HOMOSERINE DEHYDROGENASES OF SERRATIA MARCESCENS Sa-3

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Received September 9, 1971

SUMMARY - (i) At least two methionine-repressible HsDHs are present in S. marcescens Sa-3 and these account for the most (90%) of the HsDH activity, (ii) the NAD^+-activity is completely, and the $NADP^+$-HsDH strongly (75-80%) repressed by L-methionine, L-cysteine and L-homoserine, but both seem to be sensitive only to cysteine among the amino acids tested, (iii) the residual $NADP^+$-activity persisting after growth in presence of L-methionine is inhibited 80% by 10 mM L-threonine and possibly this activity is distinct from the major $NADP^+$-enzyme, and (iv) these HsDH activities are distinct from aspartokinase.

Homoserine dehydrogenase (L-homoserine: $NAD(P)^+$ oxidoreductase, E.C. 1.1.1.3; abbreviated HsDH) is an important enzyme in the biosynthesis of methionine, threonine and isoleucine. Since it is a branch point enzyme, its control is quite important in the regulation of the formation of these amino acids (1).

Both Escherichia coli K_{12} (2-4) and Salmonella typhimurium L-2 (5,6) contain two separable HsDHs; besides, the E.coli K_{12} enzymes possess aspartokinase (AK) activity (3,4). One of these HsDHs is inhibited by L-threonine (3) and its formation is repressed by a combination of L-threonine and L-isoleucine (5). Activity of the second HsDH is not affected significantly by any amino acid but its formation is completely repressed by L-methionine (4,6). In other organisms tested (1, 7-12), HsDH activity is generally repressed by L-methionine (8-10) and in all cases feed-back inhibited by threonine (8-12); often the effects are only partial. The Neurospora crassa HsDH seems to be exceptional; it is neither repressed by methionine nor inhibited by threonine (13). Instances of complete repression by methionine

are rare and in such cases the methionine-repressible activity is quantitatively a negligible (4) or a minor (6) component. We wish to report in this communication that two methionine-repressible HsDHs present in S.marcescens Sa-3 represent the bulk of HsDH activity in this organism unlike in most others.

Amino acids, NAD^+, $NADP^+$ and tris were purchased from the Sigma Chemical Company. Crystalline bovine serum albumin was from Armour Laboratories. All other chemicals were of analytical grade. The strain of S.marcescens Sa-3 and its threonine-less mutant were kindly supplied by Dr. Karlstrom (Royal Institute of Technology, Stockholm).

The bacteria were grown aerobically in shake flasks at 30° in 1% glucose-mineral salts medium of Davis (14). Threonine (100 µg/ml) was added in the case of the mutants. The cells were harvested in the late log phase. Washed cells were suspended in 5 times their weight of 20 mM potassium phosphate buffer (pH 7) containing 150 mM potassium chloride and 20 mM β-mercaptoethanol (referred to as the buffer) and sonically disrupted at 4° to 8° for 10 min in a Branson model B-12 Sonifier. The sonicate was then centrifuged for 30 min at 10,000 x g and the supernatant was dialysed against the buffer at 4°C. Protein was estimated by the method of Lowry et al. (15) using bovine plasma albumin as the standard. HsDH and AK activities were determined according to the procedures of Black and Wright (16) by the reduction of $NAD(P)^+$ with homoserine and by hydroxamate formation respectively. The reaction mixture for HsDH assays contained, in 1 ml, the following components in µmoles: Tris-HCl buffer (pH 8.4), 100; DL-homoserine, 20; EDTA, 1; $NAD(P)^+$, 0.4 and enzyme. For AK-determination, the reaction mixture contained in 1 ml (all in µmoles): aspartate (pH 8), 100; ATP, 20; $MgCl_2$, 20; NH_2OH, 400 and enzyme; incubation 25°, 1 hr. In inhibition-experiments, the reaction mixture contained, in addition to the above, the appropriate amino acids at the concentrations indicated. One unit of HsDH catalyses the formation of one µmole NAD(P)H per min and of AK, a change of

0.001 absorbance at 540 nm per hr. Specific activity (S.A.) is the number of units per mg protein unless otherwise specified.

(1) Growth and Repression. The results are shown in Table 1. None of the amino acids tested was found to inhibit the growth of the bacterium to any considerable extent. Growth with cysteine and homoserine was about 70 to 75% of the controls. The NAD-dependent HsDH is repressed completely by methionine, cysteine and homoserine whereas the $NADP^+$-HsDH activity is strongly but partially repressed by these amino acids. L-threonine repressed poorly. The following preliminary results indicate that the two activities are distinct from one another. The two HsDH

Table 1

Repression of HsDH and growth

Growth supplement		Growth*	HsDH activity				Aspartokinase	
			NAD^+		$NADP^+$			
Compound	Conc. mM		S.A.**	Repression	S.A.	Repression	S.A.	Repression
				%		%		%
None	-	245	6.6	-	6.6	-	170	-
L-threonine	1.0	250	5.0-5.8	12-25	5.0-5.8	12-25	171	0
L-methionine	1.0	240	0	100	1.8	75	173	0
L-isoleucine	1.0	256	6.6-7.2	0	6.6-7.1	0	211	-24
L-homoserine	1.0	215	0	100	1.5	80	224	-35
L-cysteine	1.0	210	0	100	0.64	90	248	-32
L-threonine +L-methionine +L-isoleucine	1.0 each	265	0	100	1.7	74	230	-46

* 16-hr growth starting from 1% inoculum. Figures represent Klett readings at 660 nm.

