SHORT COMMUNICATION

Isolation and Mapping of Glutathione Reductase-negative Mutants of *Escherichia coli* K12

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Two independent mutants defective in glutathione reductase (EC 1.6.4.2) were isolated in an *Escherichia coli* K12 strain lysogenized with bacteriophage Mu. The prophage was lost (and the ability to reduce glutathione regained) by 32% of the xylose-positive transductants when T4GT7 was used as the vector, but the markers were not cotransduced by P1. Similarly, the prophage site and *malA* were cotransduced by T4GT7 but not by P1. The *gor* gene maps between min 77 and 78 on the *E. coli* genome, and the mutation causes no growth defect.

INTRODUCTION

Glutathione is not an essential metabolite in *Escherichia coli*, since Apontoweil & Berends (1975) and Fuchs & Warner (1975) have shown that mutants unable to synthesize glutathione are not affected in growth. This suggested that mutants unable to reduce oxidized glutathione might also be unimpaired in growth. We have tested this hypothesis by devising a simple method for the isolation of Mu insertion mutants that have lost the NADPH-linked glutathione reductase (EC 1.6.4.2) activity, and have mapped the lesion, to which we have given the symbol *gor* (for glutathione oxidoreductase).

METHODS

Strains. JM2137 is F⁻ (*his gnd*)^Δ *lac araE* Str^R, derived from MC4100, JM2205 is F⁻ *leu argH xyl lysA* :: Muc⁺ *cysA* derived from PA309, JM559 is Hfr *fda*^{ts} (*his gnd*)^Δ derived from Hfr Cavalli (Henderson *et al.*, 1977). CSH25 is F⁻ *pro tyrT* (Miller, 1972) and JGC127 is F⁻ *malA dnaM zhg*::Tn10 (Glassberg *et al.*, 1979). Bacteriophage P1 was from Dr M. D. Yudkin, T4GT7 from Dr K. K. Y. Young, Muc⁺ from Dr B. J. Bachmann and MuctsAp from Professor N. Symonds (Leach & Symonds, 1979).

Media. Oxoid nutrient broth, or the minimal medium of Ashworth & Kornberg (1966), solidified with 1.5% (w/v) Oxoid agar No. 1 when necessary, was used. Tetracycline (Tc) was used at 10 mg l⁻¹ and ampicillin (Ap) at 12.5 mg l⁻¹.

Phage techniques. P1 transduction was carried out according to Lennox (1955); T4GT7 transduction according to Wilson *et al.* (1979). Lysogenization with Mu was as described by Casadaban & Cohen (1978).

Isolation of mutants. MuctsAp infected cells were diluted 10-fold into broth, incubated aerobically at 30 °C for 30 min and plated on minimal agar supplemented with ampicillin to give about 200 colonies per plate after incubation at 30 °C for 2 d.

Screening of colonies for glutathione reductase activity. Each plate was exposed to CHCl₃ vapour (about 1 ml CHCl₃) for about 30 min at room temperature and then left on the bench overnight to allow the oxidation of most of the endogenous thiols. The plates were overpoured with 5 ml of 0.4% agar containing 1.2 mM oxidized glutathione, 0.35 mM-NADPH, 0.6 mM-5,5'-dithiobis-(2-nitrobenzoic acid) (Nbs₂) in 50 mM-potassium phosphate buffer pH 8.0. Colonies showing glutathione reductase activity were scored for being yellow (and being

surrounded by a yellow zone) after 20 to 30 min. The colour faded after about 1.5 h. Colonies containing no glutathione reductase activity remained essentially colourless.

Preparation of cell-free extracts. Strains were grown in nutrient broth overnight to early-stationary phase, harvested, and resuspended in 50 mM-potassium phosphate buffer, pH 8 to about 5 mg dry mass ml⁻¹. Cells were broken with a MSE Soniprep 1500 operating at 6 µm for four periods of 15 s, and debris removed by centrifugation in an Eppendorf centrifuge for 1.5 min. The protein concentration of the supernatant fraction was measured by the Lowry method.

Measurement of glutathione reductase activity. The reaction mixture contained 1.2 mM oxidized glutathione, 0.35 mM-NADPH, 0.6 mM-Nbs₂ and 50 mM-potassium phosphate buffer pH 8 in a final volume of 1 ml at 28 °C. The reaction was started by the addition of enzyme (≤50 µg protein) and followed at 412 nm. It was linear with respect to time and protein concentration and dependent on the addition of both glutathione and NADPH.

