Characterization of α-Ketoglutarate-dependent Taurine Dioxygenase from Escherichia coli*

(Received for publication, May 23, 1997, and in revised form, June 20, 1997)

Eric Eichhorn, Jan R. van der Ploeg, Michael A. Kertesz, and Thomas Leisinger‡
From the Mikrobiologisches Institut, Swiss Federal Institute of Technology, ETH-Zentrum, CH-8092 Zürich, Switzerland

The Escherichia coli tauD gene is required for the utilization of taurine (2-aminoethanesulfonic acid) as a sulfur source and is expressed only under conditions of sulfate starvation. The sequence relatedness of the TauD protein to the α-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase of Alcaligenes eutrophus suggested that TauD is an α-ketoglutarate-dependent dioxygenase catalyzing the oxygenolytic release of sulfite from taurine (van der Ploeg, J. R., Weiss, M. A., Saller, E., Nashimoto, H., Saito, N., Kertesz, M. A., and Leisinger, T. (1996) J. Bacteriol. 178, 5438–5446). TauD was overexpressed in E. coli to ~70% of the total soluble protein and purified to apparent homogeneity by a simple two-step procedure. The apparent Mr of 81,000 of the native protein and the subunit Mr of 37,400 were consistent with a homodimeric structure. The pure enzyme converted taurine to sulfite and aminoacetaldehyde, which was identified by high pressure liquid chromatography after enzymatic conversion to ethanolamine. The reaction also consumed equimolar amounts of oxygen and α-ketoglutarate; ferrous iron was absolutely required for activity; and ascorbate stimulated the reaction. The properties and amino acid sequence of this enzyme thus define it as a new member of the α-ketoglutarate-dependent dioxygenase family. The pure enzyme showed maximal activity at pH 6.9 and retained activity on storage at −20 °C for several weeks. Taurine (Km = 55 μM) was the preferred substrate, but pantensulfonic acid, 3-(N-morpholino)propanesulfonic acid, and 1,3-dioxo-2-isoadioneethanesulfonic acid were also desulfonated at significant rates. Among the substrates tested, only α-ketoglutarate (Km = 11 μM) supported significant dioxygenase activity.

In the absence of sulfate, Escherichia coli can utilize aliphatic sulfonates as sulfur sources for growth. Sulfonates known to provide sulfur include ethanesulfonate, butanesulfonate, L-cysteate, isethionate (2-hydroxyethanesulfonate), and taurine (2-aminoethanesulfonate) (1, 2). None of these sulfonates served as sulfur source under anaerobic conditions, nor could they be utilized as a source of carbon and energy or of carbon, energy, and sulfur under either aerobic or anaerobic conditions (1). The mechanisms of sulfur assimilation from aliphatic sulfonates are unknown, but it has been shown that sulfonate/sulfur enters the assimilatory sulfate reduction pathway at the stage of sulfite (3). Recently, we have identified the tauABCD gene cluster, located at 8.5 min on the E. coli chromosome, which is specifically involved in the utilization of taurine as a sulfur source (2). Disruption of tauB, tauC, or tauD resulted in the loss of the ability to utilize taurine as a source of sulfur, but did not affect the utilization of a range of other aliphatic sulfonates as sulfur sources. The tau genes were only expressed during growth in the absence of sulfate or cysteine (2). The amino acid sequences of TauABC exhibit similarity to components of ABC-type transport systems (4). TauA has a putative signal sequence, indicating that it functions as a periplasmic binding protein, and the sequences of TauB and TauC are significantly similar to those of ATP-binding proteins and membrane components, respectively, of members of the ABC transporter superfamily. It thus appears that the proteins encoded by tauABC constitute an uptake system for taurine. TauD, the product of the tauD gene, shows 30% sequence identity to a characterized α-ketoglutarate-dependent 2,4-dichlorophenoxyacetate dioxygenase of Alcaligenes eutrophus, encoded by the tfdA gene of plasmid pJP4 (5, 6). This suggests that TauD is an α-KG-dependent dioxygenase involved in the oxygenolytic release of sulfite from taurine. Here we report the purification of the TauD protein and describe its catalytic properties, which demonstrate that it is an α-KG-dependent taurine dioxygenase.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and T4 DNA ligase were obtained from MBI Fermentas. DNase I and NADH came from Boehringer Mannheim. Fhu DNA polymerase from Stratagene, and horse liver alcohol dehydrogenase from Sigma. Lysozyme was from Fluka, and propanesulfonic acid, 3-(N-morpholino)propanesulfonic acid, and 2-bromoethanesulfonic acid (Sigma) and isethionic acid (Aldrich). All other chemicals were from Aldrich, Fluka, or Merck and were of the highest purity grade available.