** S.A. = Specific Activity (mUnits per mg protein)

activities are additive. Addition of $NADP^+$ to the reaction mixture containing NAD^+ enhances the rate of $\Delta A_{340\ nm}$. This increased rate is the sum of the rates of $\Delta A_{340\ nm}$ with each pyridine nucleotide alone. In addition, when acetone-dried cells are extracted with phosphate buffer (20 mM, pH 7), there is a differential extraction of the two activities; the proportions of the two activities vary greatly in successive extracts. In preliminary purification experiments also, the two activities are found enriched in different ammonium sulfate fractions (unpublished experiments). These results clearly indicate that there are at least two HsDHs in S.marcescens Sa-5.

Table 2

Effect of amino acids on HsDH activity

Growth supplement	Amino acid added to Reaction mixture		HsDH Activities				AK	
			NAD^+		$NADP^+$		S.A.	Inhibition
			S.A*	Inhibition	S.A.	Inhibition		
		mM		%		%		%
None	None	-	6.6	-	6.6	-	170	-
	L-threonine	10	6.6	0	6.6	0	185	-9
	L-methionine or L-isoleucine	10	6.6	0	6.6	0	170	0
	L-cysteine	10	0	100	0	100	153	10
	L-lysine	10	N.T**	-	N.T.	-	110	35
L-met (L-cys or L-homo-serine)	None		0	-	1.7	-	210	0
	L-threonine		N.T.	-	0.34	80	210	0
	L-methionine		N.T.	-	1.8	0	210	0
	L-cysteine		N.T.	-	0	100	190	10
	L-isoleucine		N.T.	-	1.2	30	210	0
	L-lysine		N.T.	-	N.T.	-	145	32

* S.A = m Units per mg protein.

** N.T = Not tested

(2) Effect of amino acids on HsDH activities. The effect of the aspartate family amino acids including L-homoserine and of L-cysteine on the activity of HsDHs was studied and the results are shown in Table 2. Among the amino acids tested, only cysteine inhibited strongly both activities from cells grown in unsupplemented media. But HsDH-activity of the cells grown in presence of L-methionine, L-homoserine or L-cysteine was strongly inhibited by L-threonine also. Isoleucine was also inhibitory. This activity is specific for $NADP^+$. This would indicate that either (i) the $NADP^+$-HsDH is modified by growth in presence of L-methionine so that it is strongly inhibited by L-threonine, or (ii) there is a third and minor HsDH which is not repressed by L-methionine but is strongly inhibited by L-threonine. This has to be checked by further purification experiments.

(3) Non-identity of AK and HsDH. Aspartokinase content of the cells was not affected, unlike HsDH activity, by growth in presence of L-methionine (Table 1). An examination of certain threonine-less mutants showed that their HsDH activity was low (from zero to 30% of the prototrophs) but the aspartokinase activity was almost the same as that of the prototroph (unpublished experiments). During the purification of aspartokinase, it was observed that (i) treatment in the absence of KCl with streptomycin to remove nucleic acids also eliminated most of HsDH activity, and (ii) dialysis in the absence of KCl also led to a considerable loss of activity. These treatments did not affect aspartokinase activity (unpublished results). The above results clearly establish that aspartokinase protein is distinct from the HsDHs in this organism.

These results indicate that the HsDHs present in S.marcescens Sa-3 are distinct from the ones previously reported (in E.coli K_{12}, S.typhimurium and other organisms). This strain seems rather unique in having a preponderance of methionine-repressible HsDHs. However, like other methionine-repressible ones, these are also not inhibited to any significant extent by any amino acid except by L-cysteine. Whether or not the very pronounced

inhibition of $NADP^+$-HsDH by threonine (noticed when cells are grown in presence of methionine) is due to a distinct $NADP^+$-HsDH or whether it is due to the same but modified $NADP^+$-HsDH is to be determined by further experiments. This residual $NADP^+$-HsDH activity (about 20 to 25% of the total $NADP^+$-HsDH activity) apparently furnishes the homoserine needed for the biosynthesis of threonine and isoleucine by cells when grown in presence of methionine.

Studies on the purification and properties of these homoserine dehydrogenases are in progress and will be reported elsewhere.

ACKNOWLEDGMENT - One of us (M.S.S.) is thankful to the CSIR and the Director of this Institute for the award of a Junior Research Fellowship.

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