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METHIONINE TRANSPORT IN S37 CELLS

SUBSTRATE-DEPENDENT FUNCTION OF AMINO ACID TRANSPORT SYSTEM A IN EXCHANGE PROCESSES

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Summary

Methionine had been observed to interact with two principal transport systems for amino acids in mammalian cells, the A and L systems. The present study of methionine transport and of exchange processes through system A arose in the course of a study to define the specificity of a transinhibition effect caused by cysteine.

Methionine uptake through two transport systems in the S37 cell was confirmed by the occurrence of a biphasic double-reciprocal plot for labeled methionine uptake. Preloading cells with methionine stimulated labeled histidine uptake through both systems A and L. Efflux of labeled methionine from cells was stimulated by histidine in a biphasic manner, so that both systems A and L can be used for exchange when methionine is the intracellular amino acid. Aminocycloheptanecarboxylic acid elicited exchange efflux of labeled methionine only through system L. α-Aminoisobutyric acid and *N*-methyl-α-aminoisobutyric acid both stimulated efflux of labeled *N*-methyl-α-aminoisobutyric acid from S37 cells. These findings are interpreted a showing that transport system A is capable of functioning as an exchange system depending upon the identity of intracellular and extracellular substrates available.

Abbreviations: C_0, extracellular concentration of an amino acid; AC7C aminocycloheptanecarboxylic acid; S37, mouse sarcoma 37 ascites tumor.

Introduction

In a previous study we had observed a transinhibition of transport systems A and L caused by intracellular cysteine [1]. In studying the specificity of that effect, we observed a stimulation of labeled histidine transport by both systems A and L when cells were preincubated with methionine. We examined the exchange effect of methionine through system A in the present study because it constituted a variance from the commonly accepted representations of transport systems A and L. System L has been thought to be the exchange system, and system A has been commonly regarded as a unidirectional active transport system.

Earlier studies suggested the interaction of methionine with both transport systems A and L. In specificity surveys employing other labeled amino acids as test substrates, methionine was able to inhibit uptake through systems A and L in the S37 cell or in the Ehrlich ascites cell [2—4]. Johnstone and coworkers [5—7] have studied methionine transport in the Ehrlich cell in some greater detail. An 'exchangeable' pool of endogenous amino acids containing valine, isoleucine, leucine, tyrosine and phenylalanine was decreased by methionine present in the medium but aspartate, threonine, serine, glutamate, glycine and alanine did not change [5]. The 'exchangeable' pool could be regarded as preferential substrates of system L, whereas alanine and glycine would be regarded as system A substrates. In a moderately high concentration range, varying the Na^+ content of the medium altered K_m, but not V, for methionine transport [6]. It has also been proposed that efflux could occur through a Na^+-dependent system, and that only this efflux was affected by gramicidine [7]. A recent study of methionine transport in WI-38 cells showed that methionine was taken up by two transport sytems. One had a high K_m for methionine and was Na^+-dependent; the other system had a low K_m for methionine and was Na^+ independent. Exchange efflux of labeled methionine was shown to occur, but no indication of which system might be involved in exchange was obtained [8]. The available data would be consistent with methionine being transported by system A and system L, and participating in exchange, but through unidentified systems.

In the present study we confirm methionine transport through systems A and L for the S37 cell and demonstrate that methionine exchange can occur through both systems A and L. We also show that methionine exchange is restricted to system L when aminocycloheptanecarboxylic acid (AC7C) is the extracellular substrate, and that intracellular labeled *N*-methyl-α-aminoisobutyric acid engages in exchange efflux when the extracellular amino acid is *N*-methyl-α-aminoisobutyric acid or α-aminoisobutyric acid. A preliminary report of a portion of this data has been made [9].

Materials and Methods

AC7C was synthesized by first adding an ethanolic solution of the cyclic ketone to aqueous potassium cyanide and ammonium carbonate to obtain the spirohydantoin, then heating the spirohydantoin with barium hydroxide as previously described [10]. *N*-[^{14}C]Methyl-α-aminoisobutyric acid, [^{3}H]histi-

dine, and [^{3}H]methionine were obtained from New England Nuclear Co. Unlabeled *N*-methyl-α-aminoisobutyric acid was obtained from Aldrich Chemical Co.