Bacterial Strains and Growth Conditions—E. coli strains DH5α (7) and BL21(DE3) (8) were grown aerobically at 37 or 30 °C in Luria-Bertani medium (7) with constant shaking (180 rpm). When necessary, kanamycin was added at 50 μg/ml and ampicillin at 100 μg/ml. Solid media were prepared by the addition of 1.5% (w/v) agar.

Taurine Desulfonation in Extracts of E. coli Cells Grown in Sulfate and Taurine—E. coli strain MC4100 (9) was grown under the conditions mentioned above in 100 ml of a modified sulfur-free M63 minimal medium (2) containing 250 μM Na2SO4 or taurine as sulfur sources. When the cultures had reached an A650 of 0.9, cells were harvested by centrifugation at 5800 × g for 20 min at 4 °C, washed once with 0.9% NaCl, and suspended in 1 ml of 20 mM Tris-HCl buffer (pH 7.0), and stored at −20 °C until further use. Cell-free extracts were obtained after the addition of 5 μg/ml lysozyme and 10 μg/ml DNase I to the cells, incubation at 30 °C for 30 min, and clarification by centrifugation at 35,000 × g for 20 min.

Construction of a tauD Expression Plasmid—The tauD gene was placed under the control of the T7 RNA polymerase promoter of vector pET-24a (+) (Novagen) in a three-step cloning procedure. First, an NdeI site at the translation start of tauD was introduced by polymerase chain reaction amplification of the tauD gene from plasmid pUC18AL4 (2, 9). The abbreviations used are: α-KG, α-ketoglutarate; HPLC, high pressure liquid chromatography; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid); MOPS, 3-(N-morpholino)propanesulfonic acid.

This paper is available on line at http://www.jbc.org

23031
10. The oligonucleotide primers used were EE1 (5’-CATGGGAGGAT- CATATGGTGGAAC-3’, with the change to introduce the NdeI site underlined) and EE2 (5’-CGGTGCTGAAAGCTTGGTTGCGA-3’). In the second step, the 1041-base pair polymerase chain reaction product was digested with NdeI and SpHl, and the resulting 226-base pair fragment encoding the N-terminal part of TauD was cloned in pME4141 (11), generating plasmid pME4140. In the third step, the final cloning of tauD in the expression vector pET-24at (+) was done by a ligation involving the vector, the 226-base pair NdeI-Sphl fragment of pME4140, and an 815-base pair Sphl-HindIII fragment encoding the C-terminal part of TauD. This resulted in plasmid pME4141, in which tauD was under the control of the T7 RNA polymerase promoter.

**Purification of TauD**—For protein production, E. coli BL21(DE3) cells harboring the tauD expression plasmid pME4141 were grown at 30 °C in a 5-liter Erlenmeyer flask containing 1000 ml of growth medium. When the culture had reached an A600 of 0.6–0.8, tauD expression was induced by the addition of isopropyl-β-D-thiogalactopyranoside to a 1 mM final concentration and incubated for a further 3 h. Cells were collected by centrifugation at 5800 × g for 20 min at 4 °C, resuspended in 50 ml of 20 mM Tris-HCl buffer (pH 7.0), and stored at −20 °C in 10-ml aliquots until further use. About 6–9 g of cells (fresh weight) were collected from 1000 ml of culture.

For cell lysis and DNA digestion, 5 µg/ml lysosome and 10 µg/ml DNase I were added to 10 ml of cell suspension, and the mixture was incubated at 30 °C with constant shaking (180 rpm) for 30 min. Crude cell-free extracts were obtained by centrifugation of the lysates at 35,000 × g for 20 min at 4 °C. The extract was then fractionated with solid ammonium sulfate at 4 °C. The TauD protein precipitate was washed with a two-step gradient of NaCl in 20 mM sodium phosphate buffer (pH 7.0). The precipitated tauD was dissolved in 1 mM sodium phosphate buffer (pH 7.0) and dialyzed for 15 h at 4 °C against 1 liter of the same buffer, with gentle stirring.

