Molecular mechanism of the intestinal biotin transport process

NABENDU S. CHATTERJEE, CHANDIRA K. KUMAR, ALVARO ORTIZ, STANLEY A. RUBIN, AND HAMID M. SAID

Medical Research Service, Veterans Affairs Medical Center, Long Beach 90822, and Department of Medicine and Physiology/Biophysics, University of California School of Medicine, Irvine 92697; and Veterans Affairs West Los Angeles, Los Angeles 90073, and Department of Medicine, University of California, Los Angeles, California 90024

Chatterjee, Nabendu S., Chandira K. Kumar, Alvaro Ortiz, Stanley A. Rubin, and Hamid M. Said. Molecular mechanism of the intestinal biotin transport process. Am. J. Physiol. 277 (Cell Physiol. 46): C605–C613, 1999.—Previous studies have characterized different aspects of the cellular/membrane mechanism and regulation of the intestinal uptake process of the water-soluble vitamin biotin. Little, however, is known about the molecular mechanisms of the uptake process. In this study, we have identified a cDNA from rat small intestine that appears to be involved in biotin transport. The open reading frame of this cloned cDNA consisted of 1,905 bases and was identical to that identified for the vitamin transporter in placental tissue. Significant heterogeneity, however, was found in the 5′ untranslated region of this clone, with three distinct variants (II, III, IV) being identified in the small intestine; the placental variant (variant I), however, was not present in the small gut. Variant II was found to be the predominant form expressed in the rat small and large intestines. Functional identity of the cloned intestinal cDNA was confirmed by stable expression in COS-7 cells, which showed a four- to fivefold increase in biotin uptake in transfected COS-7 cells compared with controls. The induced biotin uptake in transfected COS-7 cells was found to be 1) Na+-dependent, 2) saturable as a function of concentration with an apparent K_m of 8.77 µM and a V_max of 779.7 pmol·mg protein−1·3 min−1, and 3) inhibited by unlabeled biotin and pantothenic acid and their structural analogs. The distribution of complementary mRNA transcripts of the cloned cDNA along the vertical and longitudinal axes of the intestinal tract was also determined. Results of this study describe the molecular characteristics of the intestinal biotin absorption process and report the identification of a cDNA that encodes a Na+-dependent biotin uptake carrier that appears to exist in the form of multiple variants.

THE WATER-SOLUBLE VITAMIN biotin is essential for normal cellular functions, growth, and development (3, 5, 35). It acts as a coenzyme for many carboxylases involved in pathways of fatty acid biosynthesis, gluconeogenesis, and catabolism of several branched-chain amino acids and odd-chain fatty acids (3, 5, 35). Recent studies have identified additional cellular functions for biotin, which include regulation of cellular cGMP levels (7, 38, 40). Biotin deficiency in humans leads to a range of clinical abnormalities including neurological disorders, growth retardation, and skin abnormalities (3, 5, 35, 40). Deficiency of biotin during pregnancy has also been shown in several animal species to lead to embryonic growth retardation, congenital malformation, and death (38). The incidence of biotin deficiency and suboptimal levels has been reported with increased frequency in recent years. Biotin deficiency has been reported to occur in patients with inborn errors of biotin metabolism (3, 5, 35), in patients on long-term therapy with anticonvulsant agents (7, 8), where inhibition in intestinal biotin absorption is believed to be a contributing factor (31), and in patients on long-term parenteral nutrition (12, 13). Suboptimal biotin levels have also been reported during pregnancy (14), in substantial numbers of alcoholics, where impairment of the intestinal biotin absorption process is believed to be a contributing factor (32), and in patients with inflammatory bowel diseases (1, 37).

