SHORT COMMUNICATION

Some Properties of D-Mannose Isomerase from *Escherichia coli* K12

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A second-stage mutant of *Escherichia coli* K12 designated as strain 806 grew faster on D-lyxose than the mutant strain 805 previously described. Both mutants produced constitutively a novel enzyme, D-mannose isomerase, but strain 806 produced twice as much as strain 805. The enzyme could fortuitously convert D-lyxose to D-xylulose, which is a normal intermediate in the D-xylose catabolic pathway. The purified enzyme consisted of four subunits each with a molecular weight of about 40000. In 0.14 M-Na_2SO_4, the tetramer dissociated completely into dimers. While the tetramer $K_m$ values for D-mannose and D-lyxose were 80 mM and 300 mM, respectively, the dimer $K_m$ values for these two sugars were both 300 mM. The amino acid composition of the enzyme was also determined.

INTRODUCTION

Derepression has been frequently used by micro-organisms to acquire new growth abilities (see, for example, Wu, 1978; Clarke, 1978). The derepressed enzyme can usually be induced by its native substrate, and fortuitously convert the novel carbon source to a metabolizable intermediate. However, in two instances reported, the derepressed enzyme appeared to be non-functional under normal circumstances. A mutant of *Escherichia coli* K12 selected for growth on D-arabitol (Wu, 1976) produced a D-galactose dehydrogenase constitutively, but D-galactose is normally phosphorylated to D-galactose 1-phosphate in *E. coli* K12 (Kalckar et al., 1959; Yamolinsky et al., 1959). Another mutant of *E. coli* K12 selected for growth on D-lyxose (Stevens & Wu, 1976) produced a D-mannose isomerase constitutively, but again D-mannose is normally transported into *E. coli* K12 by the phosphotransferase system (Kundig et al., 1966) as D-mannose 6-phosphate (Markovitz et al., 1967). It was suggested that these enzymes were possible evolutionary remnants (Wu, 1978).

The D-galactose dehydrogenase from *E. coli* K12 was extremely unstable and thus could not be purified. On the other hand, the D-mannose isomerase from *E. coli* was somewhat more stable and has been partially purified (Stevens & Wu, 1976). In order to examine some of the properties of the latter enzyme, we have tried to purify it to near homogeneity from a second-stage mutant of *E. coli* K12 selected for faster growth on D-lyxose, which produced about twice the amount of D-mannose isomerase as compared with the original mutant. In the present paper, its subunit structure and amino acid composition are reported.

METHODS

*Bacteria*. Strain 805 of *E. coli* K12 was the original strain selected for growth on D-lyxose (Stevens & Wu, 1976). Strain 806 is described in Results.


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Chemicals. All special chemicals and enzymes used were purchased from Sigma, except: (NH₄)₂SO₄ (enzyme grade) from Schwarz/Mann; DEAE-cellulose from Bio-Rad; Sephadex G-100, G-200 and blue dextran 2000 from Pharmacia; Bacto-agar and casein acid hydrolysate from Difco; and dithiothreitol from Calbiochem.

Medium, growth conditions, enzyme assays and enzyme purification. These were as previously described (Stevens & Wu, 1976), except that the pH step used in enzyme purification was replaced by simple (NH₄)₂SO₄ fractionation.

Polyacrylamide gel electrophoresis. Since D-mannose isomerase could not penetrate the commonly used 5% (w/v) polyacrylamide gel effectively, a 2% polyacrylamide gel supported with 1% (w/v) agarose was used. The location of protein bands was established by naphthol blue-black stain, and enzyme activity in the gel was measured with the use of an automatic gel grinding fractionator (Autogeldivider; Savant Instruments). Completely dissociated subunits were obtained by incubating the enzyme at 65 °C in the presence of 1% (w/v) sodium dodecyl sulphate (SDS) and 0.5% (w/v) dithiothreitol for 4 h. SDS–polyacrylamide gel contained 0.1% SDS and 5% polyacrylamide.

Molecular weight determination. On SDS–polyacrylamide gel, molecular weights were determined by the method of Shapiro et al. (1967), using glucose oxidase (mol. wt 150000), glucose-6-phosphate dehydrogenase (52000) and myoglobin (17000) as standards. On Sephadex G-200 or G-100, molecular weights were estimated by the method of Andrews (1969, using blue dextran 2000 (for excluded volume), aldolase (mol. wt 158000), ovalbumin (45000) and chymotrypsinogen A (25000) as standards.

