Biochemical and Genetical Studies on Ribose Catabolism in Escherichia coli K12

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SUMMARY

A gene specifying ribokinase has been located on the Escherichia coli chromosome close to the reported position of an uncharacterized ribose-negative mutation. This uncharacterized mutant has been shown to lack ribose permease and thus the genes for two enzymes of ribose catabolism are close together on the chromosome.

INTRODUCTION

Two mutants of Escherichia coli K12 unable to grow on ribose as their sole carbon source have been reported in the literature (Taylor & Trotter, 1967; Anderson & Cooper, 1969) but on the information available it was not possible to decide whether the two were biochemically identical. In this paper we show that one of them (Taylor & Trotter, 1967) has a defect in ribose permease and that the chromosomal location of ribokinase is very close to the reported position of ribose permease.

METHODS

Bacteria. The two ribose-negative mutants used in these studies were AT715 (Taylor & Trotter, 1967) derived from AB259 (HfrH) and AA100 (formerly called R-1) (Anderson & Cooper, 1969) derived from PA309 (F-). Strain AT2614 (phoSI, ilv-12, SmR, F-) was used as recipient in the phage transduction experiments.

Growth of bacteria. Bacteria were grown in liquid minimal medium or nutrient broth as described by Ashworth & Kornberg (1966).

Uptake experiments. To measure ribose uptake, the cells were grown aerobically to mid-exponential phase (about 0.34 mg. dry wt/ml.) in either Oxoid nutrient broth or, to induce the enzymes of ribose catabolism, in nutrient broth supplemented with 10 mM ribose. Organisms grown in the presence of ribose were harvested, washed with 2 vol. of nutrient broth and resuspended in nutrient broth to the original volume before use. Nutrient broth-grown cells were used directly. The cell suspension was cooled to 25° and 2 ml. added to a flask containing, in 30 μl., 1 μCi of [1-14C]ribose and 0.2 μmoles of ribose (7.5 × 10⁶ c.p.m./μmole ribose). Samples (0.2 ml.) were withdrawn at known times and treated as described by Morgan & Kornberg (1969) but using 10 ml. of scintillation fluid.

Assay of enzymes. Cell-free extracts were prepared as described previously (Anderson & Cooper, 1969). Ribokinase was assayed at 30° by the method of Horecker (1967)

* All sugars are D-isomers unless otherwise specified.
and protein was determined by the Folin–Ciocalteu method (Lowry, Rosebrough, Farr & Randall, 1951) using crystalline bovine serum albumin as the standard.

Fructose 6-phosphate (F6-P) formation from ribose 5-phosphate (R5-P) was measured in a reaction mixture containing (per ml.): 50 μmoles of tris-HCl, pH 7.5; 5 μmoles of MgCl2; 0.25 μmoles of NADP; 2μg. of crystalline glucose 6-phosphate dehydrogenase; 4 μg. of crystalline phosphoglucone isomerase; 2.5 μmoles of R5-P and 400 μg. of crude bacterial protein. The change in absorbance at 340 nm. was measured at 30° on a recording spectrophotometer.

Genetic techniques. Phage P1kc transduction experiments were carried out by standard techniques (Glover, 1962). The phage lysate was added to recipients (multiplicity = 1) and suitable dilutions were spread on to appropriately supplemented glucose minimal medium. The transductants were streaked on to the appropriate plates to determine the inheritance of the unselected markers. To determine the inheritance of the PhoS marker, the transductants were grown aerobically for 36 h. in nutrient broth +0.1M-sodium phosphate, pH 7.5. A sample (0.5 ml.) of the suspension was toluenized for 10 min. at 37° then 0.2 ml. of 0.5M-tris-HCl buffer, pH 8.6, and 0.2 ml. of 0.05M-p-nitrophenyl-phosphate were added. After 5 min. incubation at 37° the reaction was stopped by the addition of 1 ml. of 1N-NaOH. The alkaline phosphatase constitutive (PhoS-) transductants gave a bright yellow colour whilst the repressible (PhoS+) transductants remained colourless.

RESULTS AND DISCUSSION

Although the mutant AT715 failed to grow on ribose as sole carbon source, it grew normally on other pentoses such as xylose and L-arabinose, and on glycolytic and gluconeogenic compounds such as glucose and lactate. This suggested that the defect in AT715 was in an enzyme specific to ribose catabolism. Since AT715 grew normally on uridine which is catabolized to yield ribose 5-phosphate (R5-P) (Kammen, 1967), it was probably in one of the enzymes which leads to the formation of internal R5-P from external ribose; that is, either ribose permease or ribokinase. The experiments reported here have been carried out to investigate these possibilities.

When ribose uptake by nutrient broth-grown organisms was measured, AB259 (the parental strain of AT715) incorporated [1-14C]ribose at a fast and constant rate, but no incorporation of radioactivity could be detected when AT715 was used (Fig. 1). When the organisms were grown in the presence of ribose, the rate of [1-14C]ribose incorporation by AB259 was increased threefold but again no uptake could be detected with AT715. In contrast, a spontaneous revertant of AT715 which had regained the ability to grow on ribose was found to incorporate radioactivity to the same extent as did AB259. Thus the inability of AT715 to grow on ribose appears to be due to the absence of a functional ribose permease.

