Cellular Uptake of Biotin: Mechanisms and Regulation\textsuperscript{1,2}

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ABSTRACT  This review describes our knowledge of biotin transport in the small intestine of humans and other mammals and presents recent findings in the area. Previous studies have shown that biotin transport across the brush border membrane of the small intestinal absorptive cells occurs via a carrier-mediated, Na\textsuperscript{+} gradient-dependent, electroneutral mechanism. Exit of biotin out of the enterocyte, i.e., transport across the basolateral membrane, also occurs via a carrier-mediated process, but the process is Na\textsuperscript{+} independent and electrogenic. Recent studies from our laboratory have shown that the uptake process of biotin in Caco-2 cells, a human-derived cultured intestinal epithelial cell line, are under the cellular regulation of both a protein kinase C– and a Ca/calmodulin–mediated pathway. In addition, the uptake process is shared by another water-soluble vitamin, pantothenic acid. For the first time, other recent studies have detected the existence of a Na\textsuperscript{+}-dependent, carrier-mediated mechanism for biotin uptake at the apical membrane of colonocytes, which could theoretically mediate absorption of the biotin synthesized by colonic microflora. This system was again found to be shared by pantothenic acid, which is also synthesized by the normal microflora of the large intestine. J. Nutr. 129: 490S–493S, 1999.

KEY WORDS: • biotin • intestinal absorption • colonic transport • transport mechanism • transport regulation

Biotin is an essential micronutrient for normal cellular function, growth and development. Humans and other mammals cannot synthesize biotin and thus must obtain the vitamin from exogenous sources via intestinal absorption. After absorption, biotin is transported to different tissues and cell types for utilization. The mechanisms of biotin transport into different cell types have been recently reviewed (Dyer and Said 1997); therefore, in this paper, we will focus our presentation on new findings regarding the mechanisms and regulation of biotin transport in mammalian small and large intestine.

The intestine is exposed to biotin from two sources, the diet and the bacterially synthesized biotin in the large intestine. We will discuss current findings on transport of biotin from these two sources in the small and large intestine, respectively.

MECHANISM AND REGULATION OF BIOTIN TRANSPORT IN THE SMALL INTESTINE: ABSORPTION OF DIETARY BIOTIN

Dietary biotin has been shown to exist in free and protein-bound forms (Lampen et al. 1942). Protein-bound biotin is digested by gastrointestinal proteases and peptidases to biocytin and biotin-containing short peptides (Wolf et al. 1984). These biotin derivatives are then converted to free biotin by the action of the enzyme biotinidase (Wolf et al. 1984). Studies in our laboratory have shown that, at least in rats, this conversion step to free biotin is essential for efficient absorption and optimal bioavailability of dietary protein-bound biotin (Said et al. 1993). The mechanism of transport of free biotin in the small intestine has been studied by us and others with the use of intact intestinal tissue preparations (Brown et al. 1986, Said et al. 1987). Transport was found to occur via a specialized, carrier-mediated process which is Na\textsuperscript{+} dependent in nature (Brown et al. 1986, Said et al. 1987). These studies, however, did not tell us about the mechanism of biotin transport across the individual membrane domain of the polarized intestinal absorptive epithelial cells, i.e., the brush border and basolateral membranes. These two membrane domains have different functions, structures and permeabilities. Thus, for detailed understanding of the intestinal absorption process, knowledge about the mechanism of the individual membrane transport event is essential. To address these issues, purified brush border membrane vesicles (BBMV)\textsuperscript{3} and basolateral membrane vesicles (BLMV) were used. Studies with purified intestinal BBMV have shown biotin transport at this membrane domain to occur via a specialized, Na\textsuperscript{+} gradient-driven, carrier-mediated system (Said et al. 1987, Said and Redha)

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\textsuperscript{3} Abbreviations used: BBMV, brush border membrane vesicles; BLMV, basolateral membrane vesicles; IBMX, 3-isobutyl-1-methylxanthine; PKA, protein kinase A; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate.