General procedures for amino acid transport experiments have been described in detail recently [11].

A non-linear curve-fitting program based on Newton's method was used in fitting biphasic kinetic data [12]. This program has been used previously for the purpose of determining the contributions of two transport systems to a simultaneous uptake process [13]. Since Newton's method was an iterative procedure in which initial estimates for parameters were refined, the ability to make reasonable initial estimates of parameters was of value in making fits when four parameters were to be obtained and there was some experimental error in the data set. We have improved our process of making the initial parameter estimates by subjecting our original data (in double-reciprocal form) to a first-degree five-point smoothing procedure [14], and then obtaining graphical estimates of the parameters according to the relationships [12,15]:

$C_1 = V_1 + V_2 = 1/I_1$
$C_2 = V_1K_2 + V_2K_1 = C_1 \times q$
$C_3 = K_1 + K_2 = C_1 \times M_1 + q$
$C_4 = C_2 \times M_2$

in which $q = (M_1 - M_2)/(I_2 - I_1)$, I_1 and I_2 were extrapolated intercepts of a double-reciprocal plot at the zero value of the abscissa, M_1 and M_2 were the slopes of the two line segments in a biphasic double-reciprocal plot, V_1 and V_2 were maximum velocities of two saturable transport processes, K_1 and K_2 were Michaelis constants for the two saturable transport processes, and C_1, C_2, C_3, and C_4 were the composite parameters fitted in the program. A fitting was done to the smoothed data, and then the parameters obtained were utilized as starting values of the parameters to fit the original data set. The final parameters C_1, C_2, C_3, C_4 obtained by fitting the original data were converted to V_1, V_2, K_1 and K_2 by inverse relationships [12]:

$$K_2 = \tfrac{1}{2}(C_3 + \sqrt{C_3^2 - 4C_4})$$

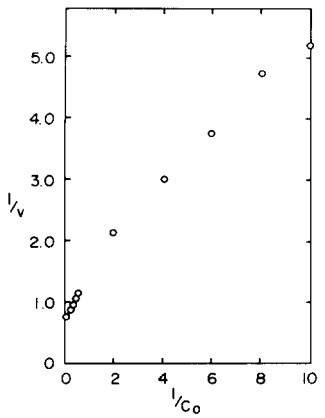
$$K_1 = \frac{C_4}{K_2}$$

$$V_2 = \frac{C_1K_2 - C_2}{K_2 - K_1}$$

$$V_1 = C_1 - V_2$$

Results

Uptake of labeled methionine across a broad concentration range yielded a biphasic double-reciprocal plot (Fig. 1). This clearly revealed the participation of two transport systems in the uptake of methionine, although the break in the plot was not quite so sharp as for histidine transport in the S37 cell [2] (compare also Fig. 2).



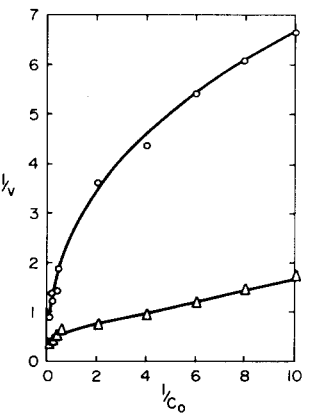


Fig. 1. Initial velocity of transport of L-[^{3}H]methionine into S37 ascites tumor cells. Cells were exposed to varying extracellular concentrations (C_0) of L-[^{3}H]methionine for 2 min at 20°C. v was estimated as half the intracellular concentration of labeled methionine attained at the end of the 2 min incubation and data are presented in double-reciprocal form. Units of the ordinate were min/mmolar; those of the abscissa mmolar^{-1}. Individual points were averages of two measurements.

