The kefC gene of *Escherichia coli* encodes a potassium efflux system that is gated by glutathione (GSH) and by GSH adducts. Independently isolated kefC mutations that result in spontaneous activation of the efflux system have been analyzed. Three mutations affect residues located adjacent to the conserved Rossman fold in the carboxyl-terminal domain. Two mutations lie in a sequence predicted to form a cytoplasmically located loop in the membrane domain of KefC. All of the mutants retain normal regulation by the YabF protein and by GSH adducts. A mutation in the Rossman fold, R416S, alters the normal regulation of KefC by GSH. In contrast to the wild-type protein, which is inactive in the presence of GSH, the R416S mutant is only active in the presence of GSH or its analogue, ophthalmic acid. Other mutations in this region or elsewhere in the protein have their spontaneous activity augmented by depletion of the GSH pool. These data identify a specific role for the carboxyl-terminal domain of KefC in regulating KefC activity and are discussed in the light of recent data that suggest that GSH adducts can bind within a Rossman fold.

The KefB and KefC potassium efflux systems play a major role in protection of *Escherichia coli* cells against the toxicity of electrophiles. The addition of electrophiles to *E. coli* cells, or the stimulation of their synthesis in the cytoplasm, elicits the activation of these two efflux systems through the formation of glutathione (GSH) adducts (1–3). KefB and KefC are independently isolated efflux systems through the formation or the stimulation of their synthesis in the cytoplasm, elicits potassium loss from the cell, occurs when the GSH is conjugated to electrophiles through the sulfhydryl group (1, 8). Slower efflux is observed when GSH is removed from cells by mutations affecting the *gshA* gene (7). KefC is, therefore, a ligand-gated transport system with both negative (GSH) and positive (GSH adducts) effectors. This feature is common in many ion channels, and the organization of the protein is also consistent with a channel-like mechanism.

KefC belongs to a family of membrane proteins that includes Na+/H+ antiports and regulatory proteins. One of the significant differences within this group of proteins is the greater complexity of organization of the KefC protein. The carboxyl-terminal domain is similar in hydrophobicity to many other transport proteins; however, there is located within it a potential voltage-gated sequence (RXXX) followed by a series of hydrophobic residues. This is a structural feature similar to that found in many mammalian voltage-gated K+ channels (10, 12). The protein lacks the distinctive P-loop that would place it in the voltage-gated K+ channel superfamily (10). Attached to the carboxyl terminus of the membrane protein via an acidic linker peptide is a “soluble” carboxyl-terminal domain, which contains a Rossman fold that has high sequence similarity to yeast glyceraldehyde-3-phosphate dehydrogenase (11). Finally, genetic evidence supports a homo-oligomeric organization for KefC (13) and the existence of a separate regulatory subunit, YabF for the *E. coli* KefC system with homologues identified for KefB and the KefX systems of *E. coli* and *Haemophilus influenzae*, respectively (10). These features reflect the complexity required to retain appropriate control over KefC activity.

Here we report the analysis of mutations that cause spontaneous activation of KefC. Five alleles have been identified; two are found in the membrane-located domain of the protein and three are close to the Rossman fold domain. Further analysis of one of the mutations in the Rossman fold motif (R416S) shows that the mutation alters the normal regulation by GSH. These data are consistent with a model in which the carboxyl-terminal domain of KefC plays a major role in regulating the activity of the protein and may be the location of the GSH-binding site.

**MATERIALS AND METHODS**

*Reagents—*All of the chemical reagents were purchased from Sigma or BDH and were of analytical grade when possible. Ophthalmic acid was obtained from Bachem Ltd. (United Kingdom). The chemicals used for preparation of complex growth medium were supplied by Oxoid. Restriction enzymes, T4 DNA polymerase, and universal sequencing primers were supplied by Boehringer Mannheim. The Qiagen plasmid preparation kits were obtained from Qiagen. Other primers designed and used in this study were purchased from Genosys Biotechnologies, Inc. or supplied by Dr. F. Carter. The Wizard PCR Preps DNA purification system was obtained from Promega. The PRISM Ready Reaction DyeDeoxy Terminator cycle sequencing kit was obtained from Applied Biosystems Ltd. The Mut-Gen Phagemid *in vitro* mutagenesis kit, version 2, was purchased from Bio-Rad.