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TABLE 1

Effect of modulators of PKC activity on the uptake of biotin by Caco-2 cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Uptake (μmol/L)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) of control</td>
<td></td>
</tr>
<tr>
<td>A Control</td>
<td>100 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>PMA 0.01</td>
<td>73 ± 5.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>57.5 ± 3.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>1</td>
<td>50.6 ± 4.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>10</td>
<td>48.9 ± 6.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B Control</td>
<td>100 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>sn-1,2-Dioctanoylgllycerol 1</td>
<td>62 ± 2.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Chelerythrin 7</td>
<td>119.4 ± 7.7</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Staurosporine 10</td>
<td>127 ± 6.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1 Cells were preincubated for 1 h at 37°C with the compound under study in Krebs-Ringer buffer, pH 7.4. 3H-Biotin (6 nmol/L) was added, and the initial rate of uptake, i.e., 3 min, was examined. Data are means ± SEM relative to simultaneously performed controls. PMA, phorbol 12-myristate 13-acetate.

1988, Said et al. 1988, Said and Derweesh 1991). Furthermore, the transport process was found to be electroneutral in nature, suggesting a coupling ratio of biotin to Na⁺ of 1:1 (Said et al. 1987, Said and Redha 1988, Said and Derweesh 1991). Exit of biotin from the intestinal absorptive epithelial cells across the basolateral membrane was examined by using purified intestinal BLMV and found to be via a Na⁺-independent, carrier-mediated system (Said et al. 1988, Said 1991).

The involvement of a carrier-mediated system in the intestinal absorption process of biotin has been found in different species including humans, rats and rabbits (Said et al. 1987, Said and Redha 1988, Said and Derweesh 1991). Therefore, the previous notion that species variation may exist in the mechanism of intestinal transport of biotin (Spencer and Brody 1964) is no longer supported.

Regional differences in the ability of the small intestine to absorb biotin have been observed and were found to vary, depending on the developmental stage of the intestine. In the intestine of adult humans and rats, biotin uptake was found to be higher in the duodenum that in the ileum (Said and Redha 1987, Said et al. 1988). This difference was found to be due to differences in the Vₘₐₓ of the biotin uptake process and not the apparent Kₘ (Said et al. 1988), suggesting a higher carrier density/activity in the proximal compared with the distal small intestine.

The transport process of biotin in the small intestine was found to be adaptively regulated by the extracellular substrate levels (Said et al. 1989). Biotin deficiency was found to lead to a specific and significant up-regulation in the substrate intestinal uptake compared with controls. On the other hand, biotin supplementation at pharmacologic amounts leads to a specific and significant down-regulation in the biotin uptake process. These changes were mediated through changes in the Vₘₐₓ but not the apparent Kₘ of the biotin uptake process, suggesting that the effect was mediated through changes in the number/activity and not the apparent affinity of the biotin uptake system.

In recent studies in our laboratory, we have focused on possible regulation of the biotin uptake process in the small intestine by specific intracellular protein kinase–mediated pathways. We used the human-derived cultured intestinal epithelial cells, Caco-2, as an in vitro model system for the enterocyte. We used Caco-2 cells because previous studies have demonstrated that these cells serve as a good in vitro model system for studying the finer details of the biotin intestinal uptake process, i.e., this cell line possesses a biotin uptake mechanism similar to that of the native enterocyte (Ma et al. 1994). We found that pretreatment of these cells for 1 h with the protein kinase C (PKC) activator phorbol 12-myristate 13-acetate (PMA) led to a concentration-dependent inhibition in biotin uptake by Caco-2 cells (Table 1). On the other hand, pretreating the cells with 4α-PMA, the negative control of PMA, did not affect biotin uptake, demonstrating the specificity of PMA action on PKC (Table 1). Similarly, pretreatment of cells with the PKC activator 1,2-dioctanoylglycerol caused a significant (P < 0.01) inhibition in biotin uptake (Table 1). In contrast, pretreatment of Caco-2 cells with the PKC inhibitors staurosporine and chelerythrin led to a small, but significant stimulation in biotin uptake (Table 1).

The mechanism through which PMA pretreatment, i.e., PKC activation, inhibits biotin uptake is not known, but the inhibition appears to be mediated through a decrease in the number/activity, but not the affinity of the functional biotin uptake carriers. This suggestion is on the basis of the observation that PMA pretreatment leads to a marked decrease in the Vₘₐₓ, but not the apparent Kₘ of the biotin uptake process, i.e., Vₘₐₓ was 255 ± 33.3 and 157.6 ± 15.3 pmol/(mg protein × 3 min); apparent Kₘ was 11.7 ± 3.1 and 10.4 ± 1.5 μmol/L for control and PMA-pretreated cells, respectively (Fig. 1).

