Movement of biotin across the rat intestinal basolateral membrane

Studies with membrane vesicles

Hamid M. SAID*

Medical Research Service, Department of Veterans' Affairs, Long Beach, CA 90822,
and Departments of Medicine and Physiology/Biophysics, University of California School of Medicine, Irvine, CA 92717, U.S.A.

The characteristics of the transport process of the water-soluble vitamin biotin across the basolateral membrane (BLM) of rat intestine, i.e. the exit of biotin from rat enterocyte, were examined using established BLM vesicles (BLMV) techniques. Results of osmolarity and temperature studies have indicated that the majority of biotin taken up by these vesicles is the result of transport of the vitamin into the intravesicular spaces, with little membrane binding. Transport of biotin in these vesicles was found to be: (1) higher in BLMV of the jejunum than those isolated simultaneously from the ileum, (2) Na^+ -independent in nature, (3) saturable as a function of concentration, with an apparent K_m of $3.51 \mu\text{M}$ and a V_{max} of $16.49 \text{ pmol/10 s per mg of protein}$, (4) inhibited by high concentrations of unlabelled biotin and its related compounds, but not by the unrelated taurocholate, (5) sensitive to the anion transport inhibitors 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid (DIDS) and 4-acetamido-4-isothiocyanostilbene-2,2'-disulphonic acid (SITS), and (6) not affected by imposing a positive or a negative intravesicular space with the use of a valinomycin-induced K^+ diffusion potential. Based on these findings, it is concluded that biotin transport across the rat intestinal BLM is mediated via a specialized carrier system which is Na^+ -independent, transports the vitamin by a process which is sensitive to the effects of anion transport inhibitors, and is electroneutral in nature.

INTRODUCTION

The water-soluble vitamin biotin is essential for normal cellular functions and growth. In addition to being an important co-enzyme for four carboxylases that are involved in key metabolic reactions [1], biotin has been shown to be involved in the regulation of gene expression of certain membrane receptors [2] and in the regulation of the level of the intracellular messenger cyclic GMP [3].

Mammals cannot synthesize biotin; therefore they must obtain the vitamin via intestinal absorption. Using the rat as an experimental animal model, we have previously characterized the mucosal-to-serosal absorption process of biotin, i.e. transport of the vitamin across the polarized enterocyte, using intestinal everted sacs [4,5]. The mucosal-to-serosal absorption process, however, represents transport of biotin across two different membrane domains: the brush border membrane (BBM) domain and the basolateral membrane (BLM) domain [6]. To understand the details of the individual transport events, we subsequently used purified BBM vesicles (BBMV) to characterize the transport process of biotin across the BBM, i.e. the entry of biotin into the enterocyte across the apical membrane, and demonstrated the existence of a carrier-mediated Na^+ -gradient-driven transport system [7]. Furthermore, we found this system to be regulated by the level of the vitamin in the diet [8]. More recent studies in our laboratory have identified certain molecular features of the BBM biotin transporter and demonstrated the existence of a histidyl residue(s) at (or near) the substrate-binding site which is essential for the function of this system [9]. In contrast to our current understanding of the mechanism, regulation and identity of the biotin transport process across the BBM, our knowledge is quite limited regarding the regulation and molecular identity of the biotin transport process across the BLM. For these studies to be performed, a suitable animal model is required, and the mech-

anism of biotin transport across the intestinal BLM of this animal model needs to be established. Thus the aim of the present study was to characterize the mechanism of biotin transport across the intestinal BLM of the rat, an animal model which, as stated above, has been used to characterize other aspects of biotin intestinal transport. I used a well-validated BLM vesicles (BLMV) technique [10–13] in the present studies.

MATERIALS AND METHODS

Materials

[8,9- $^3\text{H}(\text{U})$]Biotin (sp. radioactivity $> 35 \text{ Ci/mmol}$; radiochemical purity $> 95\%$) was purchased from New England Nuclear, Boston, MA, U.S.A. Cellulose nitrate filters ($0.45 \mu\text{m}$ pore size) were obtained from Sartorius, Hayward, CA, U.S.A. Percoll was obtained from Pharmacia Chemicals, Piscataway, NJ, U.S.A. All other chemicals and reagents were obtained from commercial sources and were of analytical quality.

