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The uptake of D-glutamic acid by *Mycobacterium avium*

It is well known since the initial work of GALE[1,2] that the transport of amino acids into the bacterial cell occurs by physical diffusion or by an active process on the part of the cell. The entry in the active process is achieved by a specific system with enzymatic properties[3,4]. Physical diffusion is not affected by temperature, nor by uncoupling agents such as NaN_3 and 2,4-dinitrophenol[5]. The two processes differ in regard to the effect of external concentration. In the active process, a Michaelis–Menten-type relationship exists between external amino acid concentration and transport[6], while the rate of entry in physical diffusion is proportional to the external concentration[1].

This communication describes the finding that a stereospecific difference exists between the uptake of D-glutamic acid and that of L-glutamic acid by *Mycobacterium avium*, and that the system responsible for the uptake of D-glutamic acid is a specific active process differing from those in which the rate-limiting catalysts participate.

D-[1-^{14}C]Glutamic acid and uniformly ^{14}C-labeled L-glutamic acid were used as labeled isomers. D-[1-^{14}C]Glutamic acid was isolated from the labeled racemate by passage through columns of Amberlite IRC-120 and 4B after decarboxylation with L-glutamic acid decarboxylase.

M. avium, non-pathogenic strain AVT, was cultured in Sauton medium and harvested near the end of the exponential phase of growth. The cells were suspended in 0.04 M phosphate buffer (pH 7.0) containing glucose (0.025 M) and labeled glutamic acid, incubated at 37° with shaking for 30 min and then centrifuged in the cold. Preliminary experiments demonstrated that there was no significant loss of labeled glutamic acid entering the cell nor the appearance of other labeled amino acids within a 30-min period. The cells were washed twice with identically constituted, non-radioactive buffer by a similar centrifugation. The cell pellet was taken up in distilled water, heated in boiling water for 5 min and then centrifuged at 15 000 × *g*. The supernatant solution was dried and redissolved in 2 ml distilled water. After removal of insoluble material, 0.5 ml of the solution was plated and the radioactivity was determined to give the value for freely extractable glutamic acid. Rates are expressed in μmoles per 10 mg dry weight of cells per 30 min.

Fig. 1 shows the relationship between the rate of uptake and the external glutamic acid concentration. The rate of uptake of L-glutamic acid increased sharply with higher concentration, but above 1 mM further increases in the external concentration produced only a small increase in the rate. The curve fits a straight line when plotted according to the method of LINEWEAVER AND BURK[7]. On the other hand, the rate of uptake of D-glutamic acid was entirely proportional to the external concentration. The results indicate that the uptake of D-glutamic acid and that of L-glutamic acid differ with regard to the system by which external glutamic acid is taken into the cell.

The uptake of D-glutamic acid was 82% inhibited by $2 \cdot 10^{-2}$ M NaN_3 and 71% by 10^{-3} M 2,4-dinitrophenol. It is evident that the uptake of D-glutamic acid is coupled to energy-producing reactions.

Although the uptake of D-glutamic acid increased linearly with the time of incubation at 37°, there was no significant increase at 4°. As a drastic difference in

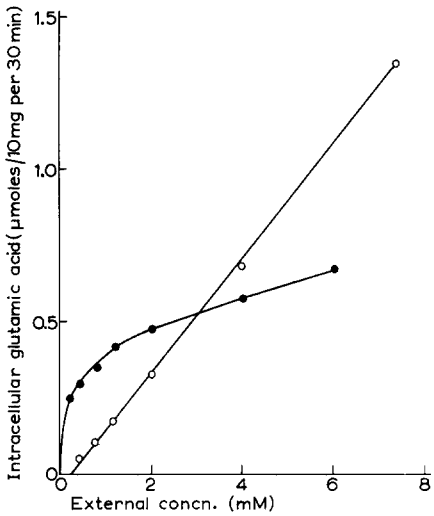

Fig. 1. The effect of external concentration on the rate of uptake of D-glutamic acid (○) or L-glutamic acid (●) by *M. avium*. The cells were incubated at 37° for 30 min with glucose and D-[^{14}C]glutamic acid or L-[^{14}C]glutamic acid. Intracellular glutamic acid was calculated from the isotope measured in hot extracts of centrifuged cells.

uptake was observed between 4° and 37°, a temperature dependence, like that of an enzyme system, is evident.

The inhibition of D-glutamic acid uptake by other amino acids was investigated (Table I) and it was found that L-aspartic acid and L-glutamic acid were very effective competitors, but D-alanine, L-alanine and D-aspartic acid were all essentially inactive in reducing the uptake of D-glutamic acid.

The uptake of D-glutamic acid resembles lysine accumulation by physical

TABLE I

THE INHIBITORY EFFECT OF OTHER AMINO ACIDS ON THE UPTAKE OF D-GLUTAMIC ACID

M. avium was incubated at 37° in phosphate buffer containing glucose, D-[^{14}C]glutamic acid (2 mM) and the indicated amino acids (4 mM). After 30 min the cells were washed twice with identically constituted, non-radioactive buffer. The cell pellet was extracted with distilled water at 100° for 5 min and centrifuged at 15 000 × *g*. The supernatant was analysed for radioactivity.

	Intracellular concentration of D-glutamic acid (μmoles/10 mg per 30 min)	*Inhibition (%) of D-glutamic acid uptake*
D-[^{14}C]Glutamic acid	0.356	—
D-[^{14}C]Glutamic acid + D-alanine	0.345	3
D-[^{14}C]Glutamic acid + L-alanine	0.356	0
D-[^{14}C]Glutamic acid + D-aspartic acid	0.314	12
D-[^{14}C]Glutamic acid + L-aspartic acid	0.083	77
D-[^{14}C]Glutamic acid + L-glutamic acid	0.093	74

diffusion[1] in its concentration dependence, but it differs fundamentally in temperature and energy dependence. The energy requirement proves it to be an active process[5]. The temperature dependence suggests the existence of an enzyme-like permeation system[4]. The experiment on the competitive effect supports the conclusion that the uptake of D-glutamic acid involves the active process. The greater affinity of structurally related L-amino acids indicates that there is a site that forms a complex with D-glutamic acid. Accordingly, it is likely that the complex associates, then dissociates reversibly, only with D-glutamic acid and that the dissociation of the complex is limited by the external concentration.

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