We also investigated the role of Ca²⁺/calmodulin and protein kinase A (PKA)–mediated pathways in the regulation of intestinal biotin uptake. Our results showed that inhibition of Ca²⁺/calmodulin–mediated pathway by calmidazolium and trifluoperazin led to a concentration-dependent inhibition in biotin uptake (Table 2). The mechanism through which calmidazolium inhibits biotin uptake is not clear but appears again to be mediated mainly via a decrease in the number/activity of the biotin uptake carriers, with less changes in their affinity. This suggestion is on the basis of the observation that calmidazolium (50 μmol/L) pretreatment led to a marked

![FIGURE 1](https://example.com/figure1.png)
**TABLE 2**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Uptake (% of control)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Control</td>
<td>100 ± 2.2 (8)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Calmidazolium</td>
<td>86.3 ± 3.0 (6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>25</td>
<td>66.9 ± 2.5 (6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>50</td>
<td>58.8 ± 3.7 (9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>100</td>
<td>46.2 ± 2.0 (6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>B Control</td>
<td>100 ± 4.0 (6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Trifluoperazine</td>
<td>70.3 ± 4.0 (3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>100</td>
<td>51.3 ± 0.5 (3)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

1 See Table 1, footnote 1.

A decrease in the V\text{max} of the biotin uptake process, with slight change in the apparent K\text{m}. V\text{max} was 214.27 ± 15.18 and 94.8 ± 5.4 pmol/(mg protein · 3 min); apparent K\text{m} was 12.2 ± 2 and 9.2 ± 1.4 μmol/L for control and calmidazolium-pretreated cells, respectively [Fig. 2]. We also tested the effect of simultaneous pretreatment of Caco-2 cells with PMA (1 μmol/L) and calmidazolium (50 μmol/L) on biotin uptake. The results showed additive inhibition in biotin uptake by such treatment; expressed as a percentage relative to simultaneously performed controls, uptake rates were 100 ± 6.5, 72.7 ± 2, 59.6 ± 9.9 and 28.9 ± 12.3 for control and in the presence of PMA, calmidazolium and PMA plus calmidazolium, respectively. No role for PKA-mediated pathway(s) in the regulation of biotin transport was evident as indicated by the lack of effect of modulators of that pathway on biotin uptake; percentage of control was 100 ± 3, 107.5 ± 6.2 and 97.7 ± 6 for control and in the presence of 1 mmol/L 3-isobutyl-1-methyl-xanthine (IBMX), 0.5 mmol/L dibutyryl cAMP, 0.1 mmol/L forskolin and 1.6 μg/mL cholera toxin, respectively.

We recently observed that the biotin transport system of intestinal epithelium interacts with pantothenic acid. Pantothenic acid caused a concentration-dependent inhibition in biotin uptake by confluent Caco-2 monolayers; the Dixon plot (Fig. 3) revealed a competitive inhibition with an inhibition constant (K\text{I}) of 12.5 μmol/L. This K\text{I} for pantothenic acid is very similar to the apparent K\text{m} of biotin uptake by these cells of 11.7 μmol/L. This suggests that the two vitamins may share a common membrane transport system in the small intestine. Furthermore, efflux of 3H-biotin from Caco-2 cells preloaded with 3H-biotin was enhanced by the presence of pantothenic acid (100 μmol/L) in the incubation medium as indicated by the significant (P < 0.01) decrease in the amount of biotin retained by these cells compared with cells incubated in the absence of pantothenic acid, i.e., retained 3H-biotin was 80.4 ± 0.9 and 117 ± 3.4 fmol/mg protein, respectively. Biotin also inhibits the uptake of pantothenic acid by these cells (data not shown), supporting the above interpretation. This type of interaction between biotin and pantothenic acid has been seen previously in the other tissues such as the blood-brain barrier (Spector and Mock 1987), the heart (Beinlich et al. 1990) and the placenta (Grassl 1992). The physiologic and nutritional implications of this interaction deserve further investigation.

**FIGURE 2**  Effect of pretreatment of Caco-2 cells with calmidazolium on biotin uptake as a function of concentration. Legend is as in Figure 1 except that calmidazolium (50 μmol/L) was used instead of phorbol 12-myristate 13-acetate (PMA).

