L-SERINE DEAMINATING ENZYMES IN *ESCHERICHIA COLI* CRUDE EXTRACTS

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Received 17 November 1969

1. Introduction

Biosynthetic L-threonine deaminase (biosynthetic TDA), degradative L-threonine deaminase (degradative TDA), and L-serine deaminase (SDA) are three related, but distinct enzymes of *E. coli* [1] of which the first is the best characterized. It requires pyridoxal phosphate as a cofactor and it deaminates both L-threonine and L-serine. The activity of biosynthetic TDA is selectively inhibited by L-isoleucine [1–4], in the biosynthesis of which it is the key enzyme and in media containing this amino acid biosynthesis of the enzyme is repressed [5].

Degradative TDA of *E. coli* is produced by "deep grown" bacteria in yeast extract-tryptone medium [1]. Its cofactor requirement is not clearly established, pyrodoxal phosphate, glutathione, and AMP have all been reported to increase its activity [1]. The degradative TDA of *Cl. tetanomorphum* is activated by pyridoxal phosphate, ADP, and mercaptoethanol [6], but its activity is not influenced by isoleucine. This enzyme also deaminates both L-threonine and L-serine [1].

SDA is optimally produced when *E. coli* are grown in yeast extract-tryptone media [7, 8]. It is a very specific enzyme deaminating only L-serine, D-serine and L-cysteine being competitive inhibitors. The role of this enzyme, which is probably a very labile molecule [8], in the physiology of the bacterial cell has not yet been clarified.

In our recent studies on the physiological role of the SDA in bacteria (to be published) it was first necessary to specify the origin of the measured L-serine deaminating activity in crude extracts, since SDA, biosynthetic TDA, and degradative TDA would all be expected to contribute to this activity. The results are presented in this communication.

2. Materials and methods

The materials and all the methods employed in this work were the same as earlier [8], with the exception that 5 μg pyridoxal phosphate was added to the 1.0 ml assay mixtures for the enzymes. For the TDA measurements L-threonine instead of L-serine was used in the assay. Under our assay conditions degradative TDA activity was not influenced either by ADP, or by other cofactors reported to be active by others [1, 6].

A wild type strain of *E. coli* K12 and its methionine dependent derivative, *E. coli* Hfr-C, (Met^-) were used in this study. But the results obtained were identical with either strain. The L-isoleucine dependent mutant *E. coli* Hfr-C, (Met^-, Ile^-) and the SDA constitutive *E. coli* Hfr-C, (Met^-), S-2 strain were isolated in our Laboratory by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis.

3. Experiments and results

Specific inhibition patterns were used to differentiate the activity of the three individual enzymes. The criteria used to distinguish the enzymes are presented in table 1. The enzyme activity which results in the deamination of L-threonine and L-serine, and which is inhibited by L-isoleucine will therefore be

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Table 1
Substrate specificity and isoleucine sensitivity of the L-serine deaminating enzymes.

Enzyme	Substrate	Effect of L-isoleucine on the enzyme activity
Biosynthetic L-threonine deaminase	L-threonine or L-serine	Inhibition
Degradative L-threonine deaminase	L-threonine or L-serine	No effect
L-Serine deaminase	L-serine	No effect

considered to originate from biosynthetic TDA; the L-serine and L-threonine deaminating activity which is not inhibited by isoleucine, from degradative TDA; and the L-serine deamination which has no equivalent threonine deamination and which is not inhibited by isoleucine, from SDA. The reasons for this differentiation of the three enzymes will now be considered.

The data of table 2 show that, as reported earlier, L-serine deaminating activity of bacteria, grown in yeast extract-tryptone medium, is insensitive to even very high concentrations of isoleucine (10^{-2} M), and has no L-threonine deamination [8]. This pattern is characteristic of SDA.

A low L-threonine deaminating activity is, however, observed in this extract. This activity is not affected by isoleucine and could therefore be considered to be due to degradative TDA. Alternatively, this threonine deaminating activity could even be considered to be a side effect of SDA.

The mutant strain *E. coli* Hfr-C, (Met$^-$), S-2, grown in yeast extract-tryptone medium, deaminates at least ten times more L-serine than the parent strain, nevertheless, its threonine deaminating activity has not increased (table 3). (The decrease in L-threonine deaminating activity of this extract and its partial isoleucine sensitivity is not relevant to our present problem and will not be considered here). The observation that L-serine deaminating activity of this strain is increased without a concomitant increase in threonine deamiantion strongly supports the conclusion that L-threonine deamination by bacteria, grown in yeast extract-tryptone medium, comes not from a side effect of SDA but from degradative TDA.