The dissolved ammonium sulfate precipitate was chromatographed on anion exchange chromatography on a 1-ml Resource Q anion-exchange column (Pharmacia Biotech Inc.) under standard assay conditions in 20 mM sodium phosphate buffer (pH 6.5), and eluted with 50-100 mM sodium phosphate buffer (pH 7.0) and 100 mM sodium phosphate buffer (pH 8.0). After equilibration (2 column volumes) and filtration on Superose 12 HR 10/30 and Superose 6 HR 10/30 columns (Pharmacia Biotech Inc.), calibrated with known M, protein standards: thyroglobulin (670,000), ferritin (440,000), catalase (232,000), aldolase (158,000), bovine serum albumin (67,000), ovalbumin (43,000), and RNase (13,700). The column was equilibrated (2 column volumes) and eluted with 50 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl at a flow rate of 0.5 ml/min.

**Analysis of Enzyme Reaction Products**—Succinate was measured by an enzyme assay using a succinic acid test kit from Boehringer Mannheim, following the manufacturer’s instructions. Bovine serum albumin was used as a standard. The native M, of TauD was estimated by gel filtration on Superose 12 HR 10/30 and Superose 6 HR 10/30 columns (Pharmacia Biotech Inc.), calibrated with known M, protein standards: thyroglobulin (670,000), ferritin (440,000), catalase (232,000), aldolase (158,000), bovine serum albumin (67,000), ovalbumin (43,000), and RNase (13,700). The column was equilibrated (2 column volumes) and eluted with 50 mM sodium phosphate buffer (pH 7.2) containing 150 mM NaCl at a flow rate of 0.5 ml/min.

**Results**

Desulfonation of Taurine in Crude Cell Extracts—Preliminary analysis of taurine desulfonation was performed with crude extract prepared from isopropyl-β-D-thiogalactopyranoside-treated cells of _E. coli_ BL21(DE3)(pME4141), which strongly overexpresses the _tauD_ gene product (Fig. 1). Desulfonation was assayed by determining the amount of sulfite formed...
during 15 min of incubation at 30 °C in 10 mM imidazole (pH 6.75) containing 10 mM taurine, 10 mM α-KG, 50 μM Fe(II)SO₄, and 100 μM l-ascorbate. Sulfite release and oxygen consumption were not detected in cell extracts prepared from uninduced cells or in extracts from induced cells incubated without taurine or α-KG. Activity in cell extracts was abolished by the addition of EDTA, and desulfonation of taurine thus required α-KG, ferrous iron, and oxygen. It therefore seemed likely that the TauD protein represents a new member of the α-KG-dependent dioxygenase enzyme family (15).

The cell extract from *E. coli* MC4100 was examined for taurine desulfonation by following taurine-dependent sulfate release. Strain MC4100 carries a single chromosomal copy of the *tau* genes, which are fully expressed during growth in sulfate-free minimal medium containing 250 μM taurine as a sulfur source (2). Extracts made from cells grown under these conditions showed a specific taurine desulfonating activity of 12.2 nmol/min/mg of protein, which corresponds to about twice the in vivo desulfonation rate required for growth at a doubling time of 60 min with taurine as a sulfur source. As expected, extracts from sulfate-grown cells showed no taurine desulfonating activity.

**Enzyme Purification**—The *tauD* gene product was purified to homogeneity from *E. coli* BL21(DE3)(pME4141) with a yield of 20% in a two-step procedure summarized in Table I. The specific taurine desulfonating activity obtained with crude extracts after overexpression was 60 times that measured in cell extracts from sulfate-grown cells showed no taurine desulfonating activity obtained with crude extracts from sulfate-grown cells showed no taurine desulfonating activity. As expected, extracts from sulfate-grown cells showed no taurine desulfonating activity.

