TRANSPORT STUDIES WITH PEPTIDES CONTAINING UNNATURAL AMINO ACIDS

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The successful use by Christensen's group (Christensen and Jones, 1962) of the unnatural, unmetabolized compounds, α-aminoisobutyric acid and cycloleucine, as amino acid transport models, has encouraged us to synthesize peptides of these amino acids and to explore their usefulness as peptide transport models. In the present investigation we have examined the uptake of unnatural tripeptides by L. casei 7469.

Three separate transport systems, one for glycine, one for L- and D-alanine and one for glycyl-L-alanine and L-alanylglycine have been demonstrated in L. casei 7469 (Leach and Snell, 1960). The finding that a tripeptide could promote growth of L. arabinosus 17-5 more effectively than either of its constituent dipeptides plus the remaining amino acid (Dunn, Humphreys and Shive, 1958) led us to investigate transport of tripeptides into L. casei 7469.

EXPERIMENTAL

α-Aminoisobutyric acid-1-C^{14} and cycloleucine-1-C^{14} (carboxyl-labeled 1-amino-cyclopentane-1-carboxylic acid) were obtained from Tracerlab, Inc., and from New England Nuclear Corp., respectively. The synthesis of glycyl-α-aminoisobutyryl-1-C^{14} L-alanine (Young, 1963),

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glycylcyloleucyl-1-C^{14}-L-alanine and glycylcyloleucyl-1-C^{14}-L-valine (Bowen, 1963) have been described elsewhere.

Lactobacillus casei 7469 was grown on the defined basal medium described by Leach and Snell (1960) supplemented with 1000 mg per liter of L-ascorbic acid and 10 mg of uracil. After 16 hours at 37°C the cells were harvested by centrifugation, washed once in salts solution (Leach and Snell, 1960) and suspended in salts solution for the experiment. Cell weights were determined from the optical density at 650 mμ of appropriate dilutions of the cell suspension by reference to a standard curve relating dry weight of cells to optical density. The incubation media contained from 1310 to 2050 μg of dry cell per ml. After 15 minutes preincubation of the cells at 37°C, glucose was added to a final concentration in the incubation medium of 0.1 % (5.5 mM); after 15 minutes further incubation either radioactive amino acid or peptide was added. Uptake of the labeled compounds was determined by the Millipore filter technique of Britten et al. (1955) using type HA filters. At desired time intervals 2.0 ml aliquots of incubation medium were removed to filters with a calibrated syringe fitted with an automatic stop. The syringe was rinsed each time with 2.0 ml of water, which was added to the filtrate. The filters were left attached to the aspirator pump for 50 minutes to remove virtually all the filtrate from the discs. The discs were cemented to 1 inch planchets, air dried and counted in a Tracerlab automatic internal gas flow counter. Aliquots of 0.1 ml of the filtrates were plated in similar planchets and counted. No correction was made for self absorption. This correction was found to be unnecessary for the filtrates. Corrections were made for radioactive filtrate absorbed by the filter discs by subtracting the activities of appropriate blanks prepared by filtering solutions containing all

components of the experimental system except cells. These blank values did not exceed 40 c.p.m.; background counting rate was about 20 c.p.m.

Results are expressed in terms of mμmoles of labeled substrate taken up per mg dry weight of cells, calculated from the radioactivity found associated with the cells on the filter disc.

RESULTS AND DISCUSSION

The uptake of glycine-1-C^{14}, α-aminoisobutyric acid-1-C^{14} and glycyl-α-aminoisobutyryl-1-C^{14}-L-alanine at time intervals up to 1 hour is shown in Figure 1A and Table 1. As the data in Table 1 for the first 5 minutes indicate, glycine is taken up twice as rapidly as α-aminoisobutyric acid and glycyl-α-aminoisobutyryl-L-alanine about 15 times as rapidly as glycine.

Table 1

Uptake of Labeled Amino Acids and Peptides from Medium by L. casei 7469 (1625 μg dry wt/ml), 37°C

Labeled substrate	Concentration μM	Rate* mμ moles/mg dry wt/hr
Glycine-1-C^{14}	38.5	6.0
α-Aminoisobutyric acid-1-C^{14}	37.75	3.5
Glycyl-α-aminoisobutyryl-1-C^{14}-L-alanine	150	86.7

*Calculated from data for first 5 minutes, represents approximately initial rate.