Methods

Intestinal BLMV were isolated from the intestines of Sprague-Dawley rats using the self-generating Percoll gradient methodology of Scalera *et al.* [10], with minor modifications. We have previously used this method in transport studies [13] and demonstrated the purity and functional suitability of the isolated BLMV by morphological (electron microscopy), enzymic (enrichment and depurification of marker enzymes in the final vesicular preparations compared with initial mucosal homogenates) and functional (transport of two model substrates, D-glucose and Ca^{2+}) criteria (details of the isolation procedure can be found in [13]). Following isolation, BLMV were suspended in a buffer (transport buffer) of 280 mM-mannitol and 20 mM-Hepes/Tris , pH 7.4 (changes in this buffer are stated in the text) and preincubated for 1 h at room temperature to load the inside

Abbreviations used: BBM, brush border membrane; BBMV, BBM vesicles; BLM, basolateral membrane; BLMV, BLM vesicles; DIDS, 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid; SITS, 4-acetamido-4-isothiocyanostilbene-2,2'-disulphonic acid.

* Address all correspondence to the author at: Medical Research Service (151), Veterans' Administration Medical Center, 5901 East Seventh Street, Long Beach, CA 90822, U.S.A.

of the vesicles. The volume of the vesicles was estimated to be $6.7 \mu\text{l}/\text{mg}$ of protein, which is similar to that reported by others [14]. Transport studies were performed at 37°C by a rapid-filtration technique [15] as described previously [7,8,13]. The reaction was started by rapid mixing of $20 \mu\text{l}$ of vesicle suspension with $80 \mu\text{l}$ of incubation buffer, which usually contained 100 mM -NaCl or -KCl , 80 mM -mannitol and 20 mM -Hepes/Tris, pH 7.4, plus biotin (labelled and unlabelled) and other constituents. At the end of the incubation the reaction was terminated by the addition of 1 ml of ice-cold stop solution (100 mM -NaCl, 100 mM -mannitol and 10 mM - KH_2PO_4 , pH 7.4). The mixture was immediately vortex-mixed, filtered on a vacuum many times and washed with 5 ml of ice-cold stop solution, a procedure universally used in such studies [16]. The filter was then transferred into a scintillation vial; 7 ml of scintillation fluid was added and the radioactivity was counted in a Beckman liquid scintillation counter. Protein concentrations in membrane suspensions were measured by the method of Lowry *et al.* [17] using BSA as the standard. As seen before with this method of preparation [13], slight quantitative variations in the absolute amount of biotin transported were noticed in some BLMV preparations. For this reason, appropriate controls were run simultaneously with each set of experiments using the same vesicular suspension to allow for proper comparison of the effects of different factors and conditions on biotin transport. All results are presented as means $\pm$ S.E.M. (pmol/unit time per mg of protein) of multiple transport measurements with vesicles from at least four rats.

RESULTS

General characteristics of biotin uptake by rat intestinal BLMV

Uptake of biotin by BLMV prepared from rat jejunum was found to be mostly the result of transport of the vitamin into an osmotically sensitive intravesicular space, with little binding to membrane surfaces. This finding is based on the results of the osmolarity study depicted in Fig. 1. In this study, biotin uptake was examined after a 60 min incubation (i.e. at equilibrium; see below) as a function of increasing the osmolarity of the incubation medium by the use of mannitol. As can be seen in Fig. 1, an inverse and linear ($r = 0.97$) correlation was found between biotin uptake and incubation medium osmolarity. Extrapolating the uptake line to infinite osmolarity intercepted the y-axis at $0.031 \text{ pmol/h per mg of protein}$, indicating that, at equilibrium and under iso-osmotic conditions, biotin uptake is mostly (93.7%) the result of transport of the substrate into the intravesicular space, with little (6.3%) binding to membranes. This conclusion was further supported by the finding that the initial rate of uptake of $0.079 \mu\text{M}$ -biotin (10 s, see below) was significantly higher ($P < 0.01$) at 37°C than at 4°C [uptake values of 0.428 ± 0.070 ($n = 6$) and 0.201 ± 0.023 ($n = 5$) $\text{pmol}/10 \text{ s per mg of protein}$ respectively].

