Histidine-2-Oxoglutarate Aminotransferase Activity in *Salmonella typhimurium*

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Recent experiments with *Pseudomonas acidovorans* and *Escherichia coli* B have indicated that a histidine-2-oxoglutarate aminotransferase activity is essential for the utilization of imidazolyl-lactate or imidazolylpyruvate as sole carbon source and L-histidine as a sole nitrogen source respectively for these two organisms (Coote & Hassall, 1969; Wickramasinghe, Hedegaard & Roche, 1967). We have examined and successfully demonstrated in *Salmonella typhimurium* strain LT2, and in several histidine auxotrophs derived from it, the ability to catalyse such a reaction.

The ability to utilize L-histidine as donor in the aminotransferase reaction was determined by the method of Lin, Pitt, Civen & Knox (1958) as modified by Spolter & Baldrige (1963). The assay measures the formation of the enol-borate complex of imidazolylpyruvate. Crude extracts show no activity in the absence of an amino acceptor, specifically 2-oxoglutarate. Oxaloacetate or pyruvate when substituted for 2-oxoglutarate provided 15 and 3.3% of the reaction velocity respectively. Unlike the reaction reported by Coote & Hassall (1969) and by Wickramasinghe *et al.* (1967), no stimulation of activity was observed by the addition of pyridoxal phosphate (final concentration 0.1 mM) to the reaction mixture. The pH optimum for the reaction is about 8.0.

We have compared the ability of cells to carry out the L-histidine-2-oxoglutarate aminotransferase reaction with their ability to carry out the eighth step in the biosynthesis of L-histidine, i.e. the aminotransferase reaction involving L-histidinol phosphate and 2-oxoglutarate (imidazolylacetol phosphate-L-glutamate aminotransferase, EC 2.6.1.9). The assay procedure used for the study of this latter reaction, the growth of strain *HisEIFAHB612* as source of the enzyme and the purification procedure for this enzyme were those reported by Martin & Goldberger (1967). Purification of the enzyme to 95% was achieved. The assay for the biosynthetic aminotransferase measures the back reaction in relation to the actual functioning of the pathway, and for the purposes of this discussion the enzyme is referred to as the biosynthetic aminotransferase or the L-histidinol phosphate-2-oxoglutarate aminotransferase. It was found that the purified biosynthetic aminotransferase would catalyse the L-histidine-2-oxoglutarate reaction. Further, the

following observations were made: that without the addition of an amino acceptor there was no formation of imidazolylpyruvate; that the pH optimum for the reaction was the same as for crude extracts; and that the relative activity with 2-oxoglutarate, oxaloacetate and pyruvate was the same as with crude extracts. Kinetic analyses of the reactions utilizing L-histidinol phosphate or L-histidine as the donor substrate in the presence of 2-oxoglutarate as the acceptor were carried out and apparent Michaelis constants determined for both substrates in the two reactions. With L-histidinol phosphate as substrate, K_m values of 0.25 mM and 0.29 mM were found for L-histidinol phosphate and 2-oxoglutarate respectively. With L-histidine as amino donor, apparent K_m values of 7.9 mM and 0.25 mM were found for L-histidine and 2-oxoglutarate respectively. The ratio of specific activities with L-histidine and L-histidinol phosphate as amino donors is 0.38 and 0.47 for the crude extracts and purified enzyme respectively.

The results reported here are consistent with the interpretation that no detectable aminotransferase activity specifically involved in the degradation of L-histidine exists in *S. typhimurium*. This conclusion is strengthened by the results presented in Table 1. If the utilization of L-histidine as the amino donor in the aminotransferase reaction is carried out by the biosynthetic aminotransferase, then one would expect the specific activity (activity/mg. of protein) of extracts of cells to be reflective of the activity of the histidine operon. The results shown are entirely consistent with this view. Strain *HisHB22* produces only a small amount of functional L-histidinol phosphate-2-oxoglutarate aminotransferase. The L-histidine-2-oxoglutarate aminotransferase activity is also low in these de-repressed cells. Strain *HisCDG63* lacks the gene coding for the biosynthetic aminotransferase, lacks demonstrable activity for all the enzymes involved in L-histidine synthesis (Loper, Grabnar, Stahl, Hartman & Hartman, 1964) and also lacks the ability to utilize L-histidine as an amino donor in the reaction involving 2-oxoglutarate as the acceptor. Strain *HisIF135* demonstrates clearly that the ability to utilize L-histidine in the aminotransferase reaction is directly related to the regulated state of the histidine operon.