*Bacterial Strains, Plasmids, and Bacteriophage—*All of the strains and plasmids used in this study are listed in Table I. Phage M13K07 used in this study was purchased from Bio-Rad. P1kc phage was used for transductions.

*Media for Growth of Strains—*The minimal medium K (where *x* indicates the approximate concentration [mM] of potassium) was used for the culture of cells for potassium transport experiments (14). Solid medium containing less than 1 mM potassium was prepared by repeated
washing of the agar in 1 M NaCl to remove contaminating potassium ions in the agar and followed by rinsing with distilled water prior to sterilization. The complex medium LK was prepared as described previously (15). The medium used in the preparation of uracil-containing single-stranded DNA was prepared as described (16). Where required, media were supplemented with ampicillin (25 μg ml⁻¹), tetracycline (25 μg ml⁻¹), chloramphenicol (12.5 μg ml⁻¹), or kanamycin (25 μg ml⁻¹).

Growth Conditions—Cells were grown at 37°C in LK complex medium (15) for use in DNA manipulations. For potassium transport studies, cells were grown in K120 minimal medium at 37°C. The medium (15) for use in DNA manipulations. For potassium transport studies, cells were grown in K120 minimal medium at 37°C.

Restriction enzyme digestion and DNA ligation were followed with the exception that single-stranded uracil-containing template DNA was prepared by M13K07 infection of CJ236 and transformed with pSM7 (16). The mutagenic primers were supplied with 5'-phosphorylation: KefCR416S (GGAGAGCAGTAA

Growth Conditions—Cells were grown at 37°C in LK complex medium (15) for use in DNA manipulations. For potassium transport studies, cells were grown in K120 minimal medium at 37°C. The medium (15) for use in DNA manipulations. For potassium transport studies, cells were grown in K120 minimal medium at 37°C.

PCR Analysis of kefC Mutants—PCR primers were designed using the published sequence of kefC (11). The primers used to amplify yabF were designed using a sequence obtained from the genome sequencing project (see Ref. 17; accession number P51577 GB.Eco110K). Primer pairs were designed to amplify yabF and kefC in 350–400-base pair products that overlapped in sequence by approximately 100 base pairs to ensure that there would be no gaps in the sequence. PCR amplification was used single colonies of the appropriate strain inoculated into the reaction mix to provide template DNA. Reactions were carried out in a total volume of 100 μl containing each primer at 0.25 μM dNTP (200 μM each) 2.5 units of Taq DNA polymerase and reaction buffer as supplied by Boehringer Mannheim) and overlaid with approximately 30 μl of mineral oil. Once optimized so that only one product was observed by agarose gel electrophoresis, the PCR reaction was purified using the Wizard PCR Prep DNA purification system prior to sequencing. The PRISM Ready Reaction DyeDeoxy Terminator cycle sequencing kit was used to perform cycle sequencing reactions on PCR products or plasmids, using the protocol outlined by the supplier of the kit. Samples were run on an Applied Biosystems 373A DNA sequencer. The cloning junctions of plasmids created in this study were verified using the universal primers supplied by Boehringer Mannheim. All sequencing reactions were performed a minimum of two times on both strands of the template.

Site-directed Mutagenesis of kefC—Plasmid pKC952 carries the entire kefC gene and 152 base pairs of upstream sequence on a HindIII-BamHI fragment cloned into pHG165 (13). The HindIII-BamHI kefC-bearing fragment from pKC952 was cloned into the phagemid vector pTZ19U (18) creating the mutagenesis vector pSM7. Site-directed mutagenesis of kefC by PCR amplification of the desired mutation. Primer KefCS420A2 (CACCCC

