glu Arg Gly Arg Asn Phe Lys Ile Ile Thr Glu Val Gln Gln Asp   44

133  GGA GAG AAC TTC ACC TGG TCC CAG TCT TAC TCT GGG GGC AAC ATC
     Gly Glu Asn Phe Thr Trp Ser Gln Ser Tyr Ser Gly Gly Asn Ile   59

178  ATG AGC AAC AAG TTC ACC ATT GGC AAA GAA TGT GAA ATG CAG ACC
     Met Ser Asn Lys Phe Thr Ile Gly Lys Glu Cys Glu Met Gln Thr   74

223  ATG GGG GGC AAG AAG TTC AAG GCA ACC GTG AAG ATG GAG GGT GGC
     Met Gly Gly Lys Lys Phe Lys Ala Thr Val Lys Met Glu Gly Gly   89

268  AAG GTG GTG GCA GAC TTC CCC AAC TAT CAC CAG ACT TCG GAG GTC
     Lys Val Val Ala Asp Phe Pro Asn Tyr His Gln Thr Ser Glu Val  104

313  GTG GGT GAC AAG TTG GTG GAG ATC TCC ACC ATC GGG GAT GTG ACC
     Val Gly Asp Lys Leu Val Glu Ile Ser Thr Ile Gly Asp Val Thr  119

358  TAC GAG CGT GTA AGC AAG AGG GTG GCT TGA GCC ACC AGG CTG GGC
     Tyr Glu Arg Val Ser Lys Arg Val Ala ***                     128

403  TCT GAG GGA CTG CAA CCC ATC AAT AAA AGT GCA CCA TGG ATC ACC
448  ACT AAA AAA AAA AAA AAA AAA
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FIG. 2. Nucleotide and deduced amino acid sequences of rat 14-kDa I-BABP cDNA. Nucleotides are numbered on the left; amino acids are numbered on the right. Asterisks denote the stop codon. Amino acids that conform to the cytosolic fatty acid-binding motif as identified by Prosite Search Version 9.2 (26) are underlined. Amino acids in boxes are those identified by Edman degradation of proteolytic fragments derived from purified ileal cytosolic I-BABP (15).

Expression of 14-kDa I-BABP cDNA in COS-7 Cells. The 14-kDa I-BABP cDNA was subcloned into the expression vector pSV·SPORT1 and transfected into COS-7 cells for transient expression. Cells were grown in 5% CO_2 at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Transfection was carried out when cells reached 70–80% confluency using LipofectAmine (GIBCO/BRL) following the manufacturer's protocol. Forty-eight hours after transfection, cells were rinsed twice with ice-cold phosphate-buffered saline and resuspended in lysis buffer (50 mM Hepes, pH 7.0/1 mg of aprotinin per ml/1% Nonidet P-40/100 mg of phenylmethylsulfonyl fluoride per ml). Lysates were used for Western blot analysis and photoaffinity binding assays.

Photoaffinity Binding Assay. Cell lysates containing 25 mg of protein were incubated with several different concentrations of 7,7-azo-[^{3}H]TC (3.75 μCi/mol) for kinetic studies or 100 μM 7,7-azo-[^{3}H]TC in the presence of various ligands for binding inhibition studies. Photolysis was performed by exposure to UV light at 25°C for 5 min using a Rayonet photoreactor RPR 100 (Southern New England Ultraviolet, Hamden, CT). After photolysis, lysates were centrifuged at 12,000 × *g* for 5 min, and supernatants were subjected to SDS/PAGE. The protein concentration of the supernatant was determined by the method of Lowry *et al.* (27).

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                MA--PTGKYEFESEKNYDEFMKRLGLPEDVIERGRNFKIITEVQQDGENFTWSQSYSGGNIMSNKFTI
Rat I-15P       **--************************************************************
Pig Gastrotropin **--******I************A**S*A*DKA**L***S**K***Q******Q*P**HSIT*T***
Rat L-FABP      *N--*S***QVQ*QE*FEP***AM*****L*QK*KDI*GVS*IVHE*KKVKLTIT*-*SKVIH*E**L
Human L-FABP    *S--*S***QLQ*QE*FEA***AI*****EL*QK*KDI*GVS*IV*N*KH*KFTITA-*SKVIQ*E**V
Rat I-FABP      **--*D*TWKVYRNE**EK**EKM*INVVKRKL*AHDNLKLTIT*E*NK**-VKES*NFRNIDVV*EL
Rat Heart FABP  **DA*V*TWKLVDS**F*DY**S**VGFATRQVASMT*PT*IIEKN*DTIT-IKTH*TFKNTEIS*QL