**TABLE I**

| Purification step | Total protein mg | Total activity units | Specific activity units/mg | Purification fold |
|------------------|------------------|----------------------|---------------------------|-------------------|
| Cell extract     | 507              | 375                  | 100                       | 0.74              |
| 50–60% (NH₄)₂SO₄| 190              | 232                  | 62                        | 1.22              |
| Resource Q       | 45               | 73.8                 | 19.7                      | 1.64              |

The dependence of tauD activity on the concentration of ferrous iron and ascorbate was investigated with pure enzyme under standard assay conditions. Enzyme activity was dependent on Fe(II) supplied as either a sulfate or chloride salt, and treatment of the enzyme with EDTA completely abolished activity. Maximal specific activity was obtained with Fe(II) concentrations between 5 and 150 μM. Other divalent metal ions, including Mg(II), Ca(II), Mn(II), Ni(II), Co(II), Cu(II), and Zn(II), could not replace ferrous sulfate at a 100 μM final concentration either as sulfate or chloride salts. Zn(II), Cu(II), and, to a lesser extent, Co(II), all added at 10–50 μM final concentrations, inhibited maximal taurine/α-KG dioxygenase activity by 80–95%. Ascorbate led to a 50% increase in activity when added at concentrations between 200 and 800 μM in an assay mixture containing 100 μM Fe(II).

**Stoichiometry of the α-KG-dependent Taurine Desulfonation Reaction**—Of the four products formed in the taurine/α-KG dioxygenase reaction (Fig. 2), succinate and sulfite were determined quantitatively and shown to be produced in equimolar amounts. This is in accordance with the expected stoichiometry of the reaction and demonstrated that oxidative decarboxylation of α-KG and desulfonation of taurine are strictly coupled. The expected consumption of 1 mol of dioxygen/mol of taurine desulfonated was experimentally verified.

The putative organic product of the TauD-catalyzed taurine oxygenation is 1-hydroxy-2-aminoethanesulfonic acid, which would decompose to aminoacetaldehyde and sulfite (Fig. 2). Attempts by gas chromatography-mass spectrometry to detect aminoacetaldehyde among the products resulting from the enzymatic desulfonation of taurine were unsuccessful. Since aminoacetaldehyde is highly reactive and readily undergoes polymerization reactions, we chose an indirect method to demonstrate qualitatively its formation from taurine. As described under “Experimental Procedures,” liver alcohol dehydrogenase was monitored by following NADH oxidation.

**Characterization of TauD**—The purified enzyme had a specific activity of 1.64 units/mg of protein. Upon storage at −20 °C at a protein concentration of 2.6 mg/ml in buffer without glycerol, >90% of the activity was lost within 3 weeks. When glycerol was added to a 16% (v/v) final concentration, the specific activity of the pure enzyme increased by 4-fold during storage at −20 °C for 10 weeks. We presume that the activation during storage reflects folding of unfolded polypeptide chain.

The effect of pH on enzyme activity was examined over a range of 4.5–10.8 using appropriate buffer systems (17). TauD exhibited a distinct activity optimum around pH 6.9.

In our initial studies with TauD, described above, we observed that the specific activity of the enzyme fell rapidly during incubation at 30 °C. A similar effect has been seen before with the related α-KG-dependent 2,4-dichlorophenoxyacetate dioxygenase (6), so we investigated it further by measuring sulfite release from taurine over 30 min by enzyme that had been preincubated in imidazole buffer at 30 °C for 15 min alone, with Fe(II)SO₄, or with Fe(II)SO₄ and ascorbate. The data (not shown) indicate that temperature itself leads to rapid enzyme inactivation, which is enhanced by ascorbate, and that the observed inactivation is not due to oxidation of the enzyme-bound ferrous iron.

**FIG. 2.** Overall reaction catalyzed by the α-KG-dependent taurine dioxygenase from *E. coli.*

![Diagram of the overall reaction catalyzed by the α-KG-dependent taurine dioxygenase](image-url)
Enzymatic reduction of the product of taurine desulfonation to ethanolamine. The formation of aminoacetaldehyde from taurine was followed by coupling the TauD-catalyzed reaction to horse liver alcohol dehydrogenase. The complete assay mixture contained (in a 1-ml volume) 1 mM taurine, 1 mM \( \alpha \)-KG, 100 \( \mu \)M Fe(II)SO\(_4\), 200 \( \mu \)M ascorbate, 175 \( \mu \)M NADH, 32 units of alcohol dehydrogenase, and 500 milliunits of TauD. NADH consumption was monitored by measuring the absorbance at 340 nm. In the controls, NADH consumption was measured in reaction mixtures lacking TauD, alcohol dehydrogenase (ADH), taurine, or \( \alpha \)-KG.