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**Table I**

| Strain | Genotype | Source |
|--------|----------|--------|
| CJ236  | dist-1, ung-1, thi-1, relA-1; pCJ105 (Cm<sup>b</sup>) | Bio-Rad<sup>a</sup> |
| Frag5  | F<sup>b</sup> , ldpABC5, thi, rha, lacZ | W. Epstein<sup>a</sup> |
| Frag 56| Frag 5, gshA::Tn10(Kan) | W. Epstein |
| MJF276 | Frag5, lacI, kefB, kefC::Tn10 | This laboratory |
| MJF335 | MJF276 gshA::Tn10(Kan) | This laboratory |
| MJF374 | MJF276 Δ[ysfF-kefC], Δ[ysfA-hisB] | This laboratory |
| MX1190 | Δ[lac-proAB], thi, supE, Δori-rectA; Tn10 [F-truD36, proAB, lacF<sup>b</sup> ÆM15] | Bio-Rad |
| TK03   | Frag5 kefC103 | W. Epstein/I. R. Booth |
| TK04   | Frag5 kefC104 | W. Epstein/I. R. Booth |
| MJF104 | TK04 kefC::Tn10 | W. Epstein/I. R. Booth |
| TK116  | Frag5 kefC116 | W. Epstein/I. R. Booth |
| TK119  | Frag5 kefC119 | W. Epstein/I. R. Booth |
| TK121  | Frag5 kefC120 | W. Epstein/I. R. Booth |
| TK121  | Frag5 kefC121 | W. Epstein/I. R. Booth |
| TK136  | Frag5 kefC136 | W. Epstein/I. R. Booth |

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<sup>b</sup> W. Epstein, Dept. of Molecular Genetics and Cell Biology, the University of Chicago, Chicago, IL.

<sup>c</sup> Also known as a phagemid.

**Plasmid Description Source/Ref.**

| Plasmid | Description | Source/Ref. |
|---------|-------------|-------------|
| pHG165  | pBR322 copy number derivative of pUC8 | Ref. 19 |
| pKC952  | HindIII-BamHI fragment carrying kefC (and retaining 152 base pairs 5' to the ATG) | Ref. 13 |
| pTZ19U<sup>c</sup> | Derivative of pUC19 with insertion of fl origin of replication | Bio-Rad |
| pSM7   | HindIII-BamHI kefC-bearing fragment of pKC952 cloned into HindIII-BamHI sites of pHG165 | This study |
| pSM9   | pSM7 encoding KefCR416S created by in vitro site-directed mutagenesis | This study |
| pSM10  | pSM7 encoding KefCS420A created by in vitro site-directed mutagenesis | This study |
| pSM12  | HindIII-EcoRI kefC-bearing fragment of pSM7 cloned into HindIII-EcoRI sites of pHG165 | This study |
| pSM14  | HindIII-EcoRI kefCR1416S-bearing fragment of pSM9 cloned into HindIII-EcoRI sites of pHG165 | This study |
| pSM15  | HindIII-EcoRI kefCS420A-bearing fragment of pSM10 cloned into HindIII-EcoRI sites of pHG165 | This study |
| pSM19  | pSM7 encoding KefCD264A created by in vitro site-directed mutagenesis | This study |
| pSM21  | pSM7 encoding KefCR1416S, S420A created by in vitro site-directed mutagenesis | This study |
| pSM26  | HindIII-EcoRI kefCS420A-bearing fragment of pSM19 cloned into HindIII-EcoRI sites of pHG165 | This study |
| pSM30  | HindIII-EcoRI kefCR1416S, S420A-bearing fragment of pSM21 cloned into HindIII-EcoRI sites of pHG165 | This study |

# Reference

1. Bio-Rad Laboratories Ltd., Hemel Hempstead, Hertfordshire, U. K.
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3. Also known as a phagemid.
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RESULTS

Mutations in the kefC locus that affect potassium retention were selected as isolates unable to grow on K<sub>10</sub> medium by ampicillin-enrichment of UV-mutagenized cells of <i>E. coli</i> Frag5. The strains grew normally when incubated in K<sub>10</sub> medium (data not shown). We have previously shown that the K<sub>0.1</sub> mutants after growth in K120 medium, filtration, washing, however, each of the mutants displayed a significantly lower extent of spontaneous K<sup>+</sup> loss than 80% of its K<sup>+</sup> pool over this period. Five strains exhibited a unique efflux profile (strains TK104 and TK121 and TK116 and TK120; (●), TK136).