In another study, I examined and compared biotin transport ($0.079 \mu\text{M}$) in BLMV isolated from the jejunum and those isolated simultaneously from the ileum of the same rats. The results showed that biotin transport is significantly greater ($P < 0.01$) in BLMV isolated from the jejunum than in those isolated from the ileum (0.285 ± 0.038 and $0.086 \pm 0.012 \text{ pmol}/10 \text{ s per mg of protein}$ respectively, $n = 8$).

Transport of biotin in BLMV as a function of time and concentration

Transport of $0.079 \mu\text{M}$ -biotin was examined as a function of time in the presence of an inwardly directed Na^+ or K^+ gradient (outside = 100 mM , inside = 0 mM). Biotin transport increased with time in a linear fashion during the initial 10 s of incubation, levelled off between 10 and 20 s and only slightly increased

thereafter (Fig. 2). At all time points examined, biotin transport was found to be similar in the presence of either a Na^+ or a K^+ gradient. Transport of a high concentration of biotin ($10 \mu\text{M}$) was also found to be similar in the presence of either a Na^+ or a K^+ gradient, and was linear for up to 12 s of incubation [in the presence of a Na^+ gradient, transport was 3.93 ± 0.49 ($n = 4$), 6.98 ± 0.64 (4), 8.28 ± 1.16 (4) and 15.0 ± 2.2 (5) pmol/mg of protein at 3, 6, 9 and 12 s respectively; in the presence of a K^+ gradient, transport was 4.84 ± 0.83 (4), 7.13 ± 0.69 (5), 9.07 ± 0.84 (5) and 15.04 ± 2.14 (5) pmol/mg of protein at 3, 6, 9 and 12 s respectively]. I adopted a 10 s incubation time in all subsequent studies, as biotin transport is linear during this time.

In another study, I examined the transport of biotin in jejunal BLMV as a function of increasing the substrate concentration (0.079 – $10 \mu\text{M}$) in the incubation medium. Incubation was performed for 10 s at 37°C . The results (Fig. 3) showed clear

Fig. 1. Uptake of biotin by rat jejunal BLMV as a function of increasing the osmolarity of the incubation medium

BLMV were preloaded with a buffer containing 280 mM -mannitol and 20 mM -Hepes/Tris, pH 7.4. Incubation was performed at 37°C for 1 h in a buffer of 100 mM -NaCl and 20 mM -Hepes/Tris, pH 7.4, containing variable amounts of mannitol to give the indicated osmolarity. Biotin ($0.079 \mu\text{M}$) was added to the incubation medium at the start of the experiments. Results are means $\pm$ S.E.M. of four to seven separate transport measurements in BLMV from four rats. $y = 0.147x + 0.031$; $r = 0.95$.

Fig. 2. Transport of biotin in rat jejunal BLMV in the presence of Na^+ and K^+ gradients as a function of time

BLMV were preloaded with a buffer of 280 mM -mannitol and 20 mM -Hepes/Tris, pH 7.4, and incubated at 37°C in a buffer of 100 mM -NaCl (■) or -KCl (□), 80 mM -mannitol and 20 mM -Hepes/Tris, pH 7.4. Biotin ($0.079 \mu\text{M}$) was added to the vesicle suspension at the start of the experiments. Results are means $\pm$ S.E.M. of four to six separate transport measurements in BLMV from four rats.

Fig. 3. Initial rate of biotin transport as a function of concentration in rat jejunal BLMV

BLMV were preloaded with a buffer of 280 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. Incubation was carried out in a buffer of 100 mM-NaCl, 80 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. Different concentrations of biotin were added to the incubation medium at the start of the experiments and incubation was carried out at 37 °C for 10 s. Results are means $\pm$ S.E.M. of eight to twelve separate transport measurements in BLMV from eight rats.

Table 1. Effects of unlabelled biotin, its related compounds, the unrelated taurocholate and the anion transport inhibitors DIDS and SITS on the transport of [3 H]biotin in rat jejunal BLMV

BLMV were preloaded with a buffer of 280 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4, and incubated at 37 °C in a buffer of 100 mM-NaCl, 80 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. [3 H]Biotin (0.079 μ M) and the compound under investigation were added to the incubation medium at the start of the experiments, and incubation was performed for 10 s. Numbers of transport measurements from at least four rats are given in parentheses. Significance was estimated using Student's *t* test; **P* < 0.01 versus control.

