Biochemical and Genetic Characterization of *nirB* Mutants of *Escherichia coli* K12 Pleiotropically Defective in Nitrite and Sulphite Reduction

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Mutants of *Escherichia coli* K12 defective in the *nirB* gene lack NADH-dependent nitrite reductase activity and reduce nitrite slowly during anaerobic growth. With one exception, these mutants require cysteine for growth. Cytochrome *c*₅₅₂ synthesis and the assimilation of ammonia are unaffected by the *nirB* mutation. The defective gene is located between the *crp* and *aroB* genes at minute 73 on the *E. coli* chromosome. Mapping and reversion studies indicate that *nirB* is identical to the previously described *cysG* gene. It is suggested that the product of the *cysG*⁺ (*nirB*⁺) gene is an enzyme required for the synthesis of sirohaem, a prosthetic group of enzymes which catalyse the six-electron reduction of nitrite to ammonia and sulphite to sulphide.

INTRODUCTION

We recently reported the isolation of mutants of *Escherichia coli* K12 which are unable to reduce nitrite to NH₄⁺ during anaerobic growth (Newman & Cole, 1978). Some of these mutants were defective in the *nirA* gene located at minute 29 on the recalibrated linkage map of Bachmann *et al.* (1976) and were therefore similar to the mutants described by Cole & Ward (1973). The product of the *nirA*⁺ gene is essential for nitrite reduction and for the synthesis or assembly of a variety of anaerobic redox enzymes which include cytochrome *c*₅₅₂, nitrate reductase, fumarate reductase and formate hydrogenlyase. The *nirA* gene is identical to the *fnr* gene described by Lambden & Guest (1976) and the pleiotropic NirR⁻ phenotype described by Chippaux *et al.* (1978) is probably also due to a defective *nirA* gene.

A second group of mutants appeared to lack only the NADH-dependent nitrite reductase activity (EC 1.6.6.4; Coleman *et al.*, 1978). It was possible, therefore, that these NirB⁻ mutants were defective in a second gene, *nirB*, which is essential for nitrite reduction and that *nirB* is the structural gene for NADH-nitrite oxidoreductase. We now report the biochemical properties of isogenic *nirB* and *nirB*⁺ strains and chromosomal location of the *nirB* gene with reference to the *rpsL*, *crp*, *cysG*, *aroB* and *mal* genes (Bachmann *et al.*, 1976). We also suggest a possible function for the *nirB*⁺ gene product.

METHODS

Organisms. The origins of the various strains of *Escherichia coli* K12 are listed in Table 1. Bacteria were stored at 4 °C as stab cultures in nutrient agar deeps (Oxoid CM1).

Media and growth conditions. Minimal agar for selective media contained (per litre distilled water): 10.5 g K₂HPO₄; 4.5 g NaH₂PO₄; 1 g trisodium citrate.2H₂O; 125 mg Na₂SO₄; 4 g glucose or other carbon

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Table 1. *Escherichia coli* strains and their origins