Humans and other mammals lack the ability to synthesize biotin and thus must obtain the vitamin from exogenous sources through absorption in the intestine. Therefore, the intestine plays a key role in determining and regulating normal biotin body homeostasis, and thus understanding the cellular and molecular mechanisms and regulation of the intestinal absorption process of biotin is of physiological and nutritional importance. Previous studies from our laboratory (11, 19–23, 25–30) and others (4) have characterized different aspects of the mechanism and regulation of the intestinal absorption process at the tissue, cellular, and membrane levels. These studies have shown, among other things, the involvement of a specialized, Na+-dependent, carrier-mediated mechanism for the biotin uptake process in the small intestine. This mechanism was found to be capable of transporting the vitamin against a concentration gradient across the intestinal brush-border membrane (20, 28–30) and was shared by the unrelated water-soluble vitamin, pantothenic acid (19). A similar uptake mechanism was also found in colonic epithelial cells and is believed to be responsible for the absorption of the bacterially synthesized biotin in the large intestine (19, 25). Studies in our laboratory have also shown that the intestinal biotin uptake process is regulated by biotin dietary levels (21) and by specific intracellular regulatory pathways (19, 25). To date, however, little is known...
about the molecular mechanism(s) of the intestinal biotin absorption process. The serendipitous cloning of the so-called Na\textsuperscript{+}-dependent multivitamin transporter (SMVT) from rat placenta (17), a transporter that also appears to transport biotin, has assisted us in our effort to address this issue. In this study, we describe the molecular characterization of the small intestinal biotin uptake process and report the cloning of a cDNA with multiple variants.

MATERIALS AND METHODS

Materials. [\textsuperscript{3}H]Biotin (sp act \(>30\) Ci/mmol; radiochemical purity \(>98\%\)) and all other radioactive materials were obtained from Dupont NEN (Boston, MA) and Amersham (Arlington Heights, IL). \[\text{L}^4\]Pantothionic acid (sp act \(>55\) mCi/mmol; radiochemical purity \(>98\%\)) was obtained from American Radiolabeled Chemicals (St. Louis, MO). All chemicals and reagents used in this study were of analytical/molecular biology grade and were obtained from commercial sources. Cellulose nitrate filters (0.45 µm pore size) used in uptake studies with isolated villus and crypt cells were purchased from Sartorius Filters (Hayward, CA). COS-7 cells were obtained from ATCC (Manassas, VA). DMEM, trypsin, fetal bovine serum (FBS), and other cell culture materials were obtained from Life Technologies (Grand Island, NY). Different kits used in this study were purchased from commercial vendors and are identified in the text. The oligonucleotides used in different experiments (Table 1) were synthesized from a commercial source. The oligonucleotides were used in different RT-PCR experiments.

Table 1. Oligonucleotides used in different RT-PCR experiments

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence (5′ to 3′)</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F*</td>
<td>P-GGAATTCCGAGGATGACTGTGGCGAGCAC</td>
<td>409 to 429</td>
</tr>
<tr>
<td>2R*</td>
<td>P-ATCTAGATCACAGGGACGTCTCTTGGAC</td>
<td>2,314 to 2,297</td>
</tr>
<tr>
<td>3R†</td>
<td>GGATATGGCGGGTTGTCAGGAGTGTGATAGGA</td>
<td>54 to 28</td>
</tr>
<tr>
<td>4F†</td>
<td>GCACCCACATCCATTGTAGGTTGCGACAC</td>
<td>1,673 to 1,695</td>
</tr>
<tr>
<td>5F†</td>
<td>CAGGGCAAAATCGGAGTTTC</td>
<td>428 to 411</td>
</tr>
<tr>
<td>6F†</td>
<td>CAGGGGAGGAGCAGCTT</td>
<td>354 to 338</td>
</tr>
<tr>
<td>7F†</td>
<td>GCCTTACCCAGCTTGGG</td>
<td>337 to 320</td>
</tr>
<tr>
<td>8F†</td>
<td>GGAAGACTGAGCCGAGA</td>
<td>23 to 42</td>
</tr>
<tr>
<td>9R†</td>
<td>GTATAGCCAGGGCCGCA</td>
<td>35 to 18</td>
</tr>
<tr>
<td>10R†</td>
<td>CAGCTACCAACGATAGGCC</td>
<td>174 to 155</td>
</tr>
<tr>
<td>11F§</td>
<td>TGTAGCCACGCCCCGAGGATAGGG</td>
<td></td>
</tr>
<tr>
<td>12R§</td>
<td>GACCTGCTACCCATGGGCTCTAGG</td>
<td></td>
</tr>
</tbody>
</table>