                GKECEMQTMGGKKFKATVKMEGG-KVVADFPNYHQ------TSEVVGDKLVEISTIGDVTYERVSKRVA
Rat I-15P       ************************-**********------**************************
Pig Gastrotropin ****DIE*I*********Q****-***VNS****H------*A*I*DG****V**V*G*S******KL*
Rat L-FABP      *E***LE**T*E*V**V*****DN*M*TT*KGIKS------VT*FN**TITNTM*L**IV*K*****I-
Human L-FABP    *E***LE**T*E*V*TV*QL**DN*L*TA*K*IKS------VT*LN**IITNTM*L**IVFK*I***I-
Rat I-FABP      *VDFAYSLAD*TELTG*LT***-N*L*GK*KRVDNGKELIAVR*IS*NE*IQTY*YEG*EAK*IF*KE-
Rat Heart FABP  *V*FDEV*ADDR*V*SV*TLD**-*L*HVQKW--DGQETTLTR*LSDG**ILTL*H*N*VST*TTEKE*
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FIG. 3. Comparison of the deduced amino acid sequence of 14 kDa I-BABP with those of rat I-15P (28), porcine gastrotropin (16), rat L-FABP (29), human L-FABP (30), rat I-FABP (31), and rat heart FABP (32). Asterisks indicate identity and dashes show introduction of spaces to maximize alignment. Sequences of maximum homology are taken from a BLAST search of a nonredundant SwissProt + Protein Identification Resource + SPUpdate + GenPept + GPUpdate data base (24) and were subject to multiple sequence alignments.

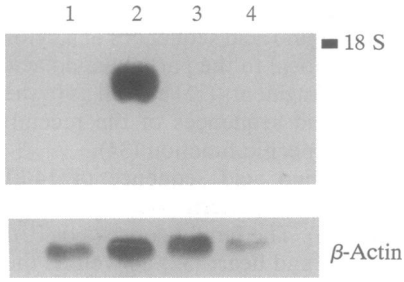

FIG. 4. Northern blot analysis of total RNA extracted from rat gastrointestinal tissues. (*Upper*) Total cellular RNA was probed with the antisense cRNA generated from *Sal* 1-digested 14-kDa I-BABP cDNA in pSPORT1 plasmid. (*Lower*) The same blot was stripped and rehybridized with a mouse β-actin antisense probe. Lanes 1, 2, 3, and 4 correspond to total RNA from stomach, ileum, jejunum, and colon, respectively.

RESULTS

Immunodetection of I-BABP. Rabbit antiserum raised against rat 14-kDa I-BABP specifically recognized a 14-kDa protein that had been purified by lysylglycocholate affinity chromatography (Fig. 1). We have previously shown that the 14-kDa I-BABP did not react with antiserum to L-FABP or I-FABP (14, 17).

Characterization of the cDNA Encoding I-BABP. Two positive cDNA clones isolated from an ileal cDNA library were completely sequenced. Both contained identical 516-bp sequences with an open reading frame encompassing 384 bp as well as 5′ (48 bp) and 3′ (84 bp) untranslated regions (Fig. 2). Translation of the nucleic acid sequence predicted a 128-amino acid polypeptide with a calculated molecular mass of 14,544 Da.

The deduced amino acid sequence of 14-kDa I-BABP (Fig. 3) was found to be identical to the sequences of our previously reported 14-kDa I-BABP proteolytic fragments (15) and to rat intestinal 15-kDa protein (I-15P) (28). Prosite analysis of 14-kDa I-BABP identified the signature pattern specific for the family of cytosolic FABP. The consensus pattern consisting of 18 amino acids, [GS]-x-[FYW]-x-[LIVMF]-x(4)-[NH]-[FY]-[DE]-x-[LIVMFY]-[LIVM]-x(2)-[LIVM], was found in the N-terminal region of 14-kDa I-BABP polypeptide (amino acids 5–22) (Fig. 3). The deduced 14-kDa I-BABP amino acid sequence was compared to the porcine gastrotropin amino acid sequence (16) and shown to have 72% homology, whereas comparisons to FABP found in rat liver (29), human liver (30), rat intestine (31), and rat heart (32) revealed homologies of 44%, 41%, 25%, and 28%, respectively (Fig. 3).