Strain	Relevant phenotype or genotype*	Source or method of isolation†
AB312	<i>leu rpsL</i> Hfr	B. Bachmann; <i>E. coli</i> Genetic Stock Center
AB2847	<i>aroB351 mal-354</i>	B. Bachmann
CB63	<i>nirB201 pro thr metB rpsL</i>	Conjugate strain 22A43 with CB201 for 3 h; select streptomycin-resistant <i>Leu</i> ⁺ recombinants and screen for <i>Nir</i> ⁻
CB68	<i>nirB201 mal leu</i> ⁺	As for CB63
CB69	<i>nirB201 ilv argA rpsL</i>	Conjugate strain 22A43 with CB201 for 3 h; select streptomycin-resistant <i>Mal</i> ⁺ recombinants and screen for <i>Nir</i> ⁻
CB88	<i>nirB203</i>	Transduce AB2847 to <i>Aro</i> ⁺ with P1 propagated on CB203
CB89	<i>nirB202 Cys</i> ⁻ <i>Mal</i> ⁻	Transduce AB2847 to <i>Aro</i> ⁺ with P1 propagated on JM72
CB98	<i>nirA1 trp his rpsL</i>	See Newman & Cole (1977)
CB201	<i>nirB201 chl</i> Cym ⁻ Hfr	NG mutagenesis of OR56
CB203	<i>nirB203</i>	NG mutagenesis of OR56
CB211	<i>nirA3</i> unknown growth requirement	NG mutagenesis of OR75
CB213	<i>nirB213</i> Cym ⁻	EMS mutagenesis of OR75
CB217	<i>nirB217</i> Cym ⁻	EMS mutagenesis of OR75
JM72	<i>cysG rpsL</i> <i>Mal</i> ⁻	M. Jones-Mortimer
KL209	Hfr with F integrated into <i>malB</i>	B. Bachmann
KLF33/JC1553	F' <i>ilv</i> ⁺ <i>argH</i> ⁺	B. Bachmann
KLF41/JC1553	F' <i>cysG</i> ⁺ <i>aroB</i> ⁺ <i>mal</i> ⁺	B. Bachmann
Lin13	DE44 (<i>bioH</i> to <i>glpD</i>) Δ	B. Bachmann
Lin72	DE14 (<i>glpR5</i> to <i>malA34</i>) Δ	B. Bachmann
OR56	Prototrophic Hfr	R. Curtiss III
OR75	Prototrophic Hfr	R. Curtiss III
W1485E	Prototroph, lysogenic for P1kc	J. R. Guest
22A43	<i>his pro ilv metB argA mal rpsL</i>	M. Jones-Mortimer

* The system of genetic nomenclature is based on the recommendations of Demerec *et al.* (1966). Cym⁻ strains respond to either cysteine or methionine.

† NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; EMS, ethyl methanesulphonate.

source (added as a 20%, w/v, sterile solution after autoclaving the bulk medium); 5 ml sulphur-free trace metals (Cole *et al.*, 1974); and 12 g Oxoid agar. Inocula for batch cultures were aerated at 37 °C for 6 to 8 h in 20 ml Lennox (1955) broth and transferred to nitrogen-free salts supplemented with Lennox broth, 20 mM-NH₄Cl and 2 mM-NaNO₂, as described by Newman & Cole (1978).

Preparation of cell-free extracts and enzyme assays. These were described previously (Newman & Cole, 1978). Harvested bacteria resuspended in TEA buffer (50 mM-Tris/HCl, 5 mM-EDTA and 5 mM-sodium ascorbate buffer, pH 8.0) were broken in the French pressure cell. TEA buffer was used for all subsequent procedures.

Genetic methods. Bacteriophage P1kc released from an overnight culture of *E. coli* W1485E was used for transduction experiments (Lambden & Guest, 1976). For conjugation experiments, early-exponential phase cultures of donor (1 ml) and recipient (10 ml) bacteria in Lennox broth were incubated at 37 °C without aeration. After 30 min (unless otherwise stated), 1 ml of the mixed culture was transferred to 9 ml minimal salts, vortexed vigorously for 1 min and plated on selective media. Strains auxotrophic for aromatic amino acids were grown on minimal agar supplemented with tryptophan, phenylalanine and tyrosine (each at 20 µg ml⁻¹) and sodium shikimate (1 µM).

Mutants unable to reduce nitrite were isolated as described by Cole & Ward (1973); the modified procedure was used to score the *Nir* phenotype of various strains (Newman & Cole, 1978). Other procedures have been described by Newman & Cole (1977, 1978).