The oligonucleotides were synthesized from a commercial source. EcoR I restriction sites are single underlined; Xho I site is double underlined. P, phosphorylation modification made at 5′ ends; F, forward; R, reverse. *Positions based on placental Na\textsuperscript{+}-dependent multivitamin transporter sequence (accession no. AF026554). †Positions based on intestinal biotin transporter (accession no. AF081204). ‡Positions based on nucleotide sequences as reported in Fig. 1A. §Rat β-actin control primer sequences (Clontech).
RT-PCR products using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Cell culture and uptake studies. Wild-type and transfected (transfection was performed by electroporation; Ref. 9) COS-7 cells were grown in DMEM containing 10% FBS. G418 (1 mg/ml) antibiotic was added to the growth medium of transfected COS-7 cells for selection and growth. Subconfluent cells were subcultured and plated onto 12-well plates at a concentration of 1 x 10^5 cells/well. All uptake studies were performed 2–5 days after confluence. Uptake was performed at 37°C in Krebs-Ringer phosphate buffer containing (in mM) 123 NaCl, 4.93 KCl, 1.23 MgSO_4, 0.85 CaCl_2, 5 glucose, 5 glutamine, 10 HEPES, and 10 MES, pH 7.4. [3H]biotin (or [14C]pantothenic acid) was added to the incubation buffer at the beginning of the experiment and uptake was terminated after 3 min of incubation (unless otherwise specified) by the addition of 1 ml of ice-cold buffer followed by immediate removal by aspiration. The monolayers were rinsed twice with ice-cold buffer, digested with 1 ml of 1 N NaOH, neutralized by HCl, harvested, and counted for radioactivity in a liquid scintillation counter. Protein contents of cell digests were estimated on parallel wells using a protein assay kit from Bio-Rad Laboratories (Hercules, CA). Uptake data presented in this paper are means ± SE of multiple separate experiments performed on at least two different occasions and are expressed as picomoles or femtomoles per milligram of protein per unit time. Statistical significance was set at the 5% level (P < 0.05) as determined by Student's t-test. Kinetic parameters of biotin uptake, i.e., maximal velocity (V_{\text{max}}) and the apparent Michaelis-Menten constant (K_{m}), were calculated using the Lineweaver-Burk plot.

RESULTS

Cloning and characterization of an intestinal cDNA. Using RT-PCR (see MATERIALS AND METHODS) and primers designed based on the ORF of SMVT (17), a product of 1.9 kb in size was identified from rat small intestine. The sequence of this cDNA was found to be identical to the ORF of SMVT (17). As to the 5' UTR, the result of our RACE-PCR showed the existence of three distinct clones with sequences of 522, 447, and 391 bp (Fig. 1A). Compared with the previously reported cDNA of the SMVT, these three clones were each found to be uniquely different at the extreme 5' UTR sequence, and all shared the absence of a 60-bp stretch in the middle of the 5' UTR sequence (Fig. 1B). These three newly identified sequences were named variants II, III, and IV, respectively, relative to the 5' UTR of SMVT that was considered variant I in this report. No sequence corresponding to the 5' UTR of SMVT (i.e., variant I) was detected in the small intestine by our RT-PCR cloning method. With regard to the 3' UTR, this region was found to be identical to that of SMVT, with the exception of a 10-bp region missing in the cloned intestinal cDNA (Fig. 1C).

Analysis of the nucleotide sequence showed that the ORF of the cloned cDNA encodes a putative polypeptide of 634 amino acids with a calculated molecular mass (M_r) of 68,596 Da. The theoretical isoelectric point was estimated to be 9.4. With the use of the Kyte-Doolittle algorithm (10) with a window of 11 amino acids, 12 membrane-spanning domains were predicted with both amino and carboxy termini to be on the cytoplasmic side of the membrane (Fig. 2). The putative polypeptide was found to carry a net positive charge of 5.4 at physiological pH, as indicated by the program Protein of the Lasergene package (DNastar, Madison, WI). The instability index of the putative polypeptide was found to be 42.03, classifying it as unstable; also, the polypeptide contained 12 histidine residues and was rich in leucine, with 15% of its amino acids being leucine. Examination of the predicted amino acid sequence also revealed several putative posttranslational modification sites: 2 protein kinase C phosphorylation sites (T16 and S235), 1 protein kinase A phosphorylation site (S322), 3 N-glycosylation sites (N488, N497, and N532), 11 O-glycosylation sites (T2, S5, T6, S10, T13, S14, S491, T504, S509, T510, S512), and 13 N-myristoylation sites (G42, G183, G193, G238, G271, G352, G361, G454, G458, G462, G472, G540, G548).