Amino acid composition. The enzyme sample (containing about 10 mg protein) was dialysed against 4 l of 0.05 M-NH₄HCO₃ for 24 h at 25 °C, and dried by lyophilization. The dried protein was collected in 6 M-HCl, sealed under vacuum, and maintained at 113 °C for 24 or 72 h. After four cycles of drying and washing, the hydrolysed amino acids were dissolved in 0.2 M-sodium citrate at pH 2.2 and analysed by a Durran Laboratories amino acid analyser. The values obtained were extrapolated to 'zero-hour' hydrolysis, except for leucine and isoleucine residues for which the maximum values were used. The amount of cysteine residue was not determined. The ratio of tryptophan to tyrosine was estimated by the method of Edelhoch (1967), with the absorption spectrum of the enzyme in 6 M-guanidine.HCl at pH 6.5 determined by a Cary-14 recording spectrophotometer.

RESULTS

Second-stage mutant. On plating lo⁹ cells of strain 805 on 0.2% (w/v) D-lyxose plates, we noticed a few large colonies formed after incubation at 37 °C for several days. One of them was purified and designated as strain 806. The doubling time of strain 806 in 0.2% D-lyxose liquid medium was about 2 h, while that of strain 805 was previously found to be around 3.1 h under identical conditions (Stevens & Wu, 1976). The only other difference found between the two strains was that strain 806 produced about twice as much D-mannose isomerase in 1% (w/v) casein acid hydrolysate medium as strain 805. The enzymes from these strains appeared completely identical, and strain 806 was therefore used to obtain purified enzyme for subsequent studies.

Homogeneity of purified enzyme. The most purified fraction of D-mannose isomerase gave one peak on Sephadex G-200, with an estimated molecular weight of about 160000, similar to that of D-xylose isomerase from Streptomyces albus (Hogue-Angeletti, 1975). In 2% polyacrylamide and 1% agarose gel, it gave one major peak containing over 90% of the protein, with a small number of low molecular weight impurities. The activity peak coincided with the major protein peak. As discussed below, the impurity might be the dissociated subunits of the enzyme.

In SDS–polyacrylamide gel, the dissociated D-mannose isomerase again gave one major protein peak, with some high molecular weight impurities, which were probably due to incomplete dissociation. The monomer molecular weight was estimated to be about 40000, similar to that of D-lyxose isomerase from Klebsiella (Aerobacter) aerogenes (Anderson & Allison, 1965).

Dissociation in Na₂SO₄. The purification procedure made use of DEAE-cellulose column chromatography (Stevens & Wu, 1976), with the purified fraction eluting as a single peak at a step of 0.14 M-Na₂SO₄ in the eluant. It was thus suspected that this salt concentration might dissociate the subunits. The purified D-mannose isomerase was then filtered on Sephadex G-100 columns, either in the absence of Na₂SO₄ or in the presence of 0.14 M-Na₂SO₄. In the
Fig. 1. Relative activity in the presence of various concentrations of Na$_2$SO$_4$ of D-mannose isomerase from E. coli K12, with D-mannose (●) or D-lyxose (○) as substrate. The maximum activities with D-mannose and D-lyxose, respectively, were 44·3 and 12·4 μmol product formed min$^{-1}$ (mg protein)$^{-1}$.

Table 1. Amino acid composition of D-mannose isomerase from E. coli K12

The numbers of amino acid residues per subunit were calculated assuming a subunit molecular weight of 40 000. For comparison, the numbers of amino acid residues for D-xylose isomerase from Streptomyces albus (Hogue-Angeletti, 1975) are also listed.

<table>
<thead>
<tr>
<th>Amino acid residue</th>
<th>D-Mannose isomerase Molar percentage</th>
<th>No. of residues</th>
<th>D-Xylose isomerase No. of residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>9.3</td>
<td>34</td>
<td>45</td>
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<tr>
<td>Thr</td>
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</tr>
<tr>
<td>Ser</td>
<td>9.1</td>
<td>33</td>
<td>9</td>
</tr>
<tr>
<td>Glx</td>
<td>12.1</td>
<td>44</td>
<td>38</td>
</tr>
<tr>
<td>Pro</td>
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</tr>
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<td>31</td>
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<tr>
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</tr>
<tr>
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<td>8</td>
</tr>
<tr>
<td>Ile</td>
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<tr>
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<td>26</td>
<td>36</td>
</tr>
<tr>
<td>Tyr</td>
<td>3.6</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>Phe</td>
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<tr>
<td>His</td>
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<tr>
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</tr>
<tr>
<td>Trp</td>
<td>3.6</td>
<td>13</td>
<td>8</td>
</tr>
</tbody>
</table>

ND, Not determined.

absence of Na$_2$SO$_4$, the enzyme was eluted in a volume corresponding to a molecular weight of about 160 000. With 0·14 M Na$_2$SO$_4$, however, it dissociated completely into dimers with a molecular weight of about 80 000.