RESULTS

Both *nirA* and *nirB* mutants were isolated as strains unable to use nitrite as sole nitrogen source during anaerobic growth: other properties of the original isolates are summarized in Table 2. The mutations were induced by treating wild-type bacteria with ethyl methane-

Table 2. Comparison of two types of mutant of *E. coli* unable to reduce nitrite

Strain	Phenotype	Formate hydrogen-lyase	Nitrite reduced during growth (mM)	Final cell density (A_{650})	NADH-nitrite oxidoreductase*	Cytochrome c_{552} †
OR56	Wild-type	+	2.0	0.88	90	35
CB201	NirB ⁻	—	0.5	0.75	2	35
CB203	NirB ⁻	+	0.4	0.75	5	55
OR75	Wild-type	+	2.0	0.73	200	33
CB211	NirA ⁻	—	< 0.1	0.94	8	< 5
CB213	NirB ⁻	+	1.1	0.82	< 2	40
CB217	NirB ⁻	+	ND	ND	< 2	13
JM72	NirB ⁻	+	< 0.1	ND	< 2	112

ND, Not determined.

* Activities are expressed as nmol NADH oxidized h⁻¹ (mg protein)⁻¹.† Concentrations are expressed as pmol (mg protein)⁻¹.

sulphonate (strains CB213 and CB217) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (CB201 CB203 and CB211). Some of the mutants might therefore carry secondary mutations unconnected with the nitrite reductase defect. Strain CB201 was resistant to 10 mM-KClO₃ during anaerobic growth and was therefore unable to catalyse the formate hydrogenlyase reaction. Strains CB201, CB213 and CB217 required either cysteine or methionine during growth in minimal media and CB211 was auxotrophic for unidentified growth factors.

Rates of synthesis of cytochrome c_{552} , glutamate dehydrogenase and glutamine synthetase and, unlike the *nirA* strains described previously, the regulation of the latter two enzymes by NH₄⁺ were similar in *nirB* mutants and wild-type bacteria. The NirB⁻ strains reduced nitrite extremely slowly during growth and washed bacterial suspensions were defective in nitrite reduction with glucose as the electron donor (Table 2). There was a close correlation between the NADH-dependent nitrite reductase activity of cell-free extracts and the rates of nitrite reduction during growth (Table 2).

Conjugation experiments to map the *nirB* gene

Data from conjugation experiments designed to determine the approximate chromosomal location of the defective gene responsible for the NirB⁻ phenotype are summarized in Table 3, together with conclusions derived from them. The first four experiments indicate that strain CB201 carries a wild-type *nirA*⁺ gene and that the defective nitrite reductase gene, designated *nirB201*, is probably located between the *his* and *ilv* operons, possibly close to *argA*. The preferential inheritance of the recipient *nirB* allele by the recombinants when streptomycin was used to select against the donor suggested that the *nirB* gene is located close to the streptomycin-resistance determinant, *rpsL*. This was confirmed in experiments with the streptomycin-resistant Hfr strain AB312 in which *nirB*⁺ was shown to be closely linked to the donor *rpsL* and *mal*⁺ genes (Table 3, experiments 5 and 6).

The plasmids KLF33 and KLF41 which carry wild-type genes covering, respectively, the 82 to 89 and 68 to 74 minute regions of the chromosome were transferred into recipient *nirB* strains (Table 3, experiments 7 and 8). All Mal⁺ transconjugants which had inherited KLF41 were Nir⁺; KLF41 therefore carries a wild-type *nirB*⁺ allele which is trans-dominant to the defective *nirB* allele. In contrast, KLF33 did not complement the *nirB* defect carried by strain CB63.

Genes adjacent to the *mal* operon have been deleted from strains Lin13 and Lin72. Although Lin72 is Nir⁺, Lin13 is Nir⁻ and is also auxotrophic for unknown growth requirements other than biotin, aromatic amino acids and cysteine. We suggest that the *nirB* gene, or another gene close to it, is absent or defective in Lin13.