Functional characteristics of the cloned cDNA. Functional identity of the ORF of the cloned intestinal cDNA was confirmed by stable transfection of the cDNA into African green monkey kidney COS-7 cells (COS-7/cDNA) followed by examination of [3H]biotin uptake and comparing the results to appropriate controls [the controls included mock electroporated COS-7 cells (COS-7/wild) and COS-7 cells transfected with an empty vector (COS-7/vector)]. Uptake of [3H]biotin (5.2 nM) was found to be four- to fivefold higher in COS-7/cDNA cells compared with uptake by COS-7/vector cells; uptake by the latter cells was found to be similar to that in the COS-7/wild cells ([79.17 ± 2.67, 17.4 ± 0.49 (P < 0.01) and 19.08 ± 1.03 (P < 0.01) fmol·mg protein^-1·3 min^-1, respectively]). The level of mRNA transcripts corresponding to the cloned intestinal cDNA in the different COS-7 cell subtypes was also determined using RT-PCR. Results showed the presence of mRNA corresponding to the cloned intestinal cDNA in COS-7/cDNA cells but not in control cells (Fig. 3).

In another study, we examined the effect of Na^+ removal from the incubation medium on the induced biotin uptake in COS-7/cDNA cells. In this experiment, Na^+ was replaced isosmotically with either K^+, Li^+, choline, Tris, or mannitol. The results showed significant (P < 0.01 for all) inhibition in biotin (5.2 nM) uptake when Na^+ was removed from the incubation medium and regardless of what was used to replace it (79.17 ± 2.67, 6.88 ± 0.55, 21.35 ± 0.76, 15.15 ± 0.42, 2.25 ± 0.25, and 1.27 ± 0.10 fmol·mg protein^-1·3

### Table 2. PCR conditions used to detect different mRNA species of the cloned intestinal cDNA

<table>
<thead>
<tr>
<th>Primer Combination</th>
<th>Annealing Temperature</th>
<th>Number of Cycles</th>
<th>Expected PCR Product, bp</th>
<th>mRNA Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>5F + 9R</td>
<td>48°C</td>
<td>35</td>
<td>463</td>
<td>Variant II</td>
</tr>
<tr>
<td>6F + 9R</td>
<td>48°C</td>
<td>35</td>
<td>389</td>
<td>Variant III</td>
</tr>
<tr>
<td>7F + 9R</td>
<td>48°C</td>
<td>35</td>
<td>372</td>
<td>Variant IV</td>
</tr>
<tr>
<td>8F + 9R</td>
<td>51°C</td>
<td>35</td>
<td>425</td>
<td>Variant I</td>
</tr>
<tr>
<td>1F + 10R</td>
<td>50°C</td>
<td>30</td>
<td>177</td>
<td>ORF</td>
</tr>
<tr>
<td>11F + 12R</td>
<td>50°C</td>
<td>23</td>
<td>764</td>
<td>β-Actin</td>
</tr>
</tbody>
</table>

Description of the primers is listed in Table 1. Annealing temperature is that used in PCR. ORF, open reading frame of the total population of the biotin transporter mRNA. Primers 11F and 12R were obtained from Clontech.

**C607 MOLECULAR ASPECTS OF INTESTINAL BIOTIN TRANSPORT**

![Downloaded from http://ajpcell.physiology.org/ by 10.220.33.2 on August 14, 2017](http://ajpcell.physiology.org/)
min⁻¹ in presence of Na⁺, K⁺, Li⁺, choline, Tris, and mannitol, respectively). In a related study, we examined the stoichiometry of the coupling between biotin and Na⁺ using the “activation method” (36), as described by us previously (25). In this method, the initial rate of biotin uptake was examined as a function of increasing the Na⁺ concentration in the incubation medium in the presence of 30 µg/ml valinomycin. Saturation was observed as a function of increasing Na⁺ concentration ([Na⁺]) with a Kₘ for Na⁺ of 18 mM (Fig. 4A). Values of log[Na⁺] were then plotted against logQ = log(v/Vₘ₅), where v is the initial rate of biotin uptake at a given Na⁺ concentration, and Vₘ₅ is the maximal uptake velocity (Fig. 4B). The Hill coefficient was then calculated and found to be 1.3, suggesting a coupling ratio between biotin and Na⁺ of 1:1.

