An impaired concentrating mechanism for amino acids in mutants of *Escherichia coli* resistant to L-canavanine and D-serine

Specific concentrating mechanisms for groups of structurally related amino acids have been demonstrated in *E. coli*[1]. We have now obtained two mutants, each deficient in a specific concentrating mechanism. One mutant is resistant to inhibition of growth by L-canavanine, the other to inhibition of growth by D-serine. These mutants appear to be impaired in their ability to concentrate the inhibitor and a group of amino acids structurally related to the inhibitor.

The canavanine-resistant mutant was obtained from wild-type *E. coli*, strain W, after ultraviolet irradiation and subsequent selection on an enriched arginine-free medium[2] containing 100 μg/ml canavanine. The growth of the wild-type strain is inhibited by 5 μg/ml canavanine. An indication that the concentrating mechanism for arginine is impaired in the mutant came from the observation that exogenously supplied arginine, which had previously been shown to suppress the formation of ornithine transcarbamylase in the wild type[3], does not suppress in the mutant. The lack of suppression can not be due to insensitivity of the enzyme-forming system to arginine, because conditions of lowered endogenous formation of arginine which increase the formation of the enzyme in the wild type[2] exert an equally marked effect in the mutant (Table I).

TABLE I

FORMATION OF ORNITHINE TRANSCARBAMYLASE AT VARIOUS INTRACELLULAR CONCENTRATIONS OF ARGININE

Cells of *E. coli* strain W were grown exponentially for 4 generations, toluenized, and their enzyme activity was determined according to the method of JONES *et al.*[8]. One enzyme unit = amount of enzyme which synthesizes 1 μmole citrulline/h.

Medium of Growth	*Enzyme units/mg dry weight bacteria*	
	Canavanine^r mutant	*Wild type*
Medium A (7)	2.1	1.6
Medium A + arginine 100 μg/ml	2.1	> 0.1
Arginine-free enriched medium (2)	12.8	12.0

The isolation of D-serine-resistant mutants has been described[4]. Earlier studies of the antagonistic effects of L-alanine and glycine on the inhibition of growth by D-serine[5] had suggested that these amino acids might interfere with the uptake of D-serine in the wild type; in the resistant mutant the concentrating mechanism for D-serine and for these two amino acids might be impaired.

The uptake of various amino acids has now been measured directly in the wild type and in the two resistant mutants (Table II). In these experiments, chloramphenicol was added to exponentially growing cultures to prevent incorporation of the labelled amino acid into protein. Radioactive amino acids were then added and their uptake by the cells was followed, using a membrane-filter technique[6]. It can be seen (Table II) that the canavanine-resistant mutant is deficient in the uptake of arginine, ornithine, and lysine whereas the D-serine-resistant mutant is deficient in

TABLE II

UPTAKE OF RADIOACTIVE AMINO ACIDS BY WILD-TYPE AND RESISTANT MUTANTS

Chloramphenicol (final concentration, 200 μg/ml) was added to cells growing exponentially at 37° in Medium A with either lactate (for the basic amino acids) or glucose (for the other amino acids) as carbon source*. After 30 min, ^{14}C-labelled amino acids (final concentrations, 10 μg/ml) were added and incubation continued for 60 min. Aliquots of the cultures were then collected on membrane filters and their radioactivities determined[6]. DL-ornithine was labelled in the α-carbon, the other amino acids were randomly labelled. The intracellular concentrations were calculated from the specific activities of the amino acids.

Amino Acid	*Uptake, μg/g wet weight bacteria*		
	Canavine mutant^r	*D-serine*^{r} *mutant*	*Wild type*
L-Arginine	262	810	1050
L-Lysine	110	703	550
DL-Ornithine	254	1350	1330
D-Serine	182	88	193
Glycine	315	23	329
L-Alanine	552	169	485

* The differences in uptake between defective and non-defective strains are more pronounced with lactate as a carbon source for the basic amino acids and with glucose for the other amino acids.

the uptake of D-serine, glycine and alanine. In regard to uptake of other amino acids, there is no difference between mutant and wild type. Canavanine appreciably depresses the uptake of arginine and of lysine in the wild type, whereas in the canavanine-resistant mutant it has only a slight effect on arginine uptake and none on the uptake of lysine. We conclude that the canavanine-resistant mutant is defective in a specific concentrating mechanism for arginine, ornithine, lysine and canavanine, and the D-serine-resistant mutant is defective in a similar mechanism for alanine, glycine and D-serine.

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