Table 3. *Conjugation data to determine the approximate location of the nirB gene*

Expt no.	Donor	Recipient	Selected phenotype	Percentage of Nir ⁺ recombinants	Conclusion
1	CB201 NirB ⁻ Chl ^R	CB98 <i>nirA</i> Chl ^S <i>trp</i> <i>his</i> <i>rpsL</i>	Chl ^R Str ^R	0	Chl defect in CB201 is unlinked to <i>nirA</i> or <i>nirB</i>
			His ⁺ Str ^R	32	<i>nirB</i> is not close to <i>nirA</i> or <i>his</i> and CB201 is <i>nirA</i> ⁺
2	CB201 NirB ⁻	22A43 Nir ⁺ <i>mal</i> <i>argA</i> <i>ilv</i> <i>rpsL</i>	Mal ⁺ Str ^R	75	<i>nirB</i> is not closely linked to <i>mal</i> , <i>argA</i> or <i>ilv</i> but could be close to <i>rpsL</i>
			Arg ⁺ Str ^R	68	
			Ilv ⁺ Str ^R	82	
3	OR75 Nir ⁺	CB63 <i>nirB</i> <i>pro</i> <i>thr</i> <i>metB</i> <i>rpsL</i>	Early Pro ⁺ Str ^R	0	<i>nirB</i> is located anti-clockwise with respect to <i>pro</i>
			Late Pro ⁺ Str ^R	0	<i>nirB</i> is located anti-clockwise with respect to <i>thr</i>
			Early Thr ⁺ Str ^R	0	
			Late Thr ⁺ Str ^R	0	<i>nirB</i> is located anti-clockwise with respect to <i>metB</i>
			Early Met ⁺ Str ^R	8	
			Late Met ⁺ Str ^R	75	
4	KL209 Nir ⁺	CB69 <i>nirB</i> <i>ilv</i> <i>argA</i> <i>rspL</i>	Ilv ⁺ Str ^R	7	<i>nirB</i> is closer to <i>argA</i> than to <i>ilv</i>
			Arg ⁺ Str ^R	61	
5	AB312 Nir ⁺ <i>rpsL</i>	CB201 <i>nirB</i> <i>rpsL</i> ⁺	Str ^R	90	<i>nirB</i> is close to <i>rpsL</i>
6	AB312 Nir ⁺ <i>leu</i>	CB68 <i>nirB</i> <i>mal</i> <i>leu</i> ⁺	Mal ⁺ Leu ⁺	83	<i>nirB</i> is close to <i>mal</i>
7	KLF33/JC1553	CB63 <i>nirB</i> <i>metB</i>	Met ⁺	0	<i>nirB</i> is not located between minutes 82 and 89, or is dominant to <i>nirB</i> ⁺
8	KLF41/JC1553	CB68 <i>nirB</i> <i>mal</i>	Mal ⁺	100	<i>nirB</i> is located between minutes 68 and 74, on the segment carried by KLF41; <i>nirB</i> ⁺ is trans-dominant to <i>nirB</i>

Transduction experiments to locate the nirB gene

The conjugation data suggested that *nirB* might be cotransducible by bacteriophage P1 with *mal*⁺ or *aroB*⁺. This was confirmed when AB2847 was transduced to Mal⁺ and Aro⁺ with phage which had been propagated in CB201: 25% of the Aro⁺ and 7% of the Mal⁺ transductants were Nir⁻ (Table 4, experiment 1). The Nir⁻ phenotypes of strains CB203, CB213, CB217 and of two other less studied isolates were also cotransducible with *aroB*⁺ and *mal*⁺ at similar frequencies (Table 4, experiment 2, and data not shown). These *nirB* defects are therefore located on the *rpsL* side of *aroB*, probably close to *cysG* (Fig. 1).

The only *cysG* mutant available was JM72: this reduced nitrite even more slowly than the new NirB⁻ mutants. Normal quantities of cytochrome *c*₅₅₂ were synthesized by JM72, but NADH-dependent nitrite reductase was inactive. Strain JM72 was therefore phenotypically NirB⁻.

