D-Amino Acid Dehydrogenase: The Enzyme of the First Step of D-Histidine and D-Methionine Racemization in Salmonella typhimurium*

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Summary. Mutants of Salmonella typhimurium deficient in D-amino acid dehydrogenase were isolated in histidine auxotrophs able to utilize D-histidine (his- dhuA). The mutants have lost the ability to utilize D-histidine and D-methionine due to mutations in the locus dadA mapped in co-transducible vicinity of the gene hemA. The dadA mutants were unable to deaminate D-histidine, D-methionine, D-alanine and several other D-amino acids to the respective keto products. In dad+ strains the enzyme activity was the highest in toluenized cells. In crude sonicates it was 5 to 10 times less. Reduction of artificial electron acceptors in the presence of D-amino acids behaved similarly. Keto product formation was strongly inhibited by cyanide. It has been concluded thereof that the deaminating enzyme is a D-amino acid dehydrogenase, the activity of which depends on structural integrity of a cell component or on a structure-bound electron acceptor. The enzyme activity was inducible by adding L- or D-alanine to growth media. The induction was the highest in media with poor carbon sources. A temperature-sensitive dadA mutant was isolated. It mapped in dadA and had thermolabile D-amino acid dehydrogenase. This has indicated that dadA is structural gene for the D-amino acid dehydrogenase.

Introduction

We have previously reported that mutations dhuA which enable histidine auxotrophs of Salmonella typhimurium to utilize D-histidine increase the activity of histidine transport system (Krajewska-Gryniewicz et al., 1971). It has been deduced thereof that wild-type S. typhimurium is able to racemize D-histidine.

A general study of D-amino acids utilization by Escherichia coli, another species of Enterobacteriaceae, was made by Kuhn and Somerville (1972). They have isolated mutants able to utilize D-histidine (dhu), D-phenylalanine (dfu), D-tyrosine (dyu), D-tryptophan (dadR), D-leucine (dlu), and D-isolcine and D-valine (dvu), as sole sources of the respective L-amino acids. With the exception of dadR, no data have been presented on biochemical changes in metabolism of the D-amino acids resulting from the mutations. DadR mutants isolated as

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1 The nomenclature rules for describing genotypes and phenotypes of Demerec et al. (1966) were followed throughout this paper. E.g. dhuA+ hisP+ mutants have Dhu+ phenotype, those with dhuA+ hisP− mutations are phenotypically Dhu−. All strains with wild-type dhuA+ locus are Dhu+.
D-tryptophan utilizers had increased activity of an enzyme deaminating D-histidine and D-phenylalanine. Mutants dhu have been isolated in *E. coli* also by Oxender (1972), who has found that they have increased activity of leucine transport system. D-Methionine can be utilized by wild-type *E. coli* (Cooper, 1966).

In this paper we describe isolation of *S. typhimurium* mutants *dadA* unable to racemize D-histidine and D-methionine. They lack D-amino acid dehydrogenase, an enzyme inducible by L-alanine, quite likely the same enzyme the activity of which is increased in *dadR* mutants.

### Materials and Methods

#### Strains

Bacterial strains used are derivatives of *S. typhimurium* LT-2, except for TK671 which is LT-2-LT-7 hybrid. They are listed in Table 1. Mutant L4 of the phage P22 (Smith and Levine, 1967) was used for transduction.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or isolation</th>
</tr>
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<tbody>
<tr>
<td>TK200</td>
<td><em>hisCBHAFIE3501 purF145</em></td>
<td>from stock collection</td>
</tr>
<tr>
<td>TK295</td>
<td>HfrK5 <em>dhuA1 hisCBHAFIE3501 hisT1504 purF145</em></td>
<td>from stock collection</td>
</tr>
<tr>
<td>TK508</td>
<td><em>dhuA1 hisCBHAFIE3501 ilvA608 purF145</em></td>
<td>from stock collection</td>
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<tr>
<td>TK603</td>
<td>HfrK5 <em>dadA1 dhuA1 hisCBHAFIE3501 hisT1504 purF145</em></td>
<td>by NTG mutagenesis of TK295</td>
</tr>
<tr>
<td>TK604</td>
<td>HfrK5 <em>dadA1 dhuA1 hisCBHAFIE3501</em></td>
<td>by transduction of TK603</td>
</tr>
<tr>
<td>TK608</td>
<td>HfrK5 <em>dhuA1 hisCBHAFIE3501</em></td>
<td>by transduction of TK604</td>
</tr>
<tr>
<td>TK617</td>
<td>HfrK5 <em>dhuA1 hisCBHAFIE3501 hisT1504</em></td>
<td>by transduction of TK295</td>
</tr>
<tr>
<td>TK622</td>
<td>HfrB1 <em>dhuA1 gal-50 hisD23 ilv-70</em></td>
<td>from stock collection</td>
</tr>
<tr>
<td>TK623</td>
<td>HfrB1 <em>dadA1 dhuA1 gal-50 hisCBHAFIE3501</em></td>
<td>by conjugation TK622 (phenocopy) × TK603</td>
</tr>
<tr>
<td>TK629</td>
<td>HfrB1 <em>dadA1 dhuA1 gal-50 hisCBHAFIE3501 leu-1210</em></td>
<td>by DES mutagenesis of TK623</td>
</tr>
<tr>
<td>TK632</td>
<td>HfrK4 <em>dhuA1 hisD3566 serA13</em></td>
<td>from stock collection</td>
</tr>
<tr>
<td>TK633</td>
<td>HfrK4 <em>dadA1 dhuA1 hisCBHAFIE3501</em></td>
<td>by conjugation TK632 (phenocopy) × TK603</td>
</tr>
<tr>
<td>TK635</td>
<td>HfrK4 <em>dadA1 dhuA1 hisCBHAFIE3501 pro-715</em></td>
<td>by DES mutagenesis of TK633</td>
</tr>
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