We also determined the kinetic parameters of the induced biotin uptake in COS-7/cDNA cells. This was performed by examining biotin uptake as a function of...
concentration (1–100 µM) by COS-7/cDNA and COS-7/vector cells. Uptake of [3H]biotin by the induced process was determined at each biotin concentration by subtracting uptake by COS-7/vector cells from the uptake by COS-7/cDNA cells (Fig. 5). Uptake of biotin by the induced process was found to be saturable as a function of concentration with an apparent K_m and V_max (calculated as described in MATERIALS AND METHODS) of 8.77 µM and 779.7 pmol·mg protein^{-1}·3 min^{-1}, respectively.

In another study, we examined the effect of the biotin structural analogs desthiobiotin, thioctic acid, diamino-biotin, biotin methyl ester, and biocytin on the induced [3H]biotin (5.2 nM) uptake in COS-7/cDNA cells. The results showed a concentration-dependent inhibition in [3H]biotin uptake by desthiobiotin, thioctic acid, and diamino-biotin with inhibition constant (K_i) values (determined by the “Dixon” method) of 16.3, 59.7, and 114.1 µM, respectively (Fig. 6). In contrast, biocytin and biotin methyl ester (100 µM each) were found to have no effect (69.04 ± 1.81, 67.36 ± 1.3, and 67.48 ± 0.46 fmol·mg protein^{-1}·3 min^{-1} for control and in the presence of biocytin and biotin methyl ester, respectively).

The effect of pantothenic acid and its structural analogs on the induced uptake of [3H]biotin (5.2 nM) in COS-7/cDNA cells was also tested. The results showed a concentration-dependent inhibition in [3H]biotin uptake by unlabeled pantothenic acid; with the use of the Dixon method, this inhibition was found to be competitive in nature with a K_i value of 4.9 µM (Fig. 7).

Similarly, structural analogs of pantothenic acid, namely dl-pantoyltaurine, d-pantethine, and pantethenyl alcohol (all at 100 µM) caused significant inhibition in [3H]biotin (5.2 nM) uptake [73.08 ± 0.5, 44.94 ± 4.46 (P < 0.01), 42.78 ± 2.59 (P < 0.01), 30.97 ± 1.02 (P < 0.01), and 34 ± 1 (P < 0.01) pmol·mg protein^{-1}·3 min^{-1} for control and in the presence of dl-pantoyltaurine, d-pantethine, and pantethenyl alcohol, respectively]. In a related study, we examined the uptake of the [14C]pantothenic acid (1.82 µM) by COS-7/cDNA cells and compared the results to uptake by control (COS-7/vector) cells. The results showed an approximately fivefold higher pantothenic acid uptake in COS-7/cDNA cells compared with control cells [32.6 ± 0.6 and 6.55 ± 0.12 (P < 0.01), pmol·mg protein^{-1}·3 min^{-1}, respectively]. The effect of Na^+ removal from the incubation medium on the induced pantothenic acid uptake by COS-7/cDNA cells was also examined as described earlier. The results showed significant (P < 0.01) inhibition for both K^+ and Li^+ in pantothenic acid uptake on Na^+ removal from the incubation medium (31.79 ± 0.57, 3.87 ± 1.2, 1.75 ± 0.77 pmol·mg protein^{-1}·3 min^{-1} in the presence of Na^+, K^+, and Li^+, respectively). We also examined the effect of unlabeled biotin (100 µM) and its structural analog thioctic acid (100 µM) on induced uptake of [14C]pantothenic acid (1.82 µM) by COS-7/cDNA cells. The results showed significant (P < 0.01 for both) inhibition in pantothenic acid uptake by both compounds (32.17 ± 0.54, 1.39 ± 0.13, and 1.26 ± 0.22 pmol·mg protein^{-1}·3 min^{-1} for control and in the presence of unlabeled biotin and thioctic acid, respectively).