Strain CB201 was transduced to *rpsL* with phage P1 that had been propagated in JM72 and the Nir and Cys phenotypes of streptomycin-resistant transductants were determined (Table 4, experiment 3). Methionine was added to selective media to satisfy the cysteine or methionine (Cym⁻) requirement of the recipient. All *rpsL* transductants were Nir⁻. Although 93% had retained the Cys⁺ Cym⁻ phenotype of the recipient, it was apparent that the defec-

Table 4. Transductions to establish the relative positions of the *nirB*, *cysG* and *aroB* genes

Expt no.	Donor	Recipient	Selected phenotype	No. scored	Unselected recombinant classes	Per-centage in each class	
1	CB201 <i>nirB201</i>	AB2847 <i>aroB mal</i>	Aro ⁺	108	Mal ⁺ Nir ⁺	21	
					Mal ⁺ Nir ⁻	8	
					Mal ⁻ Nir ⁺	50	
					Mal ⁻ Nir ⁻	21	
			Mal ⁺	108	Aro ⁺ Nir ⁺	23	
					Aro ⁺ Nir ⁻	6	
					Aro ⁻ Nir ⁺	71	
					Aro ⁻ Nir ⁻	0	
2	CB88 <i>nirB203</i>	AB2847 <i>aroB</i>	Aro ⁺	107	Nir ⁺	70	
					Nir ⁻	30	
3	JM72 <i>rpsL cysG</i>	CB201 <i>nirB201</i> Cym ⁻	Str ^R	100	Cys ⁺ Nir ⁺	0	
					Cys ⁺ Nir ⁻ *	93*	
					Cys ⁻ Nir ⁺	0	
					Cys ⁻ Nir ⁻	7	
4	OR75 <i>nir⁺ cys⁺ mal⁺</i>	JM72 <i>cysG</i> Mal ⁻	Cys ⁺	100	Nir ⁺	100	
					Nir ⁻	0	
			Mal ⁺	100	Cys ⁺ Nir ⁺	100	
					Cys ⁺ Nir ⁻	0	
					Cys ⁻ Nir ⁺	0	
					Cys ⁻ Nir ⁻	0	
5	AB2847 <i>aroB mal</i>	JM72 <i>cysG</i> Nir ⁻ Mal ⁻	Cys ⁺	105	Mal ⁺ Aro ⁺ Nir ⁺	53	
					Mal ⁺ Aro ⁺ Nir ⁻	0	
					Mal ⁺ Aro ⁻ Nir ⁺	11	
					Mal ⁺ Aro ⁻ Nir ⁻	0	
					Mal ⁻ Aro ⁺ Nir ⁺	0	
					Mal ⁻ Aro ⁺ Nir ⁻	0	
					Mal ⁻ Aro ⁻ Nir ⁺	37	
					Mal ⁻ Aro ⁻ Nir ⁻	0	
6	JM72 <i>cysG</i> Mal ⁻	AB2847 <i>aroB mal</i>	Aro ⁺	97	Mal ⁺ Cys ⁺ Nir ⁺	33	
					Mal ⁺ Cys ⁻ Nir ⁻	8	
					Mal ⁻ Cys ⁺ Nir ⁺	46	
					Mal ⁻ Cys ⁻ Nir ⁻	13	
			Mal ⁺	97	Aro ⁺ Cys ⁺ Nir ⁺	38	
					Aro ⁺ Cys ⁻ Nir ⁻	0	
					Aro ⁻ Cys ⁺ Nir ⁺	62	
					Aro ⁻ Cys ⁻ Nir ⁻	0	
7	CB88 <i>nirB203</i>	CB89 <i>nirB202 cysG</i>	Cys ⁺	269	Nir ⁺	5	
					Nir ⁻	95	

* All of these transductants were Cym⁻.

tive *cysG* allele in the donor has been cotransduced with *rpsL* at a low frequency. The absence of any Nir⁺ transductants showed that the nitrite reductase defect carried by strain JM72 is either not cotransducible with the streptomycin resistance determinant, *rpsL*, or is located extremely close to the nitrite reductase defect, *nirB201*, in strain CB201. The defect in strain JM72 was tentatively designated *nirB202*.

