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Interaction of 2-Aminobicyclo[3.2.1]octane-2-carboxylic Acid with the Amino Acid Transport Systems of the Sarcoma 37 Murine Ascites Tumor Cell†

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ABSTRACT: The relatively broad and overlapping specificities of amino acid transport systems have made the synthesis of analogues specific to single transport systems desirable. The analogue in general use as a specific substrate for transport system L has been 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH). The affinity of BCH for the binding site of system L has been shown to be less than that of the natural substrate, leucine. Earlier studies from this laboratory suggested that higher homologues in a series could have greater affinity for system L. A higher homologue of BCH, 2-aminobicyclo[3.2.1]octane-2-carboxylic acid (ABOCA), has been synthesized and studied as a substrate and competitor for amino acid transport systems of the sarcoma 37 (S37) ascites cell. ABOCA inhibited the transport system dominant in the low concentration region for histidine uptake (system L) but had no effect on the uptake of labeled *N*-methyl-α-aminoisobutyric acid (MeAIB). MeAIB had no effect on labeled ABOCA uptake in S37 cells. ABOCA inhibited the uptakes of labeled leucine and labeled BCH competitively. Leucine, histidine, and BCH inhibited the uptake of labeled ABOCA competitively. Typical L system substrates demonstrated exchange effects with labeled ABOCA. The b isomer of ABOCA demonstrated slightly greater affinity for system L than did the a isomer. We conclude that ABOCA is an analogue restricted to interaction with amino acid transport system L, that it has greater affinity for system L than does BCH, and that its selection for system L is determined principally by an apolar interaction with steric considerations secondary.

It is now well established that amino acid transport into mammalian cells occurs by multiple transport systems that possess broad and overlapping specificities. Tenenhouse & Quastel (1960) first suggested the multiplicity of amino acid transport systems, and Ahmed & Scholefield (1962) showed that competitive inhibition could be used systematically to discriminate between the individual systems. This was applied to the Ehrlich ascites cell, and two systems known as A (alanine preferring) and L (leucine preferring) were described (Oxender & Christensen, 1963). The occurrence and properties of the A and L systems as they appear in the sarcoma 37 ascites cell have been studied in this laboratory (Matthews et al., 1970, 1975, 1977; Matthews, 1972; Matthews & Zand, 1977, 1979).

The functional isolation of individual transport systems has been a practical problem of importance for progress in the area of amino acid transport, and the problem has been addressed in part by the synthesis of analogues possessing more restricted specificity than that of natural substrates. In the case of

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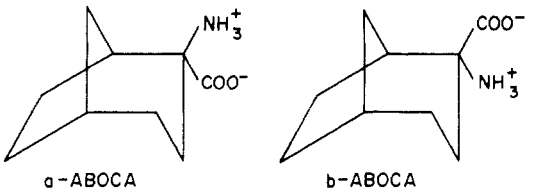

FIGURE 1: Structure of ABOCA in a and b configurations.

system L, tertiary leucine was suggested as an analogue more restricted in specificity than normal substrates (Christensen et al., 1963). Tertiary leucine was subsequently replaced with a more specific analogue, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH)[1] (Christensen et al., 1969). Although we, as others, have found BCH quite useful, one limitation has been a relatively high K_m or K_i value for its interaction with system L (Christensen et al., 1969; see data under Results). Our previous study suggested an increase in affinity for system L accompanied an increase in ring size (Matthews & Zand, 1977), and we have therefore examined the interaction of the analogue 2-aminobicyclo[3.2.1]octane-2-carboxylic acid (ABOCA) with the amino acid transport systems of the S37 cell. ABOCA is a homologue of BCH containing one additional carbon atom (Figure 1). This homologue of BCH had been utilized in earlier studies of the perturbation of nonpolar amino acid levels in brain (Zand et al., 1974) and in studies of the aminoacyl synthetase for nonpolar amino acids to accept ABOCA (Zand & Water, 1977; Water, 1976). In both of these studies, ABOCA was found to be an antagonist of nonpolar amino acids. It was therefore highly probable that ABOCA would be an effective discriminator of the L transport system, and we report herein our results supporting this activity.

Materials and Methods

The preparation of S37 cells and media, the general aspects of the design and execution of transport experiments, and the preparation of the liquid scintillator employed have been described in detail previously (Matthews & Zand, 1977, 1979).