Missense mutations in the kefC strains

The missense mutations in the strains were identified by PCR amplification of overlapping regions of kefC and direct sequencing of the products. The parent strain Frag5 was used as a positive control in the PCR-based analysis and has the same kefC sequence as that reported previously (11). The mutations were the result of single base pair mutations that were found on both strands of the template. The resulting codon change is indicated, together with the encoded amino acid alteration.

| Strain | DNA mutation (base)<sup>a</sup> | Codon change | Amino acid mutation | [K<sup>+</sup>]<sup>b</sup> |
|--------|---------------------------------|--------------|---------------------|---------------------------|
| Frag5  | None                            | None         | None                | E262K                     |
| TK103  | G784A                           | GAG → AAG    | E262K               | 274                       |
| TK136  | C1546T                          | CTG → TGT    | R516C               | 404                       |
| TK120  | C1246A                          | CTG → AGT    | R416S               | 259                       |
|        | T1258G                          | TCC → GCA    | S420A               | 358                       |
| TK116  | T1290C                          | GTA → GCA    | V427A               | 358                       |
| TK119  | T1280C                          | GTA → GCA    | V427A               | 358                       |
| TK121  | A791C                           | GAT → GCT    | D264A               | 265                       |
| TK104  | A791C                           | GAT → GCT    | D264A               | 310                       |

<sup>a</sup> Base number 1 is the adenine of the first codon of kefC (ATG).

<sup>b</sup> The K<sup>+</sup> concentration is that observed at the first time point after suspension of the cells in K<sub>10</sub> medium.

Strain TK120 carries two mutations located very close together, R416S and S420A. Site-directed mutagenesis was used to separate the mutations, and activity was studied using a construct that gives reduced KefC expression, pkC952 (13). Such a construct was desirable since high level expression of mutant proteins that cause a fast K<sup>+</sup> leak on the cell could pose a severe growth defect leading to selection for suppressor mutations. Subcloning of the 2.15-kilobase HindIII-BamHI fragment from pkC952 into the mutagenesis vector pTZ19U created a construct, pSM7, that yielded no functional KefC activity (data not shown). This enabled mutations to be introduced into this very high copy number vector without the concern that expression would be toxic to the cell. Mutagenesis was carried out as described under “Materials and Methods,” and the resulting clones were used to create separate plasmids carrying the separate R416S and S420A mutations and the two mutations in combination. The DNA was then subcloned into pHI165 to create pSM12 (wild type), pSM14 (R416S), pSM15 (S420A), and pSM30 (R416S,S420A). Plasmids were transformed into strain MJF276 (KefB KefC), and the rate of spontaneous K<sup>+</sup> loss was determined (Fig. 2). As expected, plasmids pSM12 and pkC952, which carry the same sequence, gave similar low rates of K<sup>+</sup> loss that were slightly faster than those observed in the nontransformed MJF276. Plasmid pSM15, which carries the S420A mutation, introduced a slow leak that was reproducibly faster than the MJF276/pSM12. However, rapid, spontaneous K<sup>+</sup> efflux was only observed from strains carrying the R416S mutation (MJF276/pSM14 [R416S] and MJF276/pSM30 [R416S,S420A]) (Fig. 2). The rate of K<sup>+</sup> efflux seen with the combined mutations was greater than that seen with R416S alone, and this is consistent with the slightly enhanced leak seen with S420A alone, i.e. the R416S and S420A mutations are additive with the R416S mutation dominant. The efflux profile for strain TK120 (Fig. 1), which carries the chromosomal R416S + S420A mutations, was similar to that obtained with MJF276/pSM30 (Fig. 2), confirming that the cloned lesions were responsible for the K<sup>+</sup> leak. Slight differences between the strains may arise due to a slight difference in the genetic background of MJF276 and TK120 and also by the separation in the expression of yabF and kefC in MJF276/pSM30. This suggestion was supported by analysis of MJF276/pSM26 (kefCD264A), which carries the D264A mutation in the same plasmid context as the R416S and S420A mutations.

### Footnotes

1. The location of the mutations was determined by direct sequencing of 300–400-base pair PCR products of the kefC gene. Only a single base change was observed in each of the mutants with the exception of strain TK120 (kefC120), which carries two changes R416S and S420A (Table II). Mutations were found in both the amino-terminal membrane protein domain (E262K and D264A) and in the carboxyl-terminal domain (R416S+S420A, V427A, and R516C), which carries the Rossman fold. Strains TK104 and TK121 and strains TK116 and TK119 were indistinguishable, and these pairs of strains subsequently proved to be isolelic; see below.