Totally unexpected results were obtained when JM72 was transduced with P1 that had been propagated in the wild-type, OR75. All Cys⁺ transductants were Nir⁺ and all Mal⁺ transductants were Cys⁺ Nir⁺ (Table 4, experiment 4).

The relative positions of the *nirB202*, *cysG* and *aroB*⁺ genes in strain JM72 were determined in reciprocal transductional crosses with the Aro⁻ Mal⁻ strain AB2847. Both the

donor and the recipient were Mal⁻: nevertheless, almost identical numbers of Mal⁺ and either Aro⁺ or Cys⁺ transductants were isolated on appropriate selective media (Table 4, experiments 5 and 6). This high recombination frequency suggests that one of these strains carries a defective *crp* gene at minute 72.5 rather than a defect in the *mal* operon at minute 74 on the *E. coli* linkage map. The distribution of inheritance of unselected markers was consistent with a defective *mal* gene in strain AB2847 and a defective *crp* gene in JM72. If this is correct, strain JM72 should be unable to ferment arabinose (Pastan & Perlman, 1970): this prediction was confirmed. Furthermore, all Cys⁺ transductants were also Nir⁺, and Cys⁻ recombinants were Nir⁻. The Cys⁻ and Nir⁻ defects of strain JM72 therefore appear to be due either to two defects extremely close together or to a single defect which might be a point mutation or a deletion.

The close proximity of the *nirB202* and *nirB203* mutations was established when strain CB89 *nirB202 cysG* was transduced to Cys⁺ with phage propagated in CB88 *nirB203 Cys*⁺. Only 5% of the 269 transductants screened were Nir⁺ (Table 4, experiment 7).

Simultaneous reversion of NirB mutants to Cys⁺ and Nir⁺

Samples (0.1 ml; 5×10^8 bacteria) of an overnight culture of strain CB201 were spread on glucose-minimal agar plates; some plates were seeded with a small crystal of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Cys⁺ revertants were readily isolated on both types of plate: all were Nir⁺. Similar results were obtained with Cys⁺ revertants of CB213 and CB217, but no Cys⁺ revertants of JM72 were isolated in approximately 10 independent experiments, or on the control plates from 20 transduction experiments.

Biochemical properties of isogenic nirB and nirB⁺ transductants

The nitrite reductase activity and concentration of cytochrome *c*₅₅₂ in cell-free extracts of various pairs of otherwise isogenic *nirB* and *nirB*⁺ strains were determined. In each case, the *nirB* mutant was deficient in NADH-dependent nitrite reductase activity. Furthermore, with the exception of *nirB203* strains, each mutant either required cysteine for growth, or its growth rate was faster in the presence of cysteine or methionine.

DISCUSSION

Chromosomal location of the nirB⁺ gene and its identity with cysG⁺. The determination of the relative positions of the *rpsL*, *nirB*, *cysG*, *aroB* and *mal* genes was hampered by the incorrect assumption that strain JM72 carried a defect in the *mal* operon rather than in the *crp* gene and by difficulties in scoring the Cys and Nir phenotypes of different classes of recombinant. Cys⁻ transductants were frequently lost during transduction procedures, so the observed recombination frequencies between the *cysG* and *aroB*⁺ alleles of JM72 were abnormally high. The growth rate and therefore the rate of nitrite reduction during anaerobic growth in media supplemented with nitrite were higher for Aro⁺ than for Aro⁻ strains, so it was essential to score unselected auxotrophic markers and sort recombinants into their various classes before their Nir phenotypes could be determined. Finally, there were wide variations in the response of different NirB⁻ isolates to cysteine or methionine. Thus, JM72 was an obligate cysteine auxotroph; CB201, CB213 and CB217 required either cysteine or methionine, but the growth rate of strain CB203 was inhibited by cysteine and this mutant formed normal-sized colonies on unsupplemented minimal agar. Despite these complications, the results in Table 4 establish that the NirB⁻ phenotypes are pleiotropic effects of mutations in the *cysG* gene which is located between *crp* and *aroB*, as shown in Fig. 1.

