

SUMMARY

AND

CONCLUSIONS

1. Preliminary experiments indicated the presence of the enzymes aspartokinase and homoserine dehydrogenase in the two bacterial systems studied namely, Serratia marcescens and Micrococcus glutamicus. The various plant systems screened, cotyledons of french beans (Phaseolus vulgaris) exhibited both the enzyme activities; seedlings Phaseolus mungo and Phaseolus radiatus, weak aspartokinase activity and Cicer arietinum, none. Because of the instability of the plant and M. glutamicus enzymes, it was decided to concentrate on the enzymes of S. marcescens.

2. Tracer studies performed with  $^{14}\text{C}$ -U-aspartate indicated the operation of the aspartate pathway of biosynthesis of lysine, threonine, isoleucine and methionine in S. marcescens; these amine acids contained most of the radioactivity present in the cell proteins.

3. Existence of an alternate pathway for the biosynthesis of isoleucine from glutamate was indicated by dilution experiments with glutamate-U- $^{14}\text{C}$ . Considerable conversion of labelled glutamate to  $\beta$ -methyl aspartate was also observed with fresh cell-free extracts of S. marcescens.

4. Studies on the repression and feedback inhibition of the two enzymes by the different end products showed aspartokinase to be susceptible to both inhibition and repression by lysine. Growth of bacterial cells in the presence of the effectors also indicated the presence of two isoenzymes of homoserine dehydrogenase: one susceptible to inhibition by threonine and the other repressible by methionine.

5. The lysine-sensitive aspartokinase and the two isoenzymes of homoserine dehydrogenase have been separated from one another and purified. Various properties of the three enzymes have been studied.

6. The threonine-sensitive homoserine dehydrogenase has been purified 28-fold, but the purest preparation contains two other proteins.

- (i) It requires, specifically,  $\text{NADP}^+$  for its activity.
- (ii) It has a broad pH optimum of pH 8.8 to more than pH 10.4.
- (iii)  $K_m$  for L-homoserine is 4.0 mM and for  $\text{NADP}^+$ , 0.15 mM.
- (iv) Potassium ions have an activating effect on the enzyme activity; they also protect the enzyme from threonine inhibition.

(v) The molecular weight of the enzyme is around 398,000 in the absence of threonine and 796,000 in its presence.

(vi) 8 M urea has no effect on enzyme activity.

7. The methionine-repressible homoserine dehydrogenase has been purified to homogeneity.

(i) It can act with both  $\text{NAD}^+$  and  $\text{NADP}^+$ .

(ii) It also has a broad pH optimum of 8.8 and 10.5 <sup>between</sup>

(iii)  $K_m$  values for L-homoserine are 3.5 and 3.3 mM with  $\text{NAD}^+$  and  $\text{NADP}^+$  respectively.

(iv)  $K_m$  values for  $\text{NAD}^+$  is 0.3 mM and for  $\text{NADP}^+$ , 0.1 mM.

(v) It has a molecular weight of about 155,000.

(vi) It seems to have two identical subunits, each of a molecular weight of 75,780.

(vii) Its activity is not affected by any of the end product amino acids.

8. The lysine-sensitive aspartokinase has been purified about 45-fold.

- (i) It has an optimum temperature of between 37° and 40°; the energy of activation is 16,600 cal.
- (ii) The enzyme is most active between the pH values of 7.8 and 8.2.
- (iii)  $K_m$  for L-aspartate is 50 mM and for ATP, 6.9 mM.
- (iv) The enzyme requires specifically  $Mg^{2+}$  for its activity; and has only half its maximum activity with  $Mn^{2+}$ .
- (v) The inhibition with lysine is competitive with respect to lysine.
- (vi) The enzyme is inactivated in the presence of 8 M urea.
- (vii) The molecular weight of the enzyme is approximately 280,000.