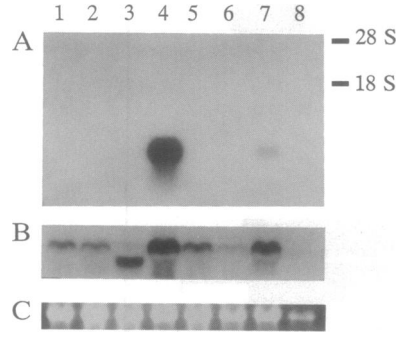

FIG. 5. Northern blot analysis of total RNA extracted from rat ileum and extraintestinal tissues. (*A*) Total cellular RNA was probed with the antisense cRNA generated from 14-kDa I-BABP cDNA in pSPORT1 plasmid. (*B*) The same blots were stripped and rehybridized with a mouse β-actin antisense probe. (*C*) Ethidium bromide stain of 18S rRNA. Lanes 1, 2, 3, 4, 5, 6, 7, and 8 correspond to total RNA from adrenal, brain, heart, ileum, kidney, liver, ovary, and testis, respectively.

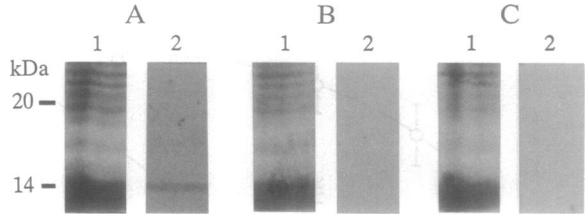

FIG. 6. Expression of 14-kDa I-BABP in COS-7 cells. Cytosolic proteins from COS-7 cells were fractionated by SDS/PAGE and stained with Coomassie blue (lanes 1) or blotted onto nitrocellulose and assayed by immunostaining with 14-kDa I-BABP antiserum (lanes 2). (*A–C*) Cytosolic proteins from cells transfected with pSV·SPORT containing 14-kDa I-BABP cDNA (*A*), cells transfected with pSV·SPORT plasmid alone (*B*), and untransfected cells (*C*), respectively.

Northern Blot Analysis. To estimate the size of I-BABP mRNA and to assess its tissue distribution of expression, Northern blot analysis on total cellular RNA was performed. The 14-kDa I-BABP cDNA antisense probe hybridized to a single transcript of ≈500 bp in RNA prepared from the ileum (Fig. 4). No transcript was detected in the RNA prepared from stomach, jejunum, or colon (Fig. 4). A similarly sized transcript was detected in the ovary (Fig. 5) with a relative level much less than in the ileum (Fig. 5). No transcript was detected in all other tissues examined (adrenal, brain, heart, kidney, liver, and testis) (Fig. 5).

Expression of the Rat I-BABP cDNA in COS-7 Cells. To confirm the biological function of the 14-kDa protein encoded by the cloned I-BABP cDNA, a pSV·SPORT1 plasmid containing the 14-kDa I-BABP cDNA insert was transfected into COS-7 cells. Cellular lysates were prepared, and lysate proteins were separated by SDS/PAGE and transblotted onto nitrocellulose. As shown in Fig. 6, a 14-kDa protein from the COS-7 cells transfected with pSV·SPORT1 containing 14-kDa I-BABP cDNA reacted with 14-kDa I-BABP antiserum, whereas no reactivity was noted with lysates prepared from COS-7 cells transfected with pSV·SPORT1 alone or untransfected cells. To demonstrate BA binding activity, COS-7 cellular lysates were incubated with 7,7-azo-[^{3}H]TC, irradiated with UV light, and then subjected to SDS/PAGE. Fig. 7 shows binding of radioactivity by a 14-kDa protein from 14-kDa I-BABP cDNA transfected COS-7 cells. This