2. Missense mutations in the kefC strains.
spontaneous K⁺ leak seen in MJF276/pSM26 was slower than that seen in TK104 (kefCD264A) (compare Fig. 1 with Fig. 2). Notwithstanding these differences, we conclude that the most important mutation in strain TK120 is the R416S mutation. YabF is the putative product of the open reading frame 5′ to kefC and is required in trans for the activity of KeF(C (10). Since the kefC mutants were identified by their loss of regulation of K⁺ efflux it was possible that the mutations affected the control by YabF. Thus, the K⁺ leak was determined in strains MJF374 (YabF⁻, KeF(C⁻) and MJF276 (YabF⁻, KeF(C)⁻) transformed with pSM12 and pSM14/R416S) (Fig. 3). No significant K⁺ efflux was observed from MJF374/pSM12 or MJF374/pSM14, but normal efflux was observed in the transformed derivatives of MJF276, which possesses YabF activity. Thus, YabF is required for the activity of both the wild-type and the mutated KeF(C proteins.

We have previously shown that the mutation in TK121 can partially suppress, by co-expression of the wild-type gene from pkC592 (13), which is equivalent to pSM12 (see “Materials and Methods”). Since the R416S mutation lies in a different domain to TK121 mutation, D264A, we sought to determine whether co-expression of the two KeF(C proteins would allow either co-dominance or suppression. Plasmids pSM14 (R416S) and pSM12 (wild type) were transformed separately into MJF104 (kefC104, kefC::Tn10), and the spontaneous K⁺ efflux was measured (Fig. 4). Expression of the wild-type kefC gene from a multicopy plasmid, pkC11, or at a reduced expression level from pSM12 partially suppressed the K⁺ loss caused by the kefC104 mutation (D264A). Similarly, transformation of TK120 (kefC120) separately with pSM12, pSM15, or pkC11 suppressed the leak caused by the mutation R416S (data not shown). Thus, both the mutated subunit carrying the D264A mutation and the subunit carrying the combined R416S,S420A mutations can be suppressed by the wild-type gene expressed in either high copy number (pkC11) or low copy number (pSM12). The ability of the KeF(C)S420A subunit to suppress the leak from TK120 in strain TK120/pSM15 suggests that this mutation does not have a major effect on the configuration of the KeF(C) protein, which is consistent with the presence of only a slow spontaneous leak from strain MJF276/pSM15 (Fig. 2). In contrast, the K⁺ leak from MJF104/pSM14, which introduced the R416S mutation, was faster than for either MJF104 or MJF104/pSM12 (Fig. 4), and thus, KeF(C)R416S failed to suppress the D264A lesion. These data indicate that suppression requires wild-type KeF(C) sequences in both the membrane and the carboxy-terminal domains.

The KeF(C) K⁺ efflux system is maintained in a closed state by GSH or its non-sulphydryl analogue, ophthalmic acid (6, 7). Cells that lack GSH leak K⁺ via KeF(B and KeF(C, and this phenotype is reminiscent of those observed with the mutants described here, which raises the possibility that the mutations affect the GSH-binding site. Thus, we sought to determine whether the K⁺ leak observed with the mutations was exacerbated in a GSH-deficient mutant. Strain MJF335 (KeF(B, KeF(C, GshA⁻) was transformed separately with pSM14 (R416S), pSM26 (D264A), pSM30 (R416S,S420A), or pSM12 (wild type), and the spontaneous K⁺ leak was determined (Fig. 5, A-C). The rate of K⁺ efflux from MJF335/pSM12 was enhanced over that seen in the isogenic GSH-sufficient strain MJF276/pSM12 and could be inhibited by inclusion of either GSH or ophthalmic acid in the growth medium used to prepare the cells (Fig. 5A). Similarly, when plasmid pSM26 (D264A) was introduced into strain MJF335 the leak was increased compared with that seen in MJF276/pSM26 to the extent that over 60% of the K⁺ pool was released in the first 60 s after suspension of the cells into K₀ medium (Fig. 5B).
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GSH or ophthalmic acid reduced the K\(^+\) leak to that seen in MJF276/pSM26 (Fig. 5B). Thus, the leak caused by the absence of glutathione was additional to the spontaneous leak caused by the D264A mutation. In contrast it was observed that the leak of K\(^+\) was slower from MJF335/pSM14 (R416S) than from MJF276/pSM14 and was enhanced by growth in the presence of GSH (Fig. 5C). Ophthalmic acid also restored spontaneous K\(^+\) efflux to strain MJF335/pSM14. Similar data were obtained with MJF335/pSM30 (R416S,S420A) (data not shown). These data clearly show that the spontaneous activity of KefCR416S is dependent upon the presence of GSH or its analogue, which is an inversion of the normal phenotype.