Substrate Range and Kinetic Constants—The enzyme showed a Michaelis-Menten-type saturation curve in response to increasing taurine concentrations, with a \( K_m \) of 55 \( \mu \)M and a \( V_{\text{max}} \) of 4.1 units/mg of protein (Fig. 4A). Among 19 potential substrates tested, methanesulfonic acid, ethanesulfonic acid, isethionic acid, 2-bromoethanesulfonic acid, \( L \)-cysteic acid, sulfo succinate, 4-aminobenzensulfonic acid, 2-(4-pyridyl)ethanesulfonic acid, and \( N \)-phenyltaurine were not utilized by TauD. 2–5% of the activity observed with taurine was observed with 1-propanesulfonic acid, 1-dodecanesulfonic acid, 4-phenyl-1-butanesulfonic acid, HEPES, and PIPES. Significant activity was supported by the compounds listed in Table II. The observed substrate range indicates that in addition to taurine, several 2-substituted ethanesulfonic acids are substrates for \( \alpha \)-KG-dependent taurine dioxygenase. Among the unsubstituted 1-alkanesulfonates, pentanesulfonic acid was the best substrate.

The \( K_m \) value for \( \alpha \)-KG was 11 \( \mu \)M (Fig. 4B). Using 30-min incubation times and 33 milliunits of enzyme, \( \alpha \)-ketoadipate, pyruvate, \( \alpha \)-ketobutyrate, \( \alpha \)-ketovalerate, \( \alpha \)-ketocaproate, \( \alpha \)-ketoisovalerate, and oxalacetate were tested as cosubstrates for taurine desulfonation. When tested at 500 \( \mu \)M to 10 mM, \( \alpha \)-keto adipate supported desulfonation at 4–10% of the rate observed with \( \alpha \)-KG, whereas the other \( \alpha \)-keto acids were inactive. A second carboxyl group is thus required for recognition of the cosubstrate by the enzyme.

**DISCUSSION**

Although several reports on the degradation of primary aliphatic sulfonates by bacteria as carbon or sulfur sources have appeared (1, 2, 18, 19), the enzymology of the desulfonation of these compounds is largely unexplored. The taurine desulfonation enzyme characterized in this study is, to our knowledge, the first pure enzyme reported that is capable of oxyg enolytic cleavage of the C–S bond of 1-alkanesulfonates. In contrast to the partially purified bacterial monoxygenase systems for alkanesulfonate desulfonation described so far (18, 19), it does not play a role in carbon metabolism, and its synthesis is regulated by the sulfur supply to the cell (2).

The biochemical properties of taurine dioxygenase allow it to be assigned to the \( \alpha \)-KG-dependent dioxygenase group of enzymes (15, 16). As a family, these enzymes catalyze a variety of reactions including hydroxylations, desaturations, and ring expansions. The family contains enzymes that are monomers, dimers, and tetramers with subunit sizes ranging between 26 and 85 kDa. Characterization of the reactions catalyzed by some of these enzymes revealed that not all show typical hydroxylations biochemistry in which \( \alpha \)-KG acts as an oxygen acceptor and one atom of molecular oxygen is found in succinate and the other in the second organic product. The classifi-
cation defined by biochemistry does not satisfactorily correlate with sequence data since the group contains both enzymes with similar biochemical characteristics but very little sequence similarity and enzymes with a high degree of amino acid sequence similarity but more diverse biochemical features. All these enzymes could have a common mechanism of action (16).

α-KG-dependent taurine dioxygenase exhibits a relatively relaxed substrate specificity, accepting not only taurine but also pentanesulfonic acid, hexanesulfonic acid, MOPS, and 1,3-dioxo-2-isoindolineethanesulfonic acid (Table II). The first three of these alternative substrates have been shown to serve as sulfur sources for *E. coli*. Since *E. coli* mutants defective in taurine dioxygenase are still able to utilize these sulfur sources, there must be at least one additional system for their metabolism as sulfur sources (2). Desulfonation by α-KG-dependent taurine dioxygenase of organosulfonates other than taurine might thus be of little or no importance for sulfur metabolism. To strengthen or refute this view, it will be interesting to explore the specificity of the TauA periplasmic binding protein, which is coexpressed with TauD, and to characterize the system or systems that catalyze desulfonation of other alkanesulfonates.