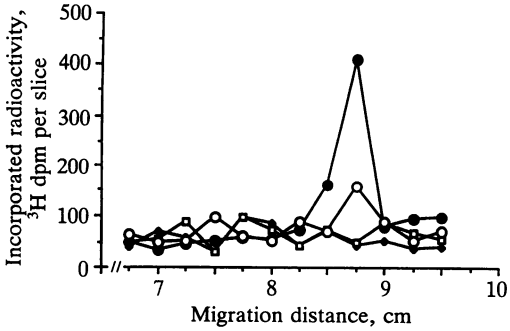

FIG. 7. BA-binding activity of 14-kDa I-BABP in COS-7 cells. Cytosolic proteins from COS-7 cells were analyzed for BA-binding activity by photoaffinity labeling with 7,7-azo-[^{3}H]TC. Following labeling, proteins were fractioned by SDS/PAGE and gel slices (2 mm) were counted for incorporation of radioactivity. ◆, Untransfected cells; □, cells transfected with pSV·SPORT1 plasmid alone; ●, cells transfected with pSV·SPORT1 containing 14-kDa I-BABP cDNA and photolabeled in the absence of 0.5 mM TC; ○, cells transfected with pSV·SPORT1 containing 14-kDa I-BABP cDNA and photolabeled in the presence of 0.5 mM TC.

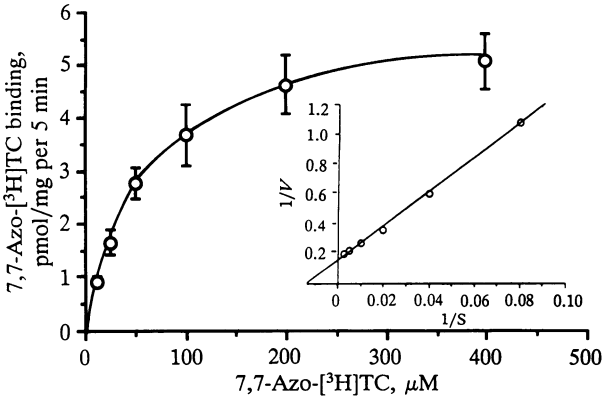

FIG. 8. Kinetics of 7,7-azo-TC binding by 14-kDa-I-BABP expressed in COS-7 cells. Cell lysates were photolabeled with increasing concentrations (12.5–400 μM) of 7,7-azo-[^{3}H]TC (3.75 Ci/mmol). The binding values (pmol/mg of protein per 5 min) represent the mean ± SE of 12 determinations using three separate preparations.

binding was almost completely inhibited by the coincubation with taurocholate during photolysis. No radioactivity was bound by proteins from untransfected cells or cells transfected with vector alone. To further characterize binding by the expressed 14-kDa protein, COS-7 cellular lysates were irradiated in the presence of increasing concentrations of 7,7-azo-[^{3}H]TC. As shown in Fig. 8, the labeling of 14-kDa protein was saturable with respect to increasing 7,7-azo-[^{3}H]TC concentrations (12.5–400 μM). Extrapolation of the kinetic curve by a double reciprocal plot yielded a K_m of 83.3 μM and V_{max} of 6.7 pmol/mg per 5 min. Finally, 7,7-azo-[^{3}H]TC binding to protein from I-BABP cDNA transfected COS-7 cells was significantly inhibited by coincubation with 0.8 mM taurocholate (>96%), taurodeoxycholate (83.1%), chenodeoxycholate (74.6%), and cholate (50.5%), indicating competition between the photoprobe and BA for the same binding site. The binding of 7,7-azo-[^{3}H]TC was also inhibited by the addition of 0.2 mM progesterone (38.5%) but not by 0.8 mM oleic acid or estradiol (Fig. 9).

DISCUSSION

Although the processes involved in transcellular transport of BA through the enterocyte have been subjected to scrutiny (14, 33), relatively little information is available regarding the molecular biology and regulation of putative BA transport and binding proteins. The present report provides the nucleic acid and deduced amino acid sequences of a cytosolic 14-kDa I-BABP that is identical to the partial amino acid sequences of 14-kDa I-BABP fragments (15) as well as to the amino acid (28) and nucleic acid sequences of the recently described I-15P of unknown specific function (34).

