TWO E. COLI GLUTAMINE SYNTHETASES WITH DIFFERENT SENSITIVITIES TO FEEDBACK EFFECTORS

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Cumulative feedback inhibition of the E. coli glutamine synthetase has been described (Woolfolk and Stadtman, 1964, 1967). The enzyme has been prepared in crystalline, homogeneous form from E. coli strain W, grown on glycerol and glutamate under conditions yielding maximal derepression (Woolfolk et al., 1966). Subsequently it was found that the enzyme derived from cells grown on glycerol and glutamate has a different susceptibility to feedback effectors than did the enzyme preparation first studied by Woolfolk and Stadtman (1964). Investigation of this difference revealed that it was not due to assay conditions, change in properties of enzyme during purification or storage, or to the method of disruption of the cells, but rather due to differences in growth conditions. This communication describes the sensitivity to various effectors of two homogeneous enzyme preparations, one derived from cells grown on glycerol and glutamate, and one from cells grown on glucose and growth limiting levels of ammonium chloride.

METHODS

Preparation of Glutamine Synthetases. E. coli strain W was grown aerobically at 37° in a 300-liter fermenter containing 21 mM glycerol, 19 mM glutamate, 1.7 mM $MgSO_4$, 14.3 mM K_2SO_4, 43 mM NaCl, and 0.1 M mixed potassium phosphates to give a final pH of 7.1. The bulk of the volume of all media was tap water. The culture medium containing limiting levels of ammonium chloride was identical except that 4 mM NH_4Cl replaced the glutamate, and 11 mM glucose was substituted for glycerol. In each case enzyme was prepared from frozen cells as previously described (Woolfolk et al., 1966). Both enzyme preparations were homogeneous on disc gel electrophoresis. These two preparations will be referred to as glutamate enzyme and ammonium chloride enzyme.

Assay of Glutamine Synthetase. Glutamine synthetase was assayed by the γ-glutamyl transfer reaction essentially as previously described (Woolfolk et al., 1966) but modified by reducing the concentrations of hydroxylamine and L-glutamine to 20 mM so that effectors competitive with ammonium ion and glutamate could be detected (Shapiro and Stadtman, unpublished data).

RESULTS

Although both enzymes catalyze the γ-glutamyl transfer reaction, and the biosynthesis of glutamine from ATP, glutamate and NH_4^+, they differ markedly in their responses to feedback effectors. Fig. 1 shows the effects of varying concentrations of 5 of the effectors on the two enzyme preparations. It can be seen that the ammonium chloride enzyme is relatively more sensitive to inhibition by glycine than is the glutamate enzyme. On the other hand, the ammonium

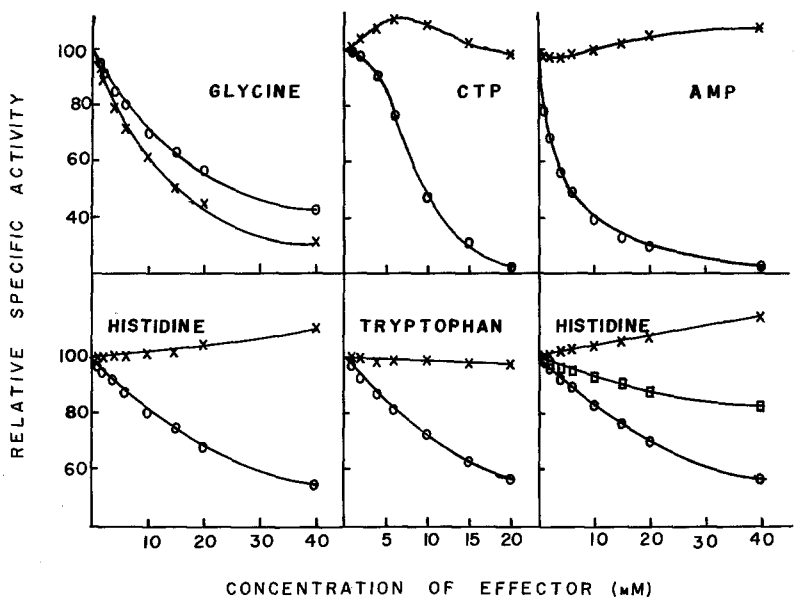

Fig. 1. Effect of varying concentrations of effectors on the two forms of glutamine synthetase. The results presented are from an experiment in which both purified preparations were examined in parallel in the same experiment, using the same assay reagents and the same effector solutions. Assays were run for 15 min. Relative specific activities were calculated by comparison with duplicate controls. O——O glutamate enzyme (1.6 μg); X——X ammonium chloride enzyme (3.0 μg); □——□ mixture of the 2 enzymes containing an equal number of units of each enzyme per ml (0.8 μg glutamate enzyme plus 1.5 μg ammonium chloride enzyme).

chloride enzyme is much less sensitive to inhibition by each of the other 4 effectors, and in fact is stimulated slightly by histidine, AMP and CTP, whereas these compounds cause appreciable inhibition of the glutamate enzyme. The lower right hand frame of Fig. 1 shows that when the two enzymes are added together their combined response to increasing histidine concentration is intermediate to that obtained with each separately.

DISCUSSION

It is evident from these results that the E. coli glutamine synthetase exists in at least 2 forms that differ in their sensitivity to various effectors. Although not definitely established it seems likely that these are metabolically interconvertible forms of a single generic species, since both enzymes are obtained as apparently homogeneous preparations by means of the same purification procedure, they are indistinguishable by disc gel electrophoresis and they possess identical amino acid compositions (Shapiro, Kingdon and Stadtman, unpublished results).

Noteworthy is the fact that when a mixture of the two enzymes is subjected to increasing concentrations of histidine, the extent of inhibition approaches a maximum of about 20%. Such partial inhibition at nearly saturating concentrations of each of eight different feedback inhibitors was previously observed with another apparently homogeneous enzyme preparation from E. coli (Woolfolk and Stadtman, 1967). In view of the present results it is obvious that this partial inhibition could be achieved by a mixed population of two enzyme forms, one of which is susceptible to almost complete inhibition by a given ligand, and the other of which is either activated, or is relatively resistant to inhibition by that ligand. However, such a mixture of enzymes can not readily account for the cumulative response to combinations of inhibitors that was observed in the earlier studies.

Other studies have shown that the E. coli glutamine synthetase is made up of 12 apparently identical subunits that are capable of complete reversible dissociation (Woolfolk, et al. 1966). Since the enzyme exists in two functionally distinct but physically similar forms the possibility exists that when these are both present in a mixture, their reversible dissociations may lead to a complex mixture of hybrid molecules of varying allosteric behaviour. Whether or not such hybridization occurs it is evident that variation in the proportions of the two enzyme forms during growth could give rise to a wide range of response to the different effectors, and could thus provide the basis of a highly flexible system for the feedback regulation of glutamine metabolism. The fact that the two forms of

glutamine synthetase reported here were isolated from cells grown under different conditions of nitrogen nutrition suggests that this phenomenon is important in cellular regulation of nitrogen metabolism in E. coli.

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