Fig. 2. Trans effects of methionine on the L and A transport systems. S37 cells were first incubated with (△) or without (○) 10 mM L-methionine present at 20°C for 1 h. After washing, the cells were incubated in varying concentrations of [^{3}H]histidine, C_0, for 2 min at 20°C. The units of the ordinate were min/mmolar; those of the abscissa mmolar^{-1}. Individual points were averages of two determinations. ———, fitting of the data as described under Materials and Methods and parameters were listed in Table I. Repetition of the experiment yielded similar results.

TABLE I

EFFECT OF METHIONINE PREINCUBATION ON KINETIC PARAMETERS FOR HISTIDINE TRANSPORT

Parameter	Control data		Methionine-loaded cells
Graphical estimates			
I_1	0.75		0.30
I_2	3.05		0.50
M_1	2.45		0.50
M_2	0.39		0.12
q	0.90		1.90
C_1	1.33		3.33
C_2	1.20		6.33
C_3	4.16		3.57
C_4	0.47		0.76
Final values from curve-fitting analysis			
C_1	1.59		3.20
C_2	1.15		6.78
C_3	5.53		4.05
C_4	0.31		0.72
K_1	0.057		0.187
V_1	0.20		1.68
K_2	5.47		3.86
V_2	1.39		1.52
K_1/V_1	0.28_5		0.11_1
(K_1/V_1) Met loaded/(K_1/V_1) control		0.39	
K_2/V_2	3.94		2.54
(K_2/V_2) Met loaded/(K_2/V_2) control		0.65	

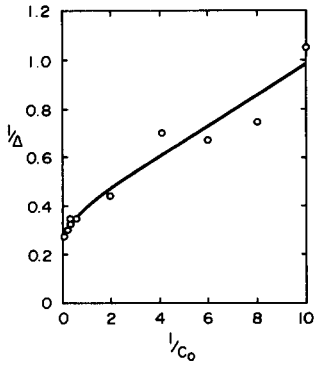


Fig. 3. Exchange efflux of [^{3}H]methionine elicited by histidine. S37 cells were first incubated in the presence of 1 mM L-[^{3}H]methionine 30 min at 37°C. They were subsequently exposed to varying concentrations (C_0) of unlabeled L-histidine for a period of 5 min at 20°C. Differences in concentration of [^{3}H]methionine remaining in cells not exposed to histidine and in cells exposed to varying concentrations of histidine (△) were determined and results presented in double-reciprocal form. The units of the ordinate were min/mmolar, those of the abscissa mmolar^{-1}. Individual points were averages of two measurements. ———, fitting of this data as described under Materials and Methods. Parameters obtained by the fitting process were: V_1 = 2.79 mM/min; K_1 = 0.178 mM; V_2 = 1.65 mM/min; K_2 = 7.56 mM.

The first indication that intracellular methionine exchanged through both transport systems A and L was obtained in the experiment of Fig. 2. The biphasic double-reciprocal plot of histidine uptake has been associated with histidine transport through systems A and L of the S37 cell [2,3,13]. Fig. 2 showed that both limbs of the double-reciprocal plot were lowered and deflected by preincubation with methionine. Our previous studies suggested that the ratio K_m/V was less sensitive to experimental error than individual kinetic parameters derived by curve-fitting analysis [12]. Note that the ratio K_m/V would be the slope of a double-reciprocal plot with only one transport system operating. The detailed parameter analysis (Table I) showed that the K_m/V ratio for both transport systems was markedly decreased by preincubation with methionine, so that both systems had functioned in exchange. The ratio of results with methionine-loaded cells to control cells was somewhat lower for system L than system A, indicating that system L was somewhat more effective in exchange.

The confirmation of methionine exchange through both systems A and L was obtained by measuring the efflux of labeled methionine from S37 cells in response to various concentrations of unlabeled histidine in the second incubation medium (Fig. 3). The biphasic nature of the plot indicated the participation of two transport systems in the exchange process. Exchange efflux of labeled intracellular histidine had given a linear response, and was through system L only [2]. Curve-fitting analysis yielded values of K_1 = 0.18 mM and K_2 = 7.6 mM, not unlike earlier estimates of K_m values for histidine transport through systems L and A, respectively [13].