Although extracts of *S. typhimurium* efficiently

Table 1. *Aminotransferase activity in strains of S. typhimurium*

All strains were grown under conditions allowing de-repression of the enzymes in the histidine operon (medium of Martin & Goldberger, 1967) except where noted. A repressed operon was obtained by raising the L-histidine concentration to 20 µg./ml. The specific activity (activity/mg. of protein) of the parental strain LT2, which remains constant whether grown under repressed or de-repressed conditions, was assigned a value of 1.0 for each substrate and all other strains were compared with it. The activity in strain LT2 with L-histidinol phosphate is 13.8 nmoles of imidazolylacetol phosphate formed/min./mg. of protein; with L-histidine as substrate it is 5.2 nmoles of imidazolylpyruvate formed/min./mg. of protein

Strain	Relative specific activity	
	L-Histidinol phosphate-2-oxoglutarate aminotransferase	L-Histidine-2-oxoglutarate aminotransferase
LT2	1.0	1.0
<i>HisHB22</i>	1.3	1.5
<i>HisCDG63</i>	0.1	0.1
<i>HisIF135</i>	11.1	9.2
<i>HisIF135</i> (repressed)	2.2	2.5

catalyse an aminotransferase reaction utilizing L-histidine as the amino donor, results obtained *in vivo* suggest that the amino nitrogen is not or is inefficiently utilized further as a nitrogen source. It has been observed previously (Magasanik, 1963) and in the present studies that *S. typhimurium* wild-type strain LT2 fails to utilize L-histidine as a nitrogen source. Strains grown on media containing limiting inorganic nitrogen, but supplemented with L-histidine as an additional nitrogen source, grow only until the ammonia is exhausted from the medium. The possibility that the amount of aminotransferase might be responsible for these observations was examined. We found that the presence or absence of the biosynthetic aminotransferase was without effect. The same result was obtained with constitutive de-repressed strains as with regulated strains or whether succinate, glycerol or glucose was used as the carbon source. Some slight increase occurred in the turbidity of cultures 'growing' on L-histidine, but there was no second rapid growth phase as has been reported for *E. coli* B under the same conditions (Wickramasinghe *et al.* 1967).

The difference between *S. typhimurium* and *E. coli* B in their ability to utilize the amino group of L-histidine (the histidase-urocanase pathway is not a factor here) as an effective nitrogen source may be due to several alternatives: (a) a pool size of L-

histidine in *E. coli* that is larger than in *S. typhimurium*; (b) an L-histidinol phosphate-2-oxoglutarate aminotransferase in *E. coli* that possesses a lower K_m for L-histidine or a higher catalytic-centre activity than does the enzyme from *Salmonella*; (c) the presence of enzymes in *E. coli*, but not in *S. typhimurium*, capable of utilizing the amino group of L-glutamate; or (d) the presence in *E. coli* of a specific aminotransferase responsible for the degradation of L-histidine.

With regard to these alternatives, certainly the concentration of L-histidine in the pools in *Salmonella* would be insufficient to provide nitrogen for rapid growth by utilizing the biosynthetic aminotransferase. This conclusion is based on the observation of Ames (1964) suggesting that in *S. typhimurium* the pool size of L-histidine, in cells suspended in the presence of glucose and under growth-limiting conditions, is maintained at approx. 1 mM, a concentration far below the K_m value for the enzyme. Thus if the pool size in *E. coli* were similar then some other explanation would be necessary. With regard to some alteration in the character of the biosynthetic aminotransferase (alternative b), the finding that the biosynthetic aminotransferase of *S. typhimurium* will utilize L-histidine as a substrate is similar to a reaction described previously for the enzyme from *Neurospora crassa* (Ames & Horecker, 1956). Although addition of pyridoxal phosphate does not influence the aminotransferase activity of the enzyme from *Salmonella* as reported here and previously by Martin & Goldberger (1967), who also demonstrated that the coenzyme is tightly bound to the purified enzyme, it is noteworthy that both the *Neurospora* enzyme (Ames & Horecker, 1956) and the *Pseudomonas* enzyme (Coote & Hassall, 1969) are stimulated by such addition. The degradative aminotransferase from *E. coli* (Wickramasinghe *et al.* 1967) is stimulated by pyridoxal phosphate addition. It might be of some interest to determine whether the biosynthetic aminotransferase from *E. coli* was stimulated by this cofactor. Alternative (c) is unlikely since glutamate can be used as a sole nitrogen source by the strains we tested.

If the last alternative (d) is the case, studies of its structure and regulation in comparison with those of the biosynthetic aminotransferase, which catalyses the same reaction, would be of considerable interest from an evolutionary point of view.

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