Preparation of ABOCA. ABOCA was synthesized by a method which has been previously described (Zand et al., 1974; Water, 1976). The ketone was obtained from Aldrich Chemical Co. The ketone was then converted to the hydantoin by treating with an equimolar amount of KCN and an excess (2×) of $(NH_4)_2CO_3$ in 1:1 (v/v) ethanol:water at 60 °C for 6 h and then allowing the mixture to stand at room temperature overnight. The yield was 75%, and the melting point was 258–260 °C. The hydantoin was hydrolyzed to ABOCA by heating at 120 °C in an autoclave for 24 h in the presence of 0.7 M barium hydroxide. The amino acid was isolated after first precipitating the Ba^{2+} with $(NH_4)_2CO_3$ and CO_2 and then concentrating the mother liquor after filtration. The product was purified by recrystallization in water:ethanol 1:1 (v/v), mp 340–350 °C sublimed. Isomers of ABOCA were separated on a preparative ion-exchange column using tartrate buffer at pH 4.25 (0.2 N Na^+) according to the procedure of Tager & Zand (1970).

Preparation of [^{14}C]*a-ABOCA.*[2] The radioactive analogue was prepared by using 2.7 mg of K^{14}CN (2 mCi) and 30.26 mg of KCN (5.0×10^{-4} M total) and the procedure previously reported for the synthesis of [^{14}C]BCH (Christensen et al., 1969).

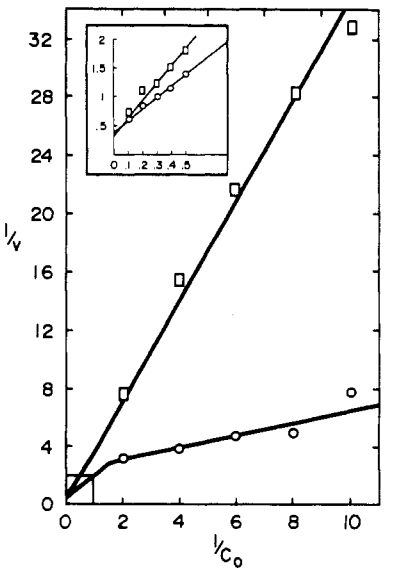

FIGURE 2: Cis inhibition of histidine transport by ABOCA. Incubations of S37 cells were conducted for 2 min at 20 °C in duplicate with varying concentrations of L-[^{3}H]histidine (C_0): control (○) and with 5 mM a-ABOCA present (□).

Results

In examining possible interactions of ABOCA with transport systems, we first tested the effect on biphasic histidine transport (Figure 2). We had previously used histidine uptake in the S37 cell as a test situation because histidine interacted with two principal transport systems, A and L, but with such disparate kinetic parameters that a double-reciprocal plot was clearly biphasic. System A dominated in the high concentration region, near the origin in the double-reciprocal plot. System L dominated in the low concentration region, farther from the origin in the double-reciprocal plot (Mattthews et al., 1970, 1975). The result of this test with a natural substrate was that the low concentration limb of the plot was greatly deflected; indeed, the plot was no longer biphasic; the plot converged with control as the origin was approached. This behavior was similar to that seen wiith other substrates preferring transport system L (Matthews et al., 1975).

We next utilized labeled ABOCA and observed concentrative uptake consistent with ABOCA behavior as a substrate rather than just as an untransported inhibitor. Cis inhibition and exchange experiments using labeled ABOCA as a substrate interacting with normal substrates yielded patterns consistent with the suggestion that ABOCA was a substrate for system L (Table I).

Interaction of ABOCA with the original defining substrate for system L, leucine, was studied in further detail, and the data (Figure 3A) showed that ABOCA is a competitive inhibitor of leucine transport and confirmed that ABOCA was a system L substrate.

It was of interest to test more rigorously the noninteraction of ABOCA with system A as suggested by the data in Figure 2. Labeled MeAIB was employed as a substrate analogue specific to system A (Christensen, 1973; Matthews et al., 1975). ABOCA at 10 times the concentration of the substrate showed no inhibition of MeAIB transport (Wilcoxon test, $p < 0.05$) (Table II). This experiment was corroborated by two broad concentration-labeled MeAIB transport experiments in which ABOCA was employed as a possible inhibitor; it showed no effect (data not shown). The converse experiment, employing ABOCA as the labeled substrate and MeAIB as the

[1] Abbreviations used: BCH, 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid; AIB, α-aminoisobutyric acid; S37, sarcoma 37 murine ascites tumor; ABOCA, 2-aminobicyclo[3.2.1]octane-2-carboxylic acid; MeAIB, *N*-methyl-α-aminoisobutyric acid.

[2] All labeled acids are labeled in the carboxyl group.