The substrate-dependent nature of exchange processes through systems A and L in the S37 cell was studied further with the aid of analogs specific to these two systems. In one experiment (repeated four times) cells were first incubated 30 min at 37°C with 1 mM [^{3}H]methionine present, then exposed to

varying concentrations of AC7C in a second incubation. A double-reciprocal plot made of the additional methionine lost from the cells, Δ, as a function of AC7C concentration, C_0, was linear. The K_m for this process averaged 0.11 mM. The relatively low K_m value obtained taken together with the earlier finding that AC7C interacted specifically with system L [11] indicated that methionine had exchanged through only system L when AC7C was the extracellular solute. In a second experiment, S37 cells were first incubated with 1 mM *N*-[^{14}C]-methyl-α-aminoisobutyric acid for 30 min at 20°C (experiment repeated twice). The cells were subsequently exposed to varying concentrations of α-aminoisobutyric acid or *N*-methyl-α-aminoisobutyric acid. *N*-Methyl-α-amino-isobutyric acid and α-aminoisobutyric acid both stimulated exchange efflux of *N*-[^{14}C]methyl-α-aminoisobutyric acid from the S37 cells. A K_m of 3.3 mM was estimated for the exchange process with extracellular α-aminoisobutyric acid present. The data obtained with extracellular *N*-methyl-α-aminoisobutyric acid indicated exchange had occurred, but was not sufficiently well-behaved to permit a reasonable parameter estimation. *N*-Methyl-α-aminoisobutyric acid has been found to be specific to system A in S37 cells [13].

Discussion

Methionine is an amino acid of considerable importance to the functioning of eukaryotic cells. It is one of the essential amino acids for protein synthesis, a primary metabolite for one-carbon metabolism, and also serves a unique role in the initiation of peptide synthesis. Its importance is further accentuated in many tumor cells, which are unable to tolerate a substitution of homocysteine for methionine due to an alteration in their metabolism [16]. It therefore seems most plausible to interpret exchange of methionine out of a tumor cell through two principal transport systems as an indication of avidity for the two transport systems rather than as an indication of expendability to metabolism.

The available data suggests that methionine interacts with amino acid transport systems A and L in various mammalian cells, and that these interactions display certain common properties. The interaction of methionine with system A has a relatively high K_m, and transport through system A is stimulated by Na^+. It was the high K_m system for methionine transport that was stimulated by Na^+ in WI-38 cells [7], and Potashner and Johnstone [6] used a relatively high methionine concentration range in studying the effect of Na^+ on transport. Methionine also is transported by system L, displaying a lower K_m for this process which is less affected by ions. This is supported by the data from WI-38 cells [8]. Considering the biphasic uptake of methionine into S37 cells (Fig. 1) and the previous data on methionine inhibition of histidine transport into S37 cells by systems A and L [2,3] our data are consistent with this view also. Methionine was shown to be active in exchange in WI-38 cells, but the transport systems supporting this effect were not identified [8]. Methionine exchange through system A has now been demonstrated for S37 cells. The data available are not sufficient to determine whether methionine exchange through system A is shared with other tissues. It is of interest to note that an early study by Heinz and Walsh [17] showed exchange of glycine and alanine in the Ehrlich cell. It appears that these two amino acids each share other transport

systems in the Ehrlich cell [18], but they use system A in common and it is probable that this represents exchange through system A demonstrated prior to segregation of the transport systems.

The exchange data suggests that a general but incomplete consistency exists for transport and exchange. *N*-Methyl-α-aminoisobutyric acid, a good A system substrate for transport, was able to exchange out of the S37 cell when A system substrates were present outside the cell. Methionine, a good substrate for transport by both systems A and L, exchanged out of the cell through both systems when histidine, an effective substrate for transport by both systems, was outside the cell. Methionine exchanged out of the cell only through system L when the extracellular amino acid selected for system L. However, histidine is known to exchange out of a cell only through system L [2]. The difference between systems A and L with regard to exchange would appear to be one of degree and range rather than kind.

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