The results with cycloleucine-1-C^{14}, glycylcycloleucyl-1-C^{14}-L-alanine and glycylcycloleucyl-1-C^{14}-L-valine appear in Figure 1B and Table 2. As shown in Table 2, glycine is taken up 6 times as rapidly as is cycloleucine in the first 5 minutes. Glycylcycloleucyl-L-alanine is taken up 15 times as rapidly as glycine in the first 12 minutes; glycylcycloleucyl-

L-valine is taken up 20 times as rapidly as glycine in the first 9 minutes. The label from the cycloleucine peptides leaves the cell more rapidly than that from the α-aminoisobutyric acid peptides; after 30 minutes the cells retain about the same amount of cycloleucine from the tripeptides as of glycine (Figure 1B), while α-aminoisobutyric acid from tripeptide remains at a high level after 1 hour (Figure 1A).

Table 2

Uptake of Labeled Amino Acids and Peptides from Medium by L. casei 7469 (2050 μg dry wt/ml), 37°C

Labeled substrate	Concentration	Rate		
	μM	mμ moles/mg dry wt/hr		
		0-5 min	0-9 min	0-12 min
Glycine-1-C^{14}	38.5	6.0	6.0	6.0
Cycloleucine-1-C^{14}	40	1.0	0.6	0.5
Glycylcycloleucyl-1-C^{14}-L-alanine	150			89.1
Glycylcycloleucyl-1-C^{14}-L-valine	150		116.3	

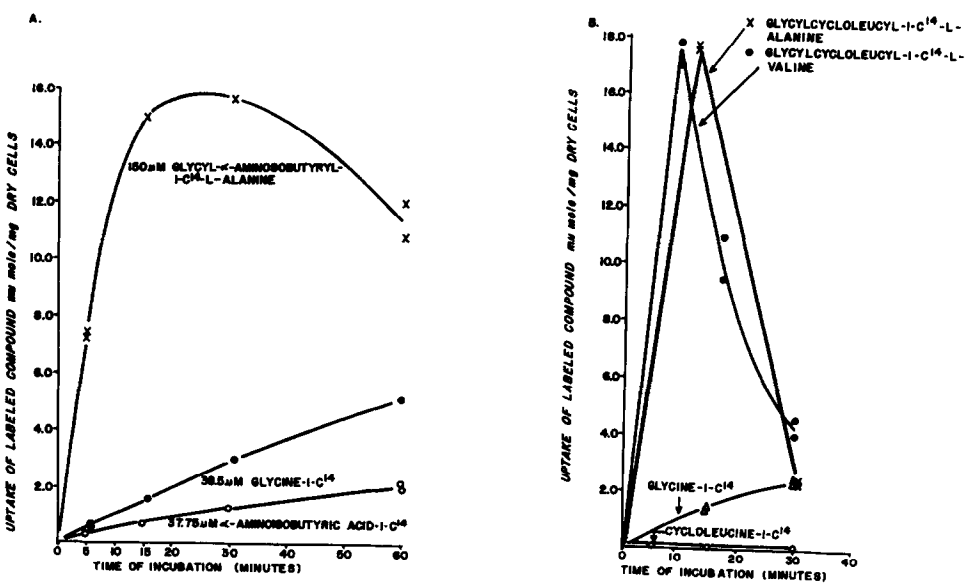

Figure 1. Uptake of tripeptides by L. casei 7469 at 37°C.
A. 1625 μg dry wt/ml.
B. 2050 μg dry wt/ml.

The dependence of tripeptide uptake on metabolic processes was tested by dividing a cell suspension of L. casei 7469 into three parts. One part was boiled for 15 minutes prior to incubation at 37°C for uptake measurement. The second part was incubated at 0°C and the third part at 37°C for uptake measurement. Neither boiled cells nor unboiled cells incubated at 0°C accumulated significant quantities of glycine-1-C^{14}, glycyl-α-aminoisobutyryl-C^{14}-L-alanine or glycylcyclololeucyl-1-C^{14}-L-alanine; the unboiled control cells actively took up all three labeled substrates.

These observations agree with those of Leach and Snell (1960). They found that glycyl-C^{14}-L-alanine and L-alanylglycine-C^{14} are taken up 20 to 30 times as rapidly as glycine-C^{14}. Although the rates of dipeptide uptake reported by Leach and Snell (1960) are greater than the tripeptide uptake rates given in the present paper, our data do not represent true initial rates.

The shapes of the uptake curves for the α-aminoisobutyric acid and cycloleucine tripeptides are similar to the curves of Leach and Snell (1960) showing accumulation of glycine-C^{14} and its dipeptides into the TCA-soluble fraction of the cells. They found that accumulation into the TCA-soluble pool fell rapidly after reaching a peak at 20 minutes, while incorporation into the TCA-insoluble protein and cell-wall fraction continued to rise slowly. The use of peptides of unnatural amino acids as tracers for transport studies appears to measure entry into the intracellular pool rather than incorporation into proteins and cell-wall material.

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