Tissue distribution of the different 5' UTR variants of the cloned intestinal cDNA. Distribution of the different variants (II-IV) of the cloned intestinal cDNA and that of SMVT (variant I) along the length of the intestinal tract was examined in this study using RT-PCR. The
Fig. 4. Uptake of biotin uptake by induced system in COS-7/cDNA cells as a function of Na⁺ concentration in incubation medium. Cells were incubated for 3 min at 37°C in Krebs-Ringer buffer, pH 7.4, containing 30 µg/ml valinomycin. Na⁺ was isosmotically replaced by K⁺. Values are means ± SE of 4–5 separate uptake determinations. A: Initial rate of biotin (5.2 nM) uptake as a function of Na⁺ concentration. B: Hill plot: log[Na⁺] plotted against logQ/logQ = log[v/V_max − v]), where v is initial rate of biotin uptake in presence of a given Na⁺ concentration and V_max is maximal uptake velocity. y = 1.3x − 1.57; r = 0.986.

Results showed that variant II was the predominant form expressed in rat small and large intestine, with the highest expression being in the small intestine compared with the large intestine (Fig. 8). Variant I was not detected in the small intestine but only in regions of the large intestine (Fig. 8). Variant III was detected in the colon and ileum but was absent from the duodenal/jejunal area (Fig. 8). Variant IV was not detected in any region of the small and large intestine under the PCR conditions used (35 cycles) in this study (Fig. 8); however, it was detected in the jejunal area only when more initial template and higher amplification cycles (40 cycles) were used (data not shown). In another study, we examined the distribution of mRNA species of the predominant intestinal variant, i.e., variant II, in other rat tissues. The result showed expression of this variant in many other tissues in the following order (relative to β-actin): kidney = heart > liver > skeletal muscle > brain = stomach = lung (Fig. 9). This pattern of distribution of variant II compared with the distribution of the ORF of SMVT in various rat tissues reported previously (17) shows similarities and differences, suggesting different patterns of distribution of the different variants in different tissues.

Distribution of mRNA transcripts complementary to the ORF of cloned cDNA along the intestinal vertical and longitudinal axes. The distribution of mRNA transcripts complementary to cloned cDNA along the vertical axis of the intestine, i.e., villus vs. crypt cells, was determined by RT-PCR using primers from the ORF and poly(A)⁺ RNA extracted from rat jejunal villus and crypt cells isolated by the Weiser method (39) as described by us before (24). Also examined was Na⁺-dependent uptake of biotin (5.2 nM) and D-glucose (0.171 µM) in the two cell types. The results showed that the level of mRNA transcripts complementary to the cloned cDNA (normalized to β-actin) was 2.6-fold higher in villus compared with crypt cells (Fig. 10). As to the Na⁺-dependent uptake of biotin and D-glucose, the results showed significantly (P < 0.01 for both) higher uptake for both substrates in villus compared with crypt cells. For biotin (5.2 nM), uptake was 191.33 ± 11.11 and 46.1 ± 4.26 pmol·mg protein⁻¹·min⁻¹, respectively; for glucose (0.17 µM), uptake was 3.31 ± 0.26 and 0.16 ± 0.06 pmol·mg protein⁻¹·min⁻¹, respectively.

For distribution of mRNA transcript complementary to the cloned cDNA along the longitudinal axis of the rat intestinal tract as well as the stomach and the liver, RT-PCR and poly(A)⁺ RNA extracted from specific tissues were used. The distribution was found to be similar along the length of the small intestine and colon and was higher than that in the liver and stomach (Fig. 11).

**DISCUSSION**

Compared with our current understanding of the mechanism and regulation of the intestinal biotin absorption process at the tissue, cellular, and membrane levels, little is known about the molecular characteristics of the absorption process. Such information is crucial for detailed understanding of the mechanism and regulation of the absorption process of this essential micronutrient in the intestine under normal physiological conditions and on how certain conditions affect
the absorption process. In this study, we used the method of RT-PCR and knowledge obtained from the recently cloned placental SMVT cDNA to address this issue. Our study has identified a cDNA clone that has an ORF identical to that of SMVT but that displayed significant heterogeneity at the 5' UTR. Three new variants (II, III, and IV) were identified in the small intestine, with variant I (the placental form) being absent in the small gut. The existence of multiple variants suggests possible involvement of alternative splicing of the transcript; it also suggests possible involvement of multiple promoters in driving transcription of the cloned intestinal cDNA. The latter suggestion is based on a substantial number of reports linking multiplicity of promoters with heterogeneity at the 5' UTR (15, 18, 33). Of the different 5' UTR variants identified in the intestine, variant II was found to be the predominant form and has a higher expression in the small intestine compared with the large intestine. This variant was also found to be expressed in other rat tissues including the kidney and the heart. Although variant I was not detected in the small intestine as mentioned earlier, some expression was found in the large intestine.