The above analysis was confirmed and extended by analysis of the chromosomal mutations affecting KefC. Each of the strains was transduced to kanamycin resistance using a gshA::Tn10(Kan) insertion mutant and the K\(^+\) leak determined in the presence and absence of GSH (Table III). For all of the mutants except TK120 (R416S,S420A) the rate of K\(^+\) loss was slower in a GSH-deficient derivative than in the GSH parent, and the leak could be reduced by growth of the cells with GSH (Table III). As observed above for MJF335/pSM14 and MJF335/pSM30, the GSH-deficient derivative of strain TK120 (R416S,S420A) exhibited a slower K\(^+\) leak than did TK120 itself (Table III), and the fast leak could be restored by growing the strain in the presence of GSH (data not shown). Thus, the reversal of the normal GSH-gating is specific to the R416S mutation.

GSH adducts activate the KefC system (7). Since the phenotype of the KefC R416S derivative is an altered interaction with GSH the effect of the mutation on activation by the GSH adduct, N-ethylsucinimidyl-S-GSH, formed by the reaction of N-ethylmaleimide with GSH (7) was investigated. The rate of N-ethylmaleimide-elicted efflux were similar for MJF276/pSM12, MJF276/pSM14, MJF276/pSM26, and MJF276/pSM30 (data not shown). It is clear that the mutation does not substantially affect the activation of KefC by GSH adducts.

DISCUSSION

The data presented here identify residues within KefC that when mutated alter the regulation of the efflux system such that it exhibits a spontaneous K\(^+\) leak. The mutations map to two regions of the protein: a well-defined region in the amino-terminal hydrophobic domain, the HALESDIE sequence; and the carboxy-terminal hydrophilic domain. All of the residues that cause a spontaneous K\(^+\) leak are highly conserved across the four members of the KefB/C family of proteins identified to date but are not as well conserved in the other members of the wider family that includes the NapA Na\(^+\)/H\(^-\) antiport family and the RosB regulatory protein. In addition we show that one type of the KefC R416S derivative is an altered interaction with the Shaker channels with the S4-S5 linker peptide (12, 21). The prediction from positive charge distribu-
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The R416S, V427A, and R516C mutations of the carboxy-terminal domain affect conserved residues but have different effects on KefC activity. Although all confer a K⁺ leak upon the KefC protein, implying loss of regulation, the effect of removing GSH is to convert the protein to the closed state in the case of R416S and to a more active state with R416C and V427A. R416 falls immediately after the putative β-α-β (Rossman) fold of the carboxy-terminal domain and is conserved in glyceraldehyde-3-phosphate dehydrogenase and in the carboxy-terminal Rossman fold of TrkA, the regulator of K⁺ uptake in E. coli (22). Analysis of the sequences of the carboxy-terminal domain in the four members of the KefB/C family shows that only the first 120 amino acids is conserved and therefore, that this region may be important for regulation of activity.

The R416S, V427A, and R516C mutations of the carboxy-terminal domain are strongly conserved in the KefB/C family of proteins. The sequence can be represented as RHELE (where X = Ala, Ser, or Thr) and therefore, has a strongly acidic nature although the D264 is not conserved in KefB (A262) and E266 is substituted in KefX (A263) of Myxococcus xanthus. However, in all four members of the KefB/C family there are always three negatively charged amino acids in this sequence that is flanked by conserved positively charged groups. Thus, the mutant analysis has clearly defined a region of KefC that is important for regulation of activity.