With respect to cosubstrate specificity, the taurine desulfonation enzyme of *E. coli* occupies an intermediate position among the α-KG-dependent dioxygenases. Besides α-KG, only α-ketoadipate, but none of the other α-keto acids tested, supported a low level of desulfonation. The range of substrates is thus more restricted than that of 2,4-dichlorophenoxyacetate dioxygenase (6), similar to the range of prolyl 4-hydroxylase (20), and less restricted than that of γ-butyrobetaine hydroxylase, which utilizes only α-KG as a cosubstrate (21).

Other features of the *E. coli* α-KG-dependent taurine dioxygenase, such as the requirement for α-KG and ferrous iron, the stimulation of activity by ascorbate, and its inhibition by divalent metal ions, are typical for α-KG-dependent dioxygenases (15, 16). Unlike lysyl hydroxylase and prolyl 4-hydroxylase, taurine dioxygenase does not appear to catalyze an uncoupled decarboxylation of α-KG in the absence of taurine. Uncoupled α-KG decarboxylation also occurs in lysyl hydroxylase and prolyl 4-hydroxylase as a side reaction during substrate hydroxylation. It is thought to lead to the oxidation of enzyme-bound Fe(II), which subsequently must be reduced by ascorbate to reactivate the enzyme. The side reaction thus results in the absolute requirement of these enzymes for ascorbate (22, 23), a property that was not observed with α-KG-dependent taurine dioxygenase.

Taurine dioxygenase exhibited 30% amino acid identity to the 2,4-dichlorophenoxyacetate dioxygenase enzyme encoded by the *tfdA* gene of plasmid pJP4 (6, 24) as well as 32 and 37% sequence identity to open reading frames of unknown function of *Saccharomyces cerevisiae* (GenBank™ accession number Z47973) and *Mycobacterium tuberculosis* (GenBank™ accession number Z77165), respectively (Fig. 5). It did not show significant similarity to other α-KG-dependent dioxygenases (16), emphasizing the genetic diversity of this group of enzymes and suggesting that they may have arisen by convergent evolution rather than from a common ancestor. While taurine dioxygenase and 2,4-dichlorophenoxyacetate dioxygenase did not react with each other’s substrates,2,3, they are both dimers, with subunit molecular masses of 32 kDa, that exhibit maximal activity at pH 6.5–7.0. The alignment of the four proteins that

[2] R. P. Hausinger, personal communication.

[3] E. Eichhorn, unpublished data.
do exhibit sequence homology (Fig. 5) revealed 4 histidine and 2 aspartate residues that are strictly conserved (marked with asterisks). Site-directed mutagenesis studies of human prolyl 4-hydroxylase (25) and lysyl hydroxylase (26) have indicated that 2 histidines and 1 aspartate constitute the endogenous ligands of the ferrous active site. In these α-KG-dependent dioxygenases and in the mechanistically related isopenicillin-N synthase, these residues are arranged in the His-Asp-Asp motif (27), whose role in metal binding is supported by the crystal structure of the manganese form of a fungal isopenicillin-N synthase (28). Although the enzymes shown in Fig. 5 are not closely related to the above enzymes at a sequence level (<9% identity), a similar iron-binding motif might be provided by histidine 99, aspartate 101, and histidine 153 of the TauD (53–57) His motif (27), whose role in metal binding is supported by the crystal structure of the manganese form of a fungal isopenicillin-N synthase (28). Although the enzymes shown in Fig. 5 are not closely related to the above enzymes at a sequence level (<9% identity), a similar iron-binding motif might be provided by histidine 99, aspartate 101, and histidine 153 of the TauD (Fig. 5). For the 2,4-dichlorophenoxyacetate dioxygenase enzyme, recent spectroscopic studies have indeed provided evidence that these residues may play a role in metal coordination (29). The function of the unknown open reading frames Y and Y′ (Fig. 5) cannot be speculated from these data, but it would be interesting to clarify if they also belong to the α-KG-dependent dioxygenase family.