Table I: Comparison of Cis Inhibition of [^{14}C]a-ABOCA Transport by Various Amino Acids with Exchange Efflux of [^{14}C]a-ABOCA Elicited by Various Amino Acids[a]

amino acid	cis inhibition (%)	ABOCA removed by exchange (%)
Trp	86	48
Phe	86	31
Leu	80	48
His	73	55
Met	63	49
Val	37	49
Ala	7	9
Thr	6	2
Ser	2	5
Gly	−5	17
Pro	−8	16

[a] In the two cis inhibition experiments, which were averaged, incubations were for 2 min at 20 °C with 0.1 mM [^{14}C]a-ABOCA and 0.5 mM competitors (L configurations) present. In the two exchange experiments, which were averaged, S37 cells were first incubated with 0.50 mM [^{14}C]a-ABOCA 30 min at 37 °C. In a second incubation, the cells were exposed to various L-amino acids at 0.50 mM for 5 min at 20 °C.

Table II: Negligible Effect of a-ABOCA as a Cis Inhibitor for MeAIB Transport[a]

	control C_i values	rank		C_i values + a-ABOCA	rank
	0.068	4		0.061	1
	0.071	5.5		0.065	2.5
	0.071	5.5		0.065	2.5
	0.072	7		0.076	8
	0.081	10		0.080	9
mean:	0.073	$T_1 = 32$	mean:	0.069	

[a] [^{14}C]MeAIB (0.50 mM) was employed in incubations for 2 min at 20 °C. When a-ABOCA was included, it was 5.0 mM. Control and inhibited samples were alternated during incubations. In the Wilcoxon analysis shown, T_1 values between 19 and 36 show no significant difference ($p < 0.05$) (Diem, 1962).

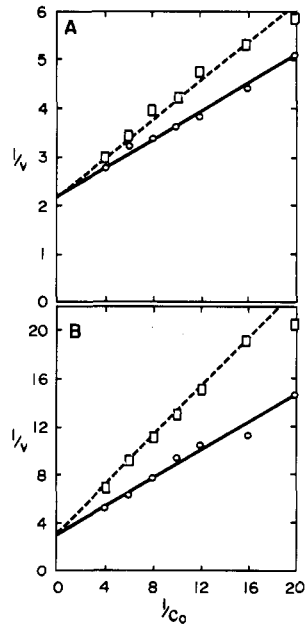

FIGURE 3: (A) Cis inhibition of leucine transport by ABOCA. Incubations of S37 cells were conducted for 2 min at 20 °C in duplicate with varying concentrations of L-[^{3}H]leucine (C_0): control (○) and with 0.1 mM a-ABOCA present (□). (B) Cis inhibition of BCH transport by ABOCA. Incubations of S37 cells were conducted for 2 min at 20 °C in duplicate with varying concentrations of [^{14}C]BCH (C_0), with a control (○), and with 0.1 mM a-ABOCA present (□).

Table III: a-ABOCA and a-BCH Compared as Cis Inhibitors of System L[a]

concn of inhibitor (mM)	% inhibition by a-BCH	% inhibition by a-ABOCA
0.10	19	35
0.50	53	69
1.0	71	78

[a] All samples contained 0.10 mM L-[^{3}H]histidine and 1 mM MeAIB. Determinations were averaged triplicates.

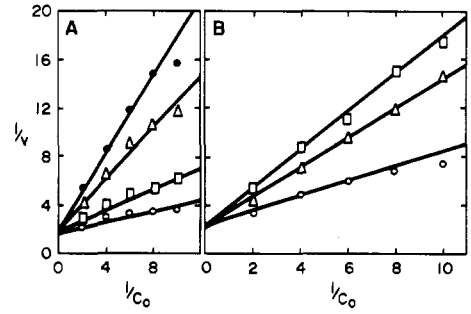

FIGURE 4: (A) Cis inhibition of ABOCA transport by BCH, histidine, and leucine. Incubations of S37 cells were conducted for 2 min at 20 °C in duplicate with varying concentrations of [^{14}C]a-ABOCA (C_0): control (○), 0.5 mM BCH (□), 0.5 mM histidine (△), and 0.5 mM leucine present (●). (B) Comparison of a-ABOCA and b-ABOCA as cis inhibitors of leucine transport. Incubations of S37 cells were conducted for 5 min at 20 °C in duplicate with varying concentrations of L-[^{3}H]leucine (C_0): control (○), 0.5 mM a-ABOCA (△), and 0.5 mM b-ABOCA present (□).

possible inhibitor, was also carried out. The mean C_i value with MeAIB included was only negligibly greater than the control value, thus demonstrating the noninteraction of ABOCA with system A (control mean = 1.897 mM, $n = 9$; mean with MeAIB present = 1.919 mM, $n = 10$).

