APPENDIX

Two Separate Genes Involved in Sulphate Transport in Escherichia coli K12

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INTRODUCTION

In the course of the experiments described above it became clear that the two cys mutations used were not identical. The cysA471 mutation was isolated by Tully (1976) as conferring resistance to chromate, the cysZ474 mutation arose spontaneously and simultaneously with a ptsI mutation, (it may be the result of an inversion with ends in these two genes). The mapping of the genes (see above, Table 3) shows that cysA and cysZ lie on opposite sides of ptsI. We therefore investigated the biochemical nature of the mutations.

METHODS

Exponential phase cells growing in minimal medium supplemented with cystine were harvested, resuspended in the sulphur-free medium of Pasternak (1962) with 10 mM-glucaronate as carbon source and incubated at 37 °C for 9 h to derepress the sulphate transport system. The cells were harvested and resuspended in 5 mM-HEPES buffer, pH 6.5, at a concentration of 0.68 mg dry mass ml⁻¹. Radioactive sulphate [³⁵SO₄⁻] was added to a final concentration of 0.2 mM and 20 μCi ml⁻¹, and the cells were incubated at room temperature. Samples were taken at 10, 60, 120 and 180 s, filtered through an Oxoid membrane filter and washed four times with 2 ml HEPES buffer. The filters were dissolved in 10 ml of scintillation fluid [0.4% 2,5-diphenyloxazole, 80% (v/v) toluene and 20% (v/v) methoxyethanol] and their radioactivity was measured in a Packard 3385 scintillation spectrometer. Background measurements were made under identical conditions and samples of [³⁵SO₄⁻] were assayed to convert the disintegrations measured to moles of sulphate ions. All other techniques were as described in the main paper.

RESULTS

Sulphate transport was assayed in the cysteine auxotrophs JM1783 (cysA) and JM1766 (cysZ) and in isogenic wild-type strains (Table 5). These strains were also tested for their growth response to thiosulphate at 20 mM, a concentration that supports the growth of mutants of Salmonella typhimurium LT2 defective in sulphate transport (Mizobuchi et al., 1962). The results are shown in Table 5. The strains are deficient in sulphate accumulation, but apparently not in the synthesis of cysteine from O-acetylserine and sulphide, since they grow when supplemented with 20 mM-S₂O³⁻.

DISCUSSION

In Salmonella typhimurium LT2 three closely-linked cistrons cysAa, Ab and Ac are required for sulphate transport (Howarth, 1958; Mizobuchi et al., 1962; Ohta et al., 1971), though none of these is the structural gene for the sulphate binding protein. A deletion of all three cistrons (Howarth, 1958) was not reported as being deficient in glucose utilization, suggesting that the known Salmonella cysA cistrons do not bracket ptsH and I. Karbonowska et al. (1977) indicate that the cysA chromate-resistant mutations of E. coli and S. typhimurium are probably in corresponding cistrons. If this is so, the ptsH and ptsI genes of Salmonella are inverted relative to cysA.

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Table 5. Properties of the sulphate transport deficient mutants

Sulphate transport was measured after 9 h of sulphur starvation as described in Methods.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pertinent genotype</th>
<th>Growth</th>
<th>Sulphate transport [pmol min⁻¹ (mg dry mass)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>JM1783</td>
<td>cys⁺</td>
<td>±</td>
<td>260</td>
</tr>
<tr>
<td>JM1781</td>
<td>cysA471</td>
<td>±</td>
<td>40</td>
</tr>
<tr>
<td>CBK110</td>
<td>cys⁺</td>
<td>±</td>
<td>275</td>
</tr>
<tr>
<td>JM1766</td>
<td>cysZ474</td>
<td>±</td>
<td>65</td>
</tr>
</tbody>
</table>

We have not assayed our cys mutants for any of the enzymes of cysteine biosynthesis, most of
the structural genes for which map elsewhere (Jones-Mortimer, 1968, 1973) but the growth of the
mutants on medium supplemented with 20 mM-thiosulphate indicates that at least one of the
isoenzymes of O-acetylserine sulphydrase (Kredich et al., 1980) is present in the strains. In
Salmonella the genes for both isoenzymes map near ptsI (Kredich et al., 1980). Wiater &
Hulanicka (1979) demonstrated a deficiency in sulphate transport in cysK mutants of E. coli.
These, like the cysK mutants of S. typhimurium are defective in O-acetylserine sulphydrase-A
(Hulanicka et al., 1975). Our (unpublished) experiments indicate that the cysZ gene cannot be
identical with cysK, since a plasmid carrying the cysK⁺ gene did not complement the cysZ
defect.

Since SO₄²⁻ ions that enter the cells can presumably be metabolized in the mutants as well as in
the wild-type strains, the observed rates of assimilation must represent the sum of transport and
metabolism.

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