The lack of a direct selection procedure for Nir⁺ strains (other than the restoration of a Cys⁺ phenotype) and the high reversion frequencies of the new NirB⁻ isolates have pre-

Fig. 1. Location of the genetic defects in various *NirB*⁻ mutants. In (a) the relative positions of genes used to locate the *nirB* defects in the minute 72 to 74 region of the *E. coli* chromosome are shown: arrows indicate the origin of transfer of the various Hfr strains used for conjugation experiments. This region is expanded in (b) to show the relative positions of four of these lesions.

vented us from determining whether they belong to one or more complementation groups. Nevertheless, the isolation of 13 *Nir*⁺ recombinants from a transduction experiment in which CB88 *nirB203* was the donor and CB89 *nirB202 cysG* the recipient indicates that the *nirB203* defect is to the right of the *nirB202* defect (Fig. 1b). The stability of JM72 to reversion to Cys⁺ or *Nir*⁺ and our failure to isolate Cys⁺Crp⁻ transductants suggest that JM72 is deleted for at least part of the *cysG* (*nirB*) region and that the postulated deletion terminates outside the *crp* gene (Fig. 1b). Furthermore, the defect carried by CB201 lies within, or extremely close to, the end of this deletion.

The biochemical basis for the pleiotropic phenotype of cysG strains. The pleiotropic-negative phenotypes of *cysG* (*nirB*) mutants are inconsistent with the proposal that *nirB* is the structural gene for NADH-nitrite oxidoreductase. It is more likely that this gene encodes a protein which must be functional for the six-electron reduction of both SO₃²⁻ to S²⁻ and NO₂⁻ to NH₄⁺. Structural similarities between sulphite reductase from *E. coli* and nitrite reductase from the fungus *Neurospora crassa* include the presence of sirohaem as well as FAD and a non-haem iron-sulphur centre (Siegel *et al.*, 1973; Greenbaum *et al.*,

1978). Although sirohaem could not be found in small samples of nitrite reductase from *E. coli* purified previously in this laboratory (Coleman *et al.*, 1978; Jackson *et al.*, 1979), a haem signal similar to that of sirohaem has recently been detected spectroscopically (R. Jackson, unpublished results). When the protein is purified to apparent homogeneity (as judged by sodium dodecyl sulphate–polyacrylamide gel electrophoresis) in stabilizing buffer, it is both highly active and orange. In the absence of stabilizing agents (FAD, EDTA and ascorbate) it rapidly becomes inactive, colourless and haem-free. We suggest, therefore, that the NADH-nitrite and NADPH-sulphite oxidoreductases from *E. coli* share a common prosthetic group, sirohaem, and that *cysG* encodes an enzyme required for sirohaem synthesis. The *hemA* product, δ -aminolaevulinic acid synthase, is also required for sirohaem synthesis (McConville & Charles, 1979), so the above hypothesis is consistent with the previously unexplained observation that *hemA* mutants of *E. coli* are unable to reduce nitrite (Cole & Brown, 1980). The Cys⁺Nir⁻ phenotype of CB203 would be compatible with this hypothesis if there are multiple genes for sirohaem synthesis in the *cysG* region, only some of which are required for inserting sirohaem into sulphite reductase. This implies that although the haem groups of sulphite and nitrite reductases are similar, they may not be identical. Alternatively, CB203 might be a leaky mutant which synthesizes sufficient sirohaem to satisfy the very low rate of cysteine biosynthesis but insufficient for nitrite reduction. A third possibility is that CB203 is defective in another nitrite reductase gene which is not involved in sirohaem synthesis (see also Abou-Jaoude *et al.*, 1979). Until the tentative identification of sirohaem as a prosthetic group of nitrite reductase and the role of the *cysG* (*nirB*) product in its synthesis have been confirmed, we suggest that the use of the *nirB* designation should be discontinued.

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