Compound	Transport (pmol/10 s per mg of protein)
Control	0.387 $\pm$ 0.036 (14)
Unlabelled biotin (25 μ M)	0.175 $\pm$ 0.011 (6)*
Desthiobiotin (25 μ M)	0.186 $\pm$ 0.019 (6)*
Biotin methyl ester (25 μ M)	0.179 $\pm$ 0.020 (7)*
Thioctic acid (25 μ M)	0.165 $\pm$ 0.008 (7)*
Taurocholate (50 μ M)	0.389 $\pm$ 0.075 (6)
DIDS (1 mM)	0.162 $\pm$ 0.012 (8)*
SITS (1 mM)	0.140 $\pm$ 0.009 (7)*

saturation in biotin transport, indicating the existence of a carrier-mediated mechanism. The kinetic parameters of this saturable process were calculated using a computerized model of the Michaelis-Menten equation as described by Wilkinson [18], and were found to be 3.51 μ M and 16.49 pmol/10 s per mg of protein for the apparent K_m and the V_{max} , respectively.

Effects of unlabelled biotin, its analogues and anion transport inhibitors on the transport of [3 H]biotin

To confirm the existence of a carrier-mediated system for biotin transport across the rat jejunal BLM, I examined the effect of adding to the incubation medium 25 μ M unlabelled biotin or its related compounds desthiobiotin, biotin methyl ester and thioctic acid, as well as the effect of the unrelated compound taurocholate (50 μ M), on the transport of 0.079 μ M-[3 H]biotin in jejunal BLMV. Incubation was performed for 10 s at 37 °C. The

results (Table 1) showed that unlabelled biotin and its related compounds, but not the unrelated taurocholate, cause significant inhibition of the transport of [3 H]biotin, confirming the existence of a specialized carrier-mediated mechanism.

I also examined the effects of the anion transport inhibitors 4,4'-di-isothiocyanatostilbene-2,2'-disulphonic acid (DIDS) and 4-acetamido-4-isothiocyanatostilbene-2,2'-disulphonic acid (SITS), at a concentration (1 mM) previously shown to inhibit anion transport [19], on the transport of biotin (0.079 μ M) into rat jejunal BLMV. The results (Table 1) showed that both inhibitors cause significant (*P* < 0.01) inhibition of biotin transport compared with simultaneously performed controls.

Effect of transmembrane electrical potential on biotin transport

In this study I examined the effect of inducing a positive or negative transmembrane electrical potential, using well-established valinomycin-induced K⁺-diffusion potential methodology [20–23], on the transport of biotin (0.079 μ M) in rat jejunal BLMV. The results with these two conditions were compared with those from simultaneously performed voltage clamp experiments. The ability of this method to induce the desired changes in transmembrane electrical potential has been shown previously in our laboratory and others [20–23]. Induction of a positive intravesicular space was done by preloading the vesicles with a buffer of 280 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4, and incubating the vesicles in a buffer of 90 mM-NaCl, 50 mM-potassium gluconate and 20 mM-Hepes/Tris, pH 7.4. Induction of a negative intravesicular space was done by preloading the vesicles with a buffer of 50 mM-potassium gluconate, 180 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4, and incubating the vesicles in a buffer of 90 mM-NaCl, 100 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4. In the voltage clamp condition, the vesicles were preloaded with a buffer of 50 mM-potassium gluconate, 180 mM-mannitol and 20 mM-Hepes/Tris, pH 7.4, and incubated in a buffer of 90 mM-NaCl, 50 mM-potassium gluconate and 20 mM-Hepes/Tris, pH 7.4. Under all conditions, valinomycin (10 μ g/mg of protein) and [3 H]biotin (0.079 μ M) were added to the incubation medium at the start of incubation. The results showed that the induction of either a positive or a negative intravesicular space did not affect biotin transport compared with the voltage clamp condition [0.239 $\pm$ 0.029 (*n* = 16), 0.261 $\pm$ 0.039 (9) and 0.257 $\pm$ 0.030 (16) pmol/10 s per mg of protein respectively]. These results suggest that transport of the anionic biotin in these vesicles is most probably electroneutral in nature.