The deduced amino acid sequence of 14-kDa I-BABP contains the signature pattern ascribed to the family of cytosolic FABP (26). The family consists of FABP found in the liver, intestine, and heart (32) as well as the adipocyte-binding protein, cellular retinol-binding protein, myelin P2 protein, and gastrotropin (16, 18, 19). It appears that rat 14-kDa I-BABP is distinct from L-FABP and I-FABP. (*i*) Our previous studies demonstrated that purified 14-kDa I-BABP failed to react with either anti-L-FABP or anti-I-FABP serum (17) but reacted with anti-14-kDa I-BABP serum, suggesting that 14-kDa I-BABP is immunologically distinct from L-FABP and I-FABP. (*ii*) The deduced amino acid sequence of 14-kDa I-BABP has only 25% similarity with rat I-FABP and 44% similarity with rat L-FABP. (*iii*) Northern blot analysis showed that 14-kDa I-BABP mRNA was abundant in the ileum; no transcript was detected in the liver or the rest of the gastrointestinal tract. In contrast to I-BABP, rat I-FABP and L-FABP genes are expressed in the epithelium throughout the small intestine (29, 31, 35). Although controversy exists regarding the relative importance of the proximal small intestine in BA absorption (1), the ileum is the only intestinal site of active BA transport which is coincident with the site of 14-kDa I-BABP mRNA expression. On the other hand, dietary fatty acids are absorbed along the whole small intestine, primarily in the proximal small intestine, suggesting that 14-kDa I-BABP plays no role in their absorption. (*iv*) The dissociation constants for the binding of taurocholate, taurodeoxycholate, cholate, chenodeoxycholate, and oleate by Z proteins (L-FABP or I-FABP) were 1700, 350, 97, 21, and 6.2 μM, respectively (36). The almost complete inhibition of 7,7-azo-TC binding to 14-kDa I-BABP expressed in COS-7 cells by taurocholate, lesser degrees of inhibition by other bile acids, and no inhibition by oleic acid in the present studies suggest that I-BABP is distinct in its binding characteristics from FABPs.

We found relatively low level expression of 14-kDa I-BABP mRNA in rat ovary. BAs are cholesterol-derived sterols and share structural similarities with steroid hormones such as progesterone and estrogen in the ovary. The ability of progesterone to partially inhibit 7,7-azo-[^{3}H]TC

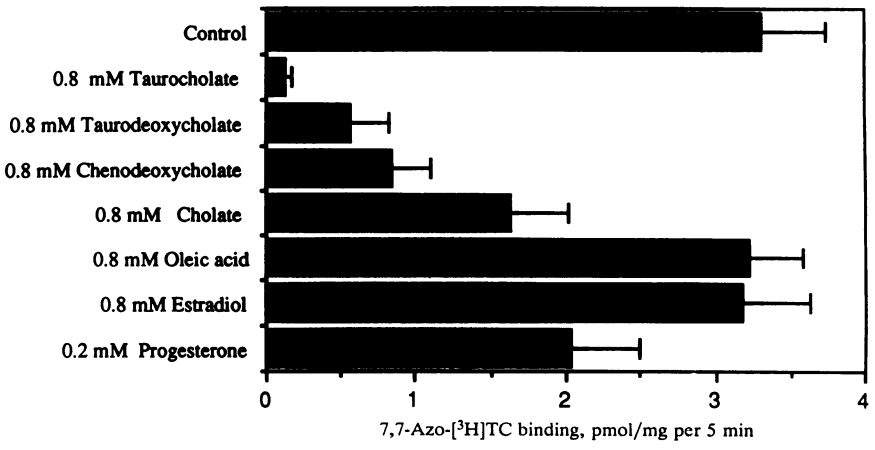

FIG. 9. Effects of agents on 7,7-azo-TC binding by the 14-kDa-I-BABP expressed in COS-7 cells. Lysates from transfected COS-7 cells were incubated with 100 μM 7,7-azo-[^{3}H]TC in the absence of any ligand as control or in the presence of several different ligands (bile salts, oleic acid, estradiol, or progesterone). The binding values (pmol/mg of protein per 5 min) represent the mean ± SE of nine determinations using three separate preparations.

binding suggests that 14-kDa I-BABP may serve a similar cytosolic transport role in the ovary.

Comparison of the deduced amino acid sequence of 14-kDa I-BABP with porcine gastrotropin revealed a high level (72%) of homology. Gastrotropin was originally reported to be a hormone that is secreted into the bloodstream and causes stimulation of gastric acid secretion (37). Subsequently, Gantz *et al.* (16) determined that porcine gastrotropin did not stimulate gastric acid output *in vivo*. However, they detected expression of gastrotropin mRNA in porcine ileum and postulated that gastrotropin may serve as an ileal ligand-binding protein for BA. Since the biological activity of gastrotropin remains controversial, it is important for future study to establish the similarity between rat gastrotropin and rat 14-kDa I-BABP.

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