The HALESDIE sequence is found at the carboxy-terminal end of GSH adducts and was photolabeled by [125I]-GSH-nitrobenzyl. Membrane-associated glyceraldehyde-3-phosphate dehydrogenase bound various GSH adducts at low concentrations of NAD⁺ and ATP (23). The residues identified by covalent linking were not in the GXXGXG motif that forms the core of the Rossman fold but at a slightly more distant site within the predicted binding site for NAD⁺ (23, 24). The Rossman fold domain of glyceraldehyde-3-phosphate dehydrogenase has also been implicated in the binding of RNA (9, 25), and it has been suggested that the fold is particularly suited to binding of both small and large molecules. These observations would be consistent with an important role for the carboxy-terminal domain of KefC in regulating channel activity and suggest that this domain might contain the binding site for GSH adducts.

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REFERENCES

1. Ferguson, G. P., Munro, A. W., Douglas, R. M., McLaggen, D., and Booth, I. R. (1993) Mol. Microbiol. 9, 1297–1303
2. Ferguson, G. P., McLaggen, D., and Booth, I. R. (1995) Mol. Microbiol. 17, 1025–1033
3. Ferguson, G. P., Chacko, A. D., Lee, C., and Booth, I. R. (1996) J. Bacteriol. 178, 3957–3961
4. Meury, J., and Kepes, A. (1982) EMBO J. 1, 339–343
5. Meury, J., and Robin, A. (1985) Eur. J. Biochem. 148, 113–118
6. Meury, J., Lebail, S., and Kepes, A. (1988) Eur. J. Biochem. 173, 33–38
7. Elmore, M. J., Lamb, A. J., Ritchie, G. Y., Douglas, R. M., Munro, A., Gajewska, A., and Booth, I. R. (1990) Mol. Microbiol. 4, 405–412
8. Bakker, E. P., Booth, I. R., Dinnhier, U., Epstein, W., and Gajewska, A. (1987) J. Bacteriol. 169, 3743–3749
9. Singh, R., and Green, M. R. (1993) Science 259, 365–368
10. Booth, I. R., Jones, M. A., McLaggen, D., Nikolaev, Y., Ness, L., Wood, C. M., Miller, S., Tito, M. E., and Ferguson, G. P. (1996) in Handbook of Biological Physics (Konings, W. N., Kaback, H. R., and Lolkema, J. S., eds) Vol. 2, pp. 693–730, North-Holland, Amsterdam
11. Munro, A., Ritchie, G. Y., Lamb, A. J., Douglas, R. M., and Booth, I. R. (1991) Mol. Microbiol. 5, 607–616
12. Pongs, O. (1983) J. Membr. Biol. 136, 1–8
13. Douglas, R. M., Ritchie, G. Y., Munro, A. W., McLaggen, D., and Booth, I. R. (1994) Mol. Microbiol. 11, 55–63
14. Epstein, W., and Kim, B. S. (1971) J. Bacteriol. 108, 639–644
15. Rowland, G. C., Giffard, P. M., and Booth, I. R. (1984) FEBS Lett. 173, 295–300
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual. 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Yura, T., Mori, H., Nagai, H., Nagata, T., Ishihama, A., Fujita, N., Isone, K., Mizobuchi, K., and Nakata, A. (1992) Nucleic Acids. Res. 20, 3305–3308
18. Mead, D. A., Szczesna-Skorupa, E., and Kemfer, B. (1986) Protein Eng. 1, 67–74
19. Stewart, G. S. A. B., Lubinsisky-Mink, S., Jackson, S., Cassel, C. G., and Kuhn, J. (1986) Plasmid 15, 172–181
20. Rowland, G. C., Giffard, P. M. & Booth, I. R. (1984) FEBS Lett. 173, 295–300
21. Jan, L. Y., and Jan, Y. N. (1992) Annu. Rev. Physiol. 54, 537–555
22. Schlosser, A., Hamann, A., Bossemeier, D., Schneider, B., and Bakker, E. P. (1993) Mol. Microbiol. 9, 533–543
23. Puder, M., and Soberman, R. J. (1997) J. Biol. Chem. 272, 10936–10940
24. Biesecker, G., Harris, J. L., Thierry, J. C., Walker, J. E., and Wonaocat, A. J. (1977) Nature 266, 328–333
25. Nagy, E., and Rigby, W. F. C. (1995) J. Biol. Chem. 270, 2755–2763