Inspection of the deduced amino acid sequence of the cloned cDNA indicates that the encoded protein carries a net positive charge of 5.4 at physiological pH, which may be important for the interaction of the polypeptide with the negatively charged biotin molecule (pK_a of biotin is 4.51). Also, multiple histidine residues were found in the deduced amino acid sequence of the predicted polypeptide, some of which may be important for the normal function of the intestinal biotin uptake process as suggested by previous studies in our laboratory with group-specific reagents (22). Further studies with site-directed mutagenesis are required to test this possibility.

To confirm the functional identity of the cloned intestinal cDNA as a biotin transporter, we stably transfected COS-7 cells with this cDNA and examined biotin transport activity. The results showed that, compared with control cells, biotin transport in the transfected cell (i.e., COS-7/cDNA cells) was significantly higher. The induced biotin uptake in COS-7 cells
was found to be $\text{Na}^+$-dependent, saturable as a function of concentration ($K_m$ of 8.77 $\mu M$), and inhibited by biotin structural analogs with a free carboxyl group in the valeric acid moiety but not by those analogs with a blocked carboxyl group. Furthermore, the induced biotin uptake in COS-7/cDNA cells was significantly inhibited by pantothenic acid and its structural analogs. Moreover, uptake of pantothenic acid itself was found to be significantly induced in COS-7/cDNA cells compared with control cells. This induced uptake of pantothenic acid was also found to be $\text{Na}^+$-dependent and was inhibited by unlabeled biotin and its structural analog thioctic acid. These findings on biotin transport and the interaction with pantothenic acid are all similar to those observed with intact intestinal epithelial cells (19). This includes an apparent $K_m$ of the induced biotin uptake in COS-7 cells similar to that of the native rat intestine of 7.57 $\mu M$ (28). These observations support the conclusion that the intestinal cDNA cloned in this study may be involved in the normal intestinal absorption process of biotin. The ability of pantothenic acid to interact with the biotin intestinal transport process has also been observed in other cells and tissues such as the blood-brain barrier and heart and was also demonstrated for placental SMVT (2, 6, 16, 17, 34).

The distribution of mRNA transcripts complementary to the ORF (open reading frame) of cloned intestinal cDNA along vertical axis of small intestine. Further studies are needed to clarify this issue. The identification of mRNA transcripts complementary to the cloned intestinal cDNA, a discrepancy in expression compared with functional transport activity was observed. While biotin transport activity is known to be higher in the proximal small intestine compared with the ileum and the colon (19, 27, 30), a similar level of expression of mRNA complementary to the cloned intestinal cDNA was found along the length of the small intestine and colon. This paradox may suggest the involvement of a cell-specific posttranslational event(s) that regulates the expression of the functional protein in the different areas of the intestinal tract. Further studies are needed to clarify this issue. The identification of mRNA transcripts complementary to the cloned intestinal cDNA in the colon corroborates the recent finding from our laboratory (25) on the existence of a functional $\text{Na}^+$-dependent biotin carrier-mediated uptake system in the colon that may be involved in the absorption of biotin bacterially synthesized by the normal microflora of the large intestine.

In summary, these studies describe the molecular characterization of the intestinal biotin absorption process and report the identification of a cDNA that appears to be involved in $\text{Na}^+$-dependent biotin uptake. Furthermore, this cDNA appears to exist in the form of multiple variants due to heterogeneity at the 5' UTR.

N. S. Chatterjee and C. K. Kumar contributed equally to this work. We extend our thanks to Dr. Toai Nguyen, Ana-Paula E. Duarte, Erica M. Miller, Qyhn Nhu Nguyen, and Scott Smith for excellent technical help.

This study was supported by grants from the Department of Veterans Affairs and by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-56061.

Address for reprint requests and other correspondence: H. M. Said, Medical Research Service-151, Veterans Affairs Medical Center, Long Beach, CA 90822 (E-mail: hmsaid@uci.edu).

Received 28 April 1999; accepted in final form 11 June 1999.
REFERENCES