**FIGURE 3** Dixon plot of the effect of pantothenic acid on biotin uptake by Caco-2 cells. Caco-2 cells were incubated at 37°C in Krebs-Ringer buffer, pH 7.4, containing 1 and 5 μmol/L 3H-biotin and different concentrations of pantothenic acid. 3H-Biotin uptake was measured after a 3-min incubation (i.e., initial rate). Data are means of three separate uptake determinations. For 1 μmol/L biotin, y = 0.71x + 0.92, r = 0.99; for 5 μmol/L biotin, y = 0.34x + 2.07, r = 0.99.

**MECHANISM OF TRANSPORT OF THE BACTERIALLY SYNTHESIZED BIOTIN IN THE LARGE INTESTINE AND ITS CELLULAR REGULATION**

It has long been recognized that the normal microflora of the large intestine synthesize considerable amounts of biotin and that a significant portion of this biotin exists in the free absorbable form (Burkholder and McVeigh 1942, Wrong et al. 1981). In vivo studies in humans, rats and minipigs have shown that the colon is indeed capable of absorbing considerable amounts of luminal biotin (Barth et al. 1986, Brown and Rosenberg 1987, Sorrell et al. 1971). Very little is known, however, about the transport mechanism involved and its cellular regulation. Using the human-
derived, nontransformed colonic epithelia cell line NCM460 (Kumar et al. 1997, Moyer et al. 1996), we have recently performed studies to address these issues (Said et al. 1997). Our results showed that biotin uptake by these cells is Na⁺ dependent in nature as indicated by the significant inhibition in biotin uptake when Na⁺ in the incubation medium is replaced with other monovalent cations or with mannitol. Na⁺ dependence was further confirmed by the finding that ouabain, an inhibitor of Na⁺-K⁺ ATPase, causes a significant inhibition in biotin (Said et al. 1997). Initial rate of biotin uptake as a function of concentration was found to include a saturable component with an apparent \( K_m \) of 19.7 ± 3.1 \( \mu \)mol/L and a \( V_{max} \) of 38.8 ± 1.9 pmol/(mg protein \cdot 3 min), respectively (Said 1991). Furthermore, uptake of \(^3\)H-biotin was found to be significantly inhibited by structural analogs with a free carboxyl group at the valeric acid moiety (such as thioctic acid and desthiobiotin), but not affected by biocytin, an analog with a blocked carboxyl group at this moiety. Collectively, these findings suggest the involvement of a specific, Na⁺-dependent, carrier-mediated system for biotin uptake by colonic epithelial cells NCM460.

After the characterization of the mechanism of biotin uptake by the human-derived colonic epithelial cells NCM460, we investigated possible regulation of the uptake process by specific intracellular protein kinase–mediated pathways (Said et al. 1997). Our results again showed that pretreatment of cells with the PKC activators PMA and with 1,2-dioctanoylglycerol led to a significant inhibition in biotin uptake. Again the effect appeared to be mediated mainly via a decrease in the \( V_{max} \) of the biotin uptake process (i.e., a decrease in the number/activity of the biotin uptake carriers) with less changes in its apparent \( K_m \) (i.e., less change in its affinity). On the other hand, no role for a PKA-mediated pathway in the regulation of biotin uptake was observed as indicated by the lack of significant effect on biotin uptake by pretreatment of NCM460 cells with modulators of this pathway (Said et al. 1997).

Similar to the findings with Caco-2 cells, biotin uptake by the NCM460 cells was also found to be inhibited in a concentration-dependent manner by pantothenic acid (unpublished observations). This observation is of potential nutritional significance because pantothenic acid is also synthesized in considerable amounts by the normal microflora of the large intestine (Wrong et al. 1981). Again, the inhibition in biotin uptake by pantothenic acid was found from the Dixon plot to be competitive in nature; the \( K_i \) of inhibition was similar to the apparent \( K_m \) of the biotin uptake process by these cells. This suggested that the two vitamins utilize the same uptake carrier in these colonic cells. This suggestion was further supported by the finding that biotin also inhibited the uptake of pantothenic acid by these cells (unpublished observations).

**ACKNOWLEDGMENTS**

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**LITERATURE CITED**