In order to concentrate this enzyme for electrophoresis, 80% saturation of (NH$_4$)$_2$SO$_4$ was used. Under that condition, the D-mannose isomerase might have dissociated further. If reassociation was not complete, small portions could have been permanently inactivated. This might account for the small amount of low molecular weight impurity in the 2% gel.

Substrate specificities of tetramers and dimers. Since this D-mannose isomerase could easily be converted under very mild conditions from tetramers to dimers, we investigated whether its activities on D-mannose and D-lyxose were different in these two states. Enzyme activities were thus measured at various concentrations of Na$_2$SO$_4$ (Fig. 1). Its activity on
D-lyxose showed a gradual decrease as the salt concentration was increased. However, on D-mannose, the activity showed a sharp drop of about 25% at a concentration of about 0.1 M Na₂SO₄, presumably indicating the transition from tetramers to dimers.

On measuring $K_m$ values for D-mannose and D-lyxose, we found an increase in the $K_m$ for D-mannose from 80 mM in the absence of Na₂SO₄ to 300 mM at 0.14 M Na₂SO₄. On the other hand, the $K_m$ for D-lyxose was the same (300 mM) in the absence of Na₂SO₄ and in the presence of 0.14 M Na₂SO₄. The $V_{max}$ values were not significantly changed under these conditions.

**Amino acid composition.** The molar percentages of amino acid residues for D-mannose isomerase from *E. coli* K12 are listed in Table 1. Aspartic acid and asparagine were not distinguished, nor were glutamic acid and glutamine. Cysteine was not determined. The numbers of residues for each subunit were calculated from the molar percentages, assuming a molecular weight of 40 000. For comparison, numbers of residues for D-xylose isomerase from *Streptomyces albus* (Hogue-Angeletti, 1975) are also listed in Table 1. The latter enzyme contained only one cysteine residue per subunit.

**DISCUSSION**

Being a possible evolutionary remnant, the D-mannose isomerase from *E. coli* K12 may provide useful information concerning the effect of time on structural genes which are completely turned off. Random mutations might in principle produce deletions of such genes, or introduce termination codons so that no useful translational products can result. However, these are drastic changes. On the other hand, one might encounter gradual alterations. Indeed, in our present study, we have discovered two relatively mild mutational consequences.

The first is that random mutations can reduce the affinity of the enzyme for its native substrate, since the $K_m$ of D-mannose isomerase from *E. coli* K12 on D-mannose (about 80 mM) was about an order of magnitude larger than those of similar enzymes from other micro-organisms, e.g. *Pseudomonas saccharophila* (Palleroni & Doudoroff, 1956), *Klebsiella (Aerobacter) aerogenes* (Anderson & Allison, 1965), *Mycobacterium smegmatis* (Hey-Fergusen & Elbein, 1970), *Xanthomonas rubrilineans* (Takasaki et al., 1964) and *Streptomyces aerocolonigenes* (Takasaki, 1967).

The second is that random mutations can reduce the dimer–dimer interaction in tetramers, since the tetrameric form of D-mannose isomerase from *E. coli* K12 could easily be dissociated into dimers under very mild conditions. Furthermore, the dimeric form had even lower affinity than the tetramer for its native substrate, suggesting that formation of tetramers might be a mechanism to increase the efficiency of the enzyme. Dissociation to monomeric forms of D-mannose isomerase required more severe conditions, a situation similar, to a certain extent, to that found for haemoglobin (Antonini & Brunori, 1970).

The amino acid composition of the *E. coli* K12 D-mannose isomerase was somewhat different from that of D-xylose isomerase from *Streptomyces albus* (Hogue-Angeletti, 1975) (Table 1), although the molecular weights and subunit structures of these two enzymes were similar. The D-mannose isomerase had many more serine residues and many fewer phenylalanine residues. It also had somewhat more isoleucine, tyrosine, histidine, lysine and tryptophan residues, and somewhat fewer aspartic acid (or asparagine), proline, alanine, leucine and arginine residues.

Further characterization of this novel D-mannose isomerase from *E. coli* K12 may involve amino acid sequencing, detailed genetic mapping, DNA sequencing, etc. In addition, it will be of utmost importance to discover the control mechanism involved in the process of derepression of enzymes which are thought to be evolutionary remnants.

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REFERENCES