REFERENCES
1. Uria-Nickelsen, M. R., Leadbetter, E. R., and Godchaux, W., III (1993) J. Gen. Microbiol. 139, 203–208
2. van der Ploeg, J. R., Weiss, M. A., Saller, E., Nashimoto, H., Saito, N., Kertesz, M. A., and Leisinger, T. (1996) J. Bacteriol. 178, 5438–5446
3. Uria-Nickelsen, M. R., Leadbetter, E. R., and Godchaux, W., III (1994) Arch. Microbiol. 161, 434–438
4. Boos, W., and Lucht, J. M. (1996) in Escherichia coli and Salmonella (Neidhardt, F. C., Ingraham, J. L., Low, B. K., Magasanik, B., Reznikoff, W. S., Riley, M., Schaechter, M., and Umbarger, H. E., eds) 2nd Ed., pp. 1175–1209, American Society for Microbiology, Washington, D. C.
5. Fukumori, F., and Hausinger, R. P. (1993) J. Bacteriol. 175, 2083–2086
6. Fukumori, F., and Hausinger, R. P. (1993) J. Biol. Chem. 268, 24311–24317
7. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
8. Studier, P. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
9. Casadaban, M. J. (1976) J. Mol. Biol. 104, 541–555
10. O’Neill, G. P., Thorbjarnardottir, S., Michelsen, U., Pålson, S., Soll, D., and Eggertason, G. (1991) J. Bacteriol. 173, 94–100
11. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103–119
12. Johnston, J. B., Murray, K., and Cain, R. B. (1975) Antonie Leeuwenhoek 41, 495–513
13. Riddles, P. W., Blakeley, R. L., and Zerner, B. (1983) Methods Enzymol. 91, 49–60
14. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
15. Abbott, M. T., and Udenfriend, S. (1974) in Molecular Mechanism of Oxygen Activation (Hayashi, O., ed) pp. 167–214, Academic Press, New York
16. Prescott, A. G. (1993) J. Exp. Bot. 44, 849–861
17. Stoll, V. S., and Blanchard, J. S. (1999) Methods Enzymol. 182, 24–38
18. Thyssse, G. J. E., and Wanders, T. H. (1974) Antonie Leeuwenhoek 40, 25–37
19. Higgins, T. P., Davey, M., Trickett, J., Kelly, D. P., and Murrell, J. C. (1996) Microbiology 142, 251–260
20. Majamaa, K., Hanauske-Abel, H. M., Günzler, V., and Kivirikko, K. I. (1984) Eur. J. Biochem. 138, 239–245
21. Ng, S.-F., Hanauske-Abel, H. M., and Englard, S. (1991) J. Biol. Chem. 266, 1526–1533
22. Puistola, U., Turpeenniemi-Hujanen, T. M., Myllylä, R., and Kivirikko, K. I. (1980) Biochim. Biophys. Acta 611, 40–50
23. Myllylä, R., Knutti-Savolainen, E. R., and Kivirikko, K. I. (1978) Biochem. Biophys. Res. Commun. 83, 441–448
24. Don, R. H., Weightman, A. J., Knackmuss, H.-J., and Timmis, K. N. (1986) J. Bacteriol. 161, 85–90
25. Myllyharju, J., and Kivirikko, K. I. (1997) EMBO J. 16, 1173–1180
26. Pirskanen, A., Kaimio, A.-M., Myllylä, R., and Kivirikko, K. I. (1996) J. Biol. Chem. 271, 9398–9402
27. Borovok, I., Landman, O., Kreisberg-Zakarin, R., Aharonowitz, Y., and Cohen, G. (1996) Biochemistry 35, 1981–1987
28. Roach, P. L., Clifton, I. J., Fülöp, V., Harloe, K., Barton, G. J., Hajdu, J., Andersson, I., Schufield, C. J., and Baldwin, J. E. (1995) Nature 375, 700–704
29. Whiting, A. K., Que, L., Jr., Saari, R. E., Hausinger, R. P., Fredrick, M. A., and McCracken, J. (1997) J. Am. Chem. Soc. 119, 3413–3414
30. Genetics Computer Group, Inc. (1995) GCG Program Manual for the Wisconsin Package, Version 8, Genetics Computer Group, Inc., Madison, WI