RESULTS AND DISCUSSION

Isolation and characterization of mutants

Strain JM2137 was lysogenized with MuctsAp and plated on minimal agar supplemented with ampicillin. The colonies obtained were replica-plated on to a new plate of the same composition, lysed with CHCl₃ and screened for the presence of glutathione reductase activity. The formation of reduced glutathione was detected by the latter's ability to convert Nbs₂ to the yellow Nbs⁻ anion, as described in the Methods. Of approximately 15 000 colonies screened, eight were obtained from two independent experiments that could not reduce glutathione by this criterion. This was probably an underestimate of the number of mutant colonies present, since the diffusion of the yellow Nbs⁻ anion could have obscured a negative colony that was close to a positive one.

Cell-free extracts were made from the wild-type and each of the mutant strains and these were assayed directly for glutathione reductase activity. The wild-type extract reduced glutathione at a rate of 6 nmol s⁻¹ (mg protein)⁻¹. None of the extracts of the mutants showed any detectable activity [less than about 0.3 nmol s⁻¹ (mg protein)⁻¹]. When a sample of the extract of the wild-type was added to the latter assays the predicted rate was obtained. Similarly, a boiled extract of the wild-type strain also showed a rate of 0.3 nmol s⁻¹ (mg protein)⁻¹. Thus, the extracts of the mutants lack glutathione reductase activity and do not contain an inhibitor of the reaction. The mutants were unimpaired in their growth on plates or in liquid culture on either rich or minimal medium.

Mapping of mutants

For convenience of mapping, the *gor* lesion of strain JM2267 was transduced using bacteriophage P1 into the multiply-marked Muc⁺ lysogen JM2205 to yield strain JM2275. This was then mated with strain JM559, a derivative of Hfr Cavalli, to obtain Leu⁺, Arg⁺, Xyl⁺ or Lys⁺ exconjugants. The donor strain was counter-selected by the addition of streptomycin or by the omission of histidine from the medium. Eighty colonies from each selection were scored for the loss of ampicillin resistance: 55% of the Xyl⁺ exconjugants were ampicillin-sensitive and 40% were Mtl⁺ but the segregation of these markers was independent. Of the other exconjugants selected less than 7% of any type had become ampicillin sensitive. These results suggested a map position close to the *xyl* gene and probably on the side away from *mtl*.

This result was confirmed by transduction with P1 and T4GT7 as vectors as shown in Table 1. Analysis of the Xyl⁺ P1 transductants indicates that the prophage is not between *xyl* and *mtl*, yet the Xyl⁺ T4GT7 transductants apparently indicate that the prophage is closer to *xyl* than is *mtl*. This result is only possible if the prophage is integrated counter-clockwise from *xyl*. A sample of 40 of the Xyl⁺ T4GT7 transductants was screened by the glutathione reductase plate assay to confirm that the Ap^s colonies were glutathione reductase positive. An independently isolated glutathione reductase negative mutation was shown to be cotransducible with *xyl* at about the same frequency. A second transduction experiment, using a

The recipient was JM2275 (*xyl mtl gor::MuctsAp lysA::Muc⁺*). The donors were CSH25 (wild-type) for the Xyl⁺ selection and JGC127 (*zhg::Tn10 dnaM malA*) for the Tc^R selection. None of the transductants were Lys⁺; the numbers in the different classes are given.

Phenotype selected	Phenotype scored		Vector	
	Mtl	Ap (gor)	P1	T4GT7
Xyl ⁺	+	S	0	2
	—	R	115	88
	—	S	0	49
	+	R	5	21
Tc ^R (zhg)	Growth at			
	Mal (malA)	41 °C (dnaM)	Ap (gor)	
	—	—	S	0
	+	—	S	2
	+	+	R	93
	—	+	R	85
	—	+	S	0
	+	+	S	0
	+	—	R	47
	—	—	R	13
				2
				178
				0
				60
				9
				37
				19
				70
				1
				0
				27
				77
				46
				89
				104

zhg::Tn10 strain (Glassberg *et al.*, 1979) as donor showed that the gene order was *malA zhg dnaM gor* (Table 1). The *gor* locus therefore appears to map in the cotransduction gap at about min 77–78 on the map of Bachmann & Low (1980).

Glutathione reductase is almost certainly evolutionarily related to lipoamide dehydrogenase (EC 1.6.4.3) to which it shows a close similarity in structure and mechanism (Perham *et al.*, 1978). However, the genes are now widely separated in the *E. coli* chromosome since *lpd* maps at about min 2 on the linkage map (Langley & Guest, 1978; Alwine *et al.*, 1973).

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