Biochimica et Biophysica Acta, 465 (1977) 426–428

BBA Report

BBA 71289

FREE CARBOXYLATE GROUPS REQUIRED FOR TRANSPORT OF NEUTRAL AMINO ACIDS BY THE EHRLICH ASCITES-TUMOR CELL

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(Received November 17th, 1976)

Summary

Although uncharged structures analogous to the carboxyl group of the amino acid molecule have served in place of that group for transport into some cells, we find that substitution either by the carboxamide group or by the chloromethyl ketone group eliminates inhibition of transport Systems A or L of the Ehrlich ascites tumor cell. Comparison of the loss of System L transport on acidification shows that the pH at which this loss occurs is correlated with pK_1' of the amino acid substrate, suggesting that transport is terminated by protonation of the site-bound carboxylate group.

The methyl esters of leucine and alanine can inhibit competitively two different components of the uptake of histidine by S37 ascites tumor cells [1], apparently similar to Systems A and L of the Ehrlich cell [2,3]. The chloromethyl ketone analogs of tyrosine and phenylalanine have also been shown to compete with the latter two amino acids for their uptake by *Bacillus subtilis*, although by a different system [4,5]. These results suggest that only the α-carbonyl portion, and not the entire free carboxyl group, is needed for recognition of the amino acid, at least to a degree to cause inhibition of transport. If these analogs actually serve as transport substrates, the interesting possibility is raised that neutral amino acids might be transported in the cationic form, $RCH(NH_3^+)COOH$, or as the comparatively rare species, $RCH(NH_2)COOH$.

We have tested the amides of glycine, leucine, and phenylalanine, also the chloromethyl ketone analog of phenylalanine (a generous gift of Dr. Elliott N. Shaw, University of the State of New York at Stony Brook) as inhibitors of the model substrates, 2-(methylamino)-isobutyric acid and 2-aminonorbornane-2-carboxylic acid, by the Ehrlich cell. The tests of transport were carried out for 30 s in Krebs-Ringer phosphate medium, modified to contain only 0.5 mM $CaCl_2$. In all respects the propagation and

handling of the Ehrlich cell were as described previously [6].

The commercial preparations of the amides showed significant inhibition of neutral amino acid uptake; but removal of contaminating quantities of free amino acids by treatment with Dowex 1-8X anion-exchange resin led to complete loss of effectiveness for all three. The chloromethyl ketone analog was also without significant effect (Table I). Similar results were obtained when phenylalanine was used as substrate. An attempted purification of the esters of phenylalanine and leucine with the same resin led to their hydrolysis and retention as the free amino acids. Therefore, we failed to bring these preparations to a purity at which their effects could not be explained by the content of the corresponding free amino acid.

Fig. 1 shows our observation that transport by System L declines with

TABLE I

EFFECTS OF AMIDES AND A CHLOROMETHYL KETONE ANALOG OF NEUTRAL AMINO ACIDS ON THE UPTAKE OF AMINO ACIDS BY THE EHRLICH CELL

In experiment No. 1 the pH of the medium was 7.4 and the substrate was 2-(methylamino)-isobutyric acid. In experiment No. 2 and 3 the pH of the medium was 6.2 and the substrate was 2-aminonorbornane-2-carboxylic acid. Under these conditions uptake may be attributed to System L. At pH 7.4, entry as the uncharged amine leads to stimulation of uptake of the norbornane amino acid presumably by exchange via System L. In all the cases the substrate was present at a 20 μM concentration containing tracer amounts of the ^{14}C isotope, the uptake for 30 s being observed, and expressed as the final distribution ratio attained.

Experiment	Compound	Uptake of model amino acid
1	None	1.08
	50 mM glycine amide	1.11
2	None	15.1
	0.1 mM phenylalanine	3.4
	10 mM phenylalanine amide	15.3
	10 mM leucine amide	16.1
3	None	14.1
	2 mM phenylalanine-chloromethylketone	13.6

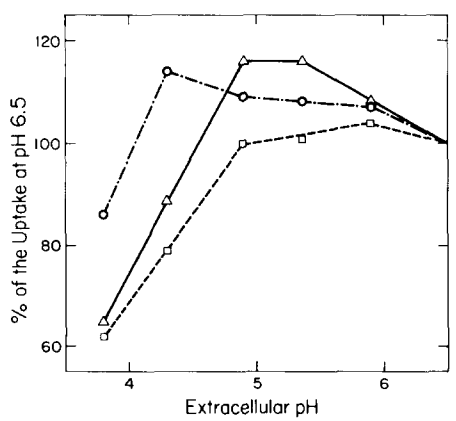

Fig. 1. Effect of lowering the pH on transport of three amino acids by System L. Uptake measured for 30 s at 37°C from a Krebs-Ringer medium in which choline replaces Na^+, and in which 20 mM ϵ-amino-caproic acid provides the buffer system. Curve marked by circles, 25 μM L-phenylalanine for which v/s = 32.7 min^{-1}; curve marked with triangles, 25 μM L-valine, for which v/s = 4.3 min^{-1}; curve marked with squares, the norbornane amino acid 50 μM for which v/s = 28.9 min^{-1}, all at pH 6.5. The rise in velocity seen in passing from pH 7.4 to pH 5 is also a structure-determined variable among substrates.

falling pH at some point below pH 5. Decline for the three amino acids tested here falls in the order, the norbornane amino acid, valine and phenylalanine, separated by intervals of roughly 0.2 pH units. This is the same order taken by the pK'_1 values of these amino acids, 2.8, 2.3 and 1.8. We interpret this result to mean that the carboxyl groups tend to become protonated in the environment of the transport function, perhaps in carrier-bound form, this protonation occurring more readily than in free solution, and being unfavorable to transport. Other explanations are possible.

These results strongly suggest that the free α-carboxyl group is needed in the carboxylate form for transport into the Ehrlich cell via Systems A and L.

Support is gratefully acknowledged from the Institute of Child Health and Human Development, Grant HDO1233, U.S.P.H.S. and Juan March Foundation, Madrid, Spain.

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