In support of the view that the AT715 lesion affects a ribose permease, we find that the mutant can still synthesize ribokinase. It can be seen from Table 1 that nutrient broth-grown AT715 contains ribokinase at about half the specific activity of its parent, AB259. This ribokinase produced R5-P since crude extracts of AT715 form F6-P as a result of the non-oxidative reactions of the pentosephosphate pathway when either R5-P or ribose + ATP were supplied as substrate. Although growth in the presence of ribose led to an increase in the ribokinase activity of AB259, that of AT715 was unchanged suggesting that external ribose could not be converted to the internal inducer.
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in this mutant. Thus the ribose-negative AT715 differs from the ribose-negative AA100, which is unable to grow on ribose because it lacks ribokinase (Anderson & Cooper, 1969). This view is further supported by the observation that the ribokinase-negative mutant can take up ribose (Fig. 1). The initial rate of ribose uptake by AA100 was identical to that of its parent, PA309, but subsequently the rate decreased rapidly in the mutant whilst that of the parental strain remained constant. It is likely that this

![Graph](image)

Fig. 1. Accumulation of [1-14C]ribose by nutrient broth-grown Escherichia coli strains AT715 (△), AA100 (▲), PA309 (○), AB259 (●). For experimental details, see text.

Table 1. Ribokinase activity in AT715 and AB259

Ribokinase activity was measured according to Horecker (1967) as the ATP-dependent removal of ribose. The reaction mixture at 30° contained in 1 ml.: tris-HCl buffer, pH 8.0 (100 μmoles); KCl (50 μmoles); MgCl2 (5 μmoles); ATP (5 μmoles); Escherichia coli protein (20 to 1000 μg.) and ribose (1 μmole).

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Ribose phosphorylated (μmoles/mg. protein per min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient broth</td>
<td>0.038 AB259</td>
</tr>
<tr>
<td>Nutrient broth + ribose</td>
<td>0.166 AT715</td>
</tr>
</tbody>
</table>

Table 2. Order of the ilv, rbsK and phoS loci

<table>
<thead>
<tr>
<th>P1 donor...</th>
<th>AA100</th>
<th>+</th>
<th>rbsK</th>
<th>+</th>
<th>phoS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient...</td>
<td>AT2614</td>
<td>ilv-12</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Percentage of Ilv+ transductants that are:

<table>
<thead>
<tr>
<th>Rbs⁻ Pho⁻</th>
<th>Rbs⁻ Pho⁺</th>
<th>Rbs⁺ Pho⁻</th>
<th>Rbs⁺ Pho⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>20</td>
<td>20</td>
<td>7</td>
</tr>
</tbody>
</table>
difference is due to the inability of AA100 to phosphorylate the internal ribose and thereby further metabolize it. When PA309 and AA100 were grown on nutrient broth + ribose there was a threefold increase in the rate of $[1^{-14}C]$ ribose incorporation by PA309 but, surprisingly, no increase for AA100. The reason for this is not clear at the moment, but it could be that in the absence of ribokinase the organisms are unable to form the inducer for ribose permease synthesis. An alternative explanation, that the permease and kinase are functionally linked for the uptake of ribose seems unlikely, since we have other mutants which also lack ribokinase but which take up ribose very rapidly to a high level since they form ribose permease constitutively (R. A. Cooper, unpublished observations).

These results show that the two ribose-negative mutants AT715 and AA100 are biochemically distinct. Since the genetic location of the AT715 lesion, identified in the present work as ribose permease (rbsP), is known (Taylor & Trotter, 1967) it was of particular interest to determine the chromosomal location of the ribokinase gene (rbsK) to see whether the two genes were closely linked.

Preliminary studies on the location of rbsK by interruption of conjugation experiments indicated that it was close to 74 min. on the Escherichia coli linkage map (Taylor & Trotter, 1967) and cotransduction studies showed appreciable linkage of rbsK to ilv (73%) and a lower linkage to metE (2.5%). The position of the rbsK locus was determined more precisely by a three-factor cross infecting strain AT2614 with Ptkc grown on the Rbs$^-$ donor. Ilv transductants were selected and subsequently analysed for the inheritance of the unselected markers Rbs$^-$ and Pho$^+$. Among 250 Ilv$^+$ transductants 73% had inherited the Rbs$^-$ marker and 27% the Pho$^+$ marker. As Table 2 shows, the Rbs$^+$Pho$^+$ recombinants were the least frequent category among the four possible phenotypes. If we assume that those transductants formed as a result of a minimum of four crossovers will occur significantly less frequently than those transductants formed as a result of a minimum of two crossovers, these results suggest that rbsK is situated between phoS and ilv. Since rbsP is also located between phoS and ilv, and the high linkage of rbsK to ilv (73%) is identical to the reported linkage of rbsP to ilv (Taylor & Trotter, 1967), rbsK and rbsP must be very close together on the Escherichia coli chromosome.

Considering the physiological roles of ribose permease and ribokinase it seems unlikely that their close chromosomal relationship is fortuitous. It is possible that they belong to a single unit of transcription and this possibility is being investigated at the present time.

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REFERENCES