It was also desirable to compare ABOCA with BCH in that ABOCA appeared to be similar to BCH in being restricted to interaction with only one of the two principal neutral amino acid transport systems, system L. ABOCA and BCH were first compared as inhibitors of system L using low concentration labeled histidine as the test substrate, with MeAIB included to eliminate minor A system activity (Table III). ABOCA demonstrated greater inhibition of system L than BCH did. A cis inhibition experiment was then conducted in which BCH was the labeled substrate and ABOCA was the inhibitor (Figure 3B). ABOCA was a competitive inhibitor of BCH, exhibiting a smaller K_i value (0.11 mM) than the BCH K_m value (0.17 mM). The converse experiment was carried out in which labeled ABOCA was the substrate and BCH, histidine, and leucine were employed as cis inhibitors (Figure 4A). These three amino acids all behaved as competitive inhibitors, and the K_m for ABOCA uptake was 0.13 mM while the K_i values were the following: BCH, 0.50 mM; histidine, 0.19 mM; leucine, 0.13 mM. These results suggested that ABOCA had a greater affinity for system L than BCH did.

In order to answer the question of isomer preference, it was necessary to determine whether or not there was any discrimination of transport system L between the a and b isomers of ABOCA. A comparison of the two stereoisomers as inhibitors of leucine transport suggested that the b isomer had slightly greater affinity for system L than the a isomer did (Figure 4B). The K_m for leucine transport in this experiment was 0.23 mM, while the K_i value for a-ABOCA was 0.35 mM and the K_i for b-ABOCA was 0.29 mM. A similar experiment using a low concentration range of labeled histidine as substrate

yielded similar results (data not shown).

Discussion

The results of this study have unequivocally established that ABOCA is a substrate of system L and is not a substrate or competitor of system A. In addition, the data support our original hypothesis in that ABOCA exhibits a greater affinity for system L than does BCH. The results demonstrating that ABOCA has a greater affinity than does BCH for the L system are consistent with our earlier suggestion of the dominance of flexible apolar interactions over precise steric considerations in selectivity of substrates by the L system (Matthews & Zand, 1977). Steric factors, however, cannot be totally ignored since there is a definite preference for the ABOCA isomer in which the carboxyl function is exo to the methylene bridge. The assignment of structure and absolute configuration of the ABOCA isomers has been reported earlier (Chacko et al., 1978), thereby permitting the present assessment of preference by system L for the exo carboxyl isomer. This result parallels the findings reported for BCH (Christensen et al., 1969) in which the isomer possessing this configuration was favored. The results of the present study suggest that ABOCA may be preferable to BCH as an amino acid analogue for discrimination of amino acid transport systems.

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Equilibria and Kinetics of Ligand Binding to the Human Erythrocyte Glucose Transporter. Evidence for an Alternating Conformation Model for Transport†

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ABSTRACT: Cytochalasin B (CB), *n*-propyl β-D-glucopyranoside (PG), and 4,6-*O*-ethylidene-D-glucose (EG) are known to bind asymmetrically to the human erythrocyte glucose transporter. The first two compounds bind to the inner (cytoplasmic) surface of the transporter, while the latter binds to the outer surface. Equilibrium measurements of the inhibition of CB binding to the glucose transporter reported herein indicate that the ternary complexes of CB transporter with EG, PG, or D-glucose are not formed. Moreover, measurements of CB binding in the presence of both EG and PG or in the presence of high concentrations of D-glucose show that a ternary complex of transporter and sugars bound simultaneously on both sides of the membrane probably does not occur. Finally, the kinetics of dissociation of radiolabeled CB from the transporter in the presence of CB, glucose, PG, and EG have been determined. With the exception of the case of EG, the kinetics fit a simple scheme of rate-limiting unimolecular dissociation, and in no instance do they suggest the existence of a ternary complex of sugar, CB, and transporter. These data are consistent with a model for transport in which the substrate binding site exists alternately at the cytoplasmic and external faces of the membrane, as the result of protein conformational change.

A model for the facilitated diffusion of glucose in erythrocytes, which we call the alternating conformation model, is shown schematically in Figure 1. According to this model, which was described by Vidaver (1966), translocation of the occupied or unoccupied substrate site across the membrane is effected by a conformational change of the transporter. This model accounts for sugar specificities of the transporter that differ at the cytoplasmic and external surfaces of the membrane (Barnett et al., 1975). It also accounts for most of the results from steady-state kinetic studies, including the phenomena of the uphill transport of one sugar induced by a

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