The very high L-serine deaminase content of this

Table 2
Deaminase activity of *E. coli* Hfr-C, (Met$^-$) extracts. *

Substrate, 4×10^{-2} M	L-Isoleucine –	10^{-2} M	10^{-3} M	10^{-4} M
Bacteria grown in yeast extract – tryptone medium				
L-Serine	296.0	296.0	298.0	298.0
L-Threonine	28.3	28.3	27.7	30.0
Bacteria grown in minimal medium				
L-Serine	37.2	18.1	16.2	19.7
L-Threonine	22.8	0.5	3.7	24.8

* Bacteria were sonically disrupted in 0.2 M potassium phosphate buffer, pH 7.5, and the crude extract used for enzyme assay. Protein content of the 1 ml assay mixtures: (a) Bacteria grown in yeast extract medium, 75 μg; (b) bacteria grown in minimal medium, 214 μg. Enzyme activity is expressed as nmoles of ketoacids produced at 30°C by 10 mg protein/60 min.

Table 3
Deaminase activity of *E. coli* Hfr-C, (Met$^-$), S-2 extracts.*

Substrate 4×10^{-2} M	L-Isoleucine –	10^{-2} M	10^{-3} M	10^{-4} M
Bacteria grown in yeast extract –tryptone medium				
L-Serine	3880.0	3800.0	3800.0	3950.0
L-Threonine	14.1	7.6	8.4	15.2
Bacteria grown in minimal medium				
L-Serine	457.0	447.0	447.0	471.0
L-Threonine	10.7	0.4	1.2	10.5

* Bacteria were sonically disrupted in 0.2 M potassium phosphate buffer, pH 7.5, and the crude extract used for enzyme assay. Protein content of the 1 ml assay mixtures: (a) bacteria grown in yeast extract medium, serine assay 2.45 μg, threonine assay 900 μg; (b) bacteria grown in minimal medium, serine assay 24.6 μg, threonine assay 1350 μg. Enzyme activity is expressed as nmoles of ketoacids produced at 30°C by 10 mg protein/60 min.

Table 4
Deaminase activity of *E. coli* Hfr-C, (Met^-, Ile^-) extracts.*

Substrate, 4×10^{-2} M	L-Isoleucine –	10^{-2} M	10^{-3} M	10^{-4} M
Bacteria grown in yeast extract – tryptone medium				
L-Serine	233.0	225.0	202.0	229.0
L-Threonine	24.2	18.2	23.0	19.1
Bacteria grown in minimal medium				
L-Serine	11.1	12.5	10.5	10.1
L-Threonine	1.0	0.7	1.1	1.2

* Bacteria were sonically disrupted in 0.2 M potassium phosphate buffer, pH 7.5, and the crude extract used for enzyme assay. Protein content of the 1 ml assay mixtures: (a) bacteria grown in minimal medium, 1610 μg. Enzyme activity is expressed as nmoles of ketoacids produced at 30°C by 10 mg protein/60 min.

extract permitted us to measure SDA activity exclusively since, after appropriate dilution, serine deaminating activity of the degradative TDA dose not influence the assay and its presence can be completely neglected.

The data of table 2 show that extracts of bacteria grown in minimal medium deaminate L-threonine and L-serine equally well. Moreover, a part of L-serine deamination, and L-threonine deamination in such extracts is inhibited by isoleucine. This activity pattern indicates the presence of the biosynthetic TDA. The serine deamination which is resistent to the presence of isoleucine has no equivalent threonine activity. It seems therefore reasonable to suggest that SDA is present in addition to the biosynthetic TDA in bacteria grown on minimal medium.

The presence of SDA in bacteria, grown on minimal medium, can also be demonstrated with mutant bacteria. The strain *E. coli* Hfr-C, (Met^-, Ile^-) grows well when supplied with α-ketobutyrate instead of isoleucine, i.e. it is a biosynthetic TDA-less mutant. The data of table 4 show that L-serine deaminating activity of this mutant in minimal medium is low, and cannot be influenced by isoleucine. The biosynthetic TDA content of this extract is only about 1/20 that of the parent strain. It is very probable therefore, that after the mutational loss of the biosynthetic TDA we are observing here pure SDA activity.

Mutant bacteria grown in yeast extract-tryptone medium, as well as the parent strain, deaminate serine and threonine, which demonstrates that the mutation resulting in the loss of the biosynthetic TDA does not influence the intracellular level of SDA and degradative TDA. Our conclusion therefore that the serine deamination measured in the presence of isoleucine in bacteria grown in minimal medium, is due exclusively to SDA seems to be confirmed.

4. Summary

(a) The measured L-serine deaminating activity of a crude bacterial extract may originate from L-serine deaminase, from biosynthetic L-threonine deaminase, or from degradative L-serine deaminase. Nevertheless, the contribution of the individual enzymes can be determined.

(b) About a half of the L-serine deaminating activity of wild type *E. coli* bacteria, grown in synthetic minimal medium, originates from L-serine deaminase and about half from biosynthetic L-threonine deaminase.

(c) Ninety percent of L-serine deaminating activity of wild type *E. coli* bacteria, grown in yeast extract-tryptone medium, originates from L-serine deaminase, and the remainging ten percent from the degradative L-threonine deaminase.

(d) Conditions have been established in which threonine deaminases are eliminated and the activity of L-serine deaminase alone could be measured, even in crude extracts.

Acknowledgement

We would like to acknowledge the assistence of Dr. I.B.Holland in the preparation of the manuscript.

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