The Significance of the Substrate Specificity of T2r+--induced Deoxycytidylate Deaminase

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SUMMARY

In contrast to the deoxycytidylate deaminase from animal tissues, the deaminase induced by T2r+ infection of Escherichia coli does not deaminate 5-hydroxymethyl-deoxycytidylate. The depletion of this essential T-even phage deoxyribonucleic acid precursor is thus prevented. Although there is an apparent difference in substrate specificity between these deoxycytidylate deaminases, their ability to regulate the formation of deoxyuridylate by feedback control is quite similar.

The discovery of deoxycytidylate deaminase (1, 2) provided an additional pathway for the synthesis of deoxouridine 5'-phosphate in animal tissues, a pathway apparently not available to most bacteria. However, on infection of Escherichia coli (4, 5) with T2 bacteriophage and Bacillus subtilis with SP-8 (6), a marked increase in deoxycytidylate deaminase occurs. As in the case of the enzyme from animal tissues (7-9), the phage deaminase is subject to feedback control by deoxycytidine triphosphate and deoxythymidine triphosphate (10, 11), signifying the important role this enzyme must play in regulating the supply of deoxythymidine 5'-phosphate for deoxyribonucleic acid synthesis. It has now become apparent that the T-even phage possesses a control mechanism, in addition to induced enzyme synthesis and feedback regulation, which may greatly aid in enhancing the efficiency of its nucleotide interconversions. As shown previously (12, 13), the substrate specificity of deoxycytidylate deaminase from animal tissues although restricted to a deoxyribonucleoside 5'-phosphate was apparently not impaired by substituents in position 5 of the pyrimidine ring. Thus no great difference in the $K_m$ and $V_{max}$ for the 5-methyl, 5-hydroxymethyl, and 5-halogenated derivatives of dCMP was observed. However, in the case of the T2-deaminase assayed in crude extracts or with an enzyme purified about 200-fold, the deamination of 5-hydroxymethyl-dCMP could not be shown although excellent activity was obtained with dCMP and 5-methyl-dCMP (Fig. 1). As indicated by the arrow in Fig. 1, addition of chick embryo deoxycytidylate deaminase to the reaction mixture containing T2-deaminase and 5-hydroxymethyl-dCMP resulted in the immediate deamination of the 5-hydroxymethyl dCMP. Thus a fundamental difference in the specificity of these two enzymes is indicated which is further emphasized by the results in Table I. It is seen here that while the chick embryo enzyme showed little difference in specificity toward the various derivatives of dCMP tested, the T2 enzyme showed a graded reduction in activity as the size of the 5-substituent, from bromine on, increased, until with 5-hydroxymethyl-dCMP no activity at all was obtained. Although the latter compound was not deaminated, it apparently combined with the enzyme since it was found to inhibit the deamination of dCMP. The above results would suggest that the active site of the phage-induced enzyme is restricted in its specie...
Hydrogen Donor Specificity of Cobamide-dependent Ribonucleotide Reductase and Allosteric Regulation of Substrate Specificity*

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SUMMARY

An essentially pure preparation of the cobamide-dependent ribonucleotide reductase from Lactobacillus leichmannii can utilize the purified thioredoxin system from Escherichia coli B as hydrogen donor in place of dihydrolipoate in the reduction of the triphosphates of cytidine, guanosine, adenosine, and uridine. The substrate specificity pattern of L. leichmannii reductase is determined by A1P and various deoxyribonucleoside triphosphates, apparently acting as allosteric effectors.

Recent studies have shown that the reductive conversions of ribonucleotides to deoxyribonucleotides in Lactobacillus leichmannii and Escherichia coli B are catalyzed by enzyme systems of widely differing properties (1-6). Ribonucleotide reductase

It should be reported that Flaks and Cohen (20) isolated small amounts of hydroxymethyl-dUMP from T2-infected cells and indicated that dCMP deaminase may have been responsible for this effect. But in view of the very active deoxycytidylate deaminase in E. coli, the hydroxymethyl-dCMP may have risen by the following pathway: hydroxymethyl-dCMP → hydroxymethyl-deoxycytidine → hydroxymethyl-deoxyuridine → hydroxymethyl-dUMP.

REFERENCES

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