DISCUSSION

The present study examined biotin transport across the BLM of rat intestine using BLMV preparations isolated by an established Percoll density-gradient methodology [10]. The purity and functional suitability of BLMV isolated by this procedure has been demonstrated previously by us and others [10–13]. Uptake of biotin by rat BLMV was found by osmolarity and temperature studies to be mostly the result of transport of the vitamin into the inside of vesicles, with little binding to the BLM itself. When transport of biotin was examined in BLMV isolated simultaneously from the jejunum and ileum of the same rats, significantly higher biotin transport was found in the former intestinal segment compared with the latter. This finding corresponds well with previous findings with intact intestinal tissue preparations [4,5] as well as with BBMV [7], which showed biotin transport in adult rats to be significantly higher in the jejunum than in the ileum.

Unlike the transport of biotin across the BBM of the rat enterocyte, which was found to be Na⁺-gradient-dependent and

capable of accumulating the substrate against a concentration gradient (as demonstrated by the 'overshoot' phenomenon) [7], biotin transport across the rat BLM was found in the present study to be Na^+ -independent and incapable of moving the substrate against a concentration gradient in the intravesicular spaces. These results also allow the conclusion that the accumulation of biotin against a concentration gradient observed in the intact tissue preparation [4] is the work of the BBM transporter.

The transport of biotin in rat BLMV was found to be carrier-mediated, as indicated by the saturability of the transport process as a function of concentration. The apparent K_m of this carrier was found to be lower than that reported for the biotin transporter in the BBM domain of the rat enterocyte (reported to be in the range 7.9–9.3 μM) [7,24], indicating that the BLM transporter has a higher affinity towards its substrate than does the BBM transporter. Further confirmation for the existence of a specialized carrier-mediated system for biotin transport across the BLM was provided by the findings that unlabelled biotin and its related compounds, but not the unrelated taurocholate, caused significant inhibition of the transport of [^3H]biotin. The ability of the inhibitors of anion transport carriers, DIDS and SITS, to inhibit biotin transport lends additional support for the existence of a carrier-mediated process.

Transport of a substrate by a non-carrier-mediated process is not markedly influenced by temperature. Thus, if we assume that the data in Table 1 giving the residual transport of a physiological concentration of [^3H]biotin in the presence of high pharmacological concentrations of unlabelled biotin, biotin-related compounds, DIDS and SITS to represent the transport by the non-carrier-mediated diffusion process, and then determine the transport in the temperature study by the carrier-mediated component (total transport minus non-carrier-mediated transport), the transport at 4 °C will be approx. 0.061 pmol/10 s per mg of protein and that at 37 °C will be 0.288 pmol/10 s per mg of protein. These results indicate that the carrier-mediated transport of biotin is approx. 4.72 times greater at 37 °C than that at 4 °C, demonstrating the temperature-dependence of the carrier-mediated process.

Alteration in transmembrane electrical potential (positive or negative) by the use of an established methodology [20–23] was found to have no effect on the transport of the anionic biotin (pK_a of biotin = 4.65 [25]). This finding also indicates that, under the experimental conditions used in this study, the transport process of biotin across the rat intestinal BLM is electroneutral in nature. This finding and the above-described finding of the significant inhibition of biotin transport by DIDS and SITS raise the possibility of the involvement of an anion-exchange transport mechanism for biotin transport across this membrane domain of the rat enterocyte. Further studies, however, are required to confirm this suggestion.

The characteristics of the biotin transport process across the intestinal BLM of this animal model are similar to those reported previously across the human intestinal BLM [26] with regard to Na^+ -independence, inability to accumulate the substrate against

a concentration gradient and the involvement of a specialized carrier-mediated system in the process. It is different, however, in being insensitive to the manipulations of transmembrane electrical potential.

In summary, the present study demonstrates that biotin transport across the BLM of the rat intestine is via a carrier-mediated Na^+ -independent system which transports the substrate by an electroneutral process.

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