A Novel L-Cysteine/Cystine C-S-Lyase Directing [2Fe-2S] Cluster Formation of Synechocystis Ferredoxin*

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Iron-sulfur (Fe-S) clusters are found as functional units in numerous electron-transferring proteins. They are essential in basic biological processes such as oxidative phosphorylation, photosynthesis, and nitrogen fixation. More recently, they have also been identified as components of enzymes not concerned with redox reactions (1). Lately they have been implicated in gene regulation (2).

Despite these important and diverse functions and thorough biochemical investigations of Fe-S proteins, the biosynthetic steps leading to Fe-S cluster assembly have not been established. While it seems likely that enzyme-catalyzed reactions are involved in the formation of Fe-S clusters in vivo, the chemical reconstitution of apoproteins with free ferrous iron and sulfide in the presence of thiols (3) has been most intensively exploited for cluster build-up in vitro. Nevertheless, several proteins have been suggested as candidates in the biosynthetic process. Rhodanese (4) and 3-mercaptopuruvate sulfurtransferase (5) were effective in purified in vitro systems, comprising apoprotein, thiol, ferrous ion, and the respective sulfur-containing substrate, thiosulfate, or mercaptopyruvate. Analysis of yeast respiration-deficient mutants suggested a role for the BCS1 gene product (6) in the synthesis of functional Rieske protein. Studies on nif mutants of Azotobacter vinelandii (7) identified nifS as essential for nitrogenase activity. More recently, nifS was cloned and expressed in Escherichia coli; the gene product (NifS) was characterized as a pyridoxal phosphate-containing L-cysteine desulfurase, which yielded alanine and elemental sulfur as products (8); with dithiothreitol present in the reaction mixture, sulfide was produced instead of sulfur. Further work showed that the apo- form of the nitrogenase iron protein component could be activated by NifS-catalyzed reassembly of its [4Fe-4S] cluster in vitro (9). NifS was also successfully used for the in vitro reconstitution of the [4Fe-4S] cluster of a mutant FNR protein (10) as well as of the [2Fe-2S] cluster of SoxR (11), both E. coli proteins. Most recently, a NifS-like protein was isolated from E. coli and found to reactivate apodihydroxy acid dehydratase in vitro (12).

The significance of these various findings depends on the nature of the in vivo sulfur source for Fe-S clusters. Feeding experiments with E. coli assigned this role to cysteine (13). Cysteine sulfur was also incorporated into the [2Fe-2S] cluster of ferredoxin in isolated, intact chloroplasts (14). In the chloroplast system, ferredoxin cluster formation was found to be a stroma-located process, consisting of two separate steps: first, the NADPH-stimulated liberation of sulfide from cysteine followed by the ATP-dependent sulfide incorporation into ferredoxin (15).

With the cyanobacterium Synechocystis as a procaryotic model for the chloroplast organelle we now established an apo-edoxin conversion assay. The procaryotic system was chosen because of the relative ease of obtaining large quantities of cell material without the problem of compartmentalization. The endogenous [2Fe-2S] ferredoxin was adopted as the Fe-S protein studied because of its abundance and the well characterized chemical procedures of reversible cluster removal (16). Moreover, the physiological existence of apoferredoxin has been demonstrated for the chloroplast system, where cluster assembly occurs in the stroma after proteolytic processing of preferredoxin that is imported from the cytosol (17).

We here report on the set up of the assay, the purification and characterization of the converter protein ( provisionally named C-DES because of its cysteine desulhydrase activity), and the stoichiometry of the cluster formation reaction.

EXPERIMENTAL PROCEDURES

Materials—l-Cysteine and 1-propargylglycine were from Fluka; l-allylglycine, l-vinylglycine, and pyridoxal phosphate were from Sigma; d-cysteine was from Novabiochem; and l-cystine was from SERVA. Glutathione and glutathione reductase were purchased from Boehringer Mannheim. Other chemicals were of the highest purity commercially available. l-[U-14C]Cysteine (302.2 mCi/mmol) was obtained from Du Pont NEN. Sephadex G-25 and G-75, DEAR-Sephadex A-25, Q-Sepharose FF, Mono P HR5/5, and Polybuffer 74 were from Pharmacia; Ultrogel AcA 44 and Dowex 50 WX8 were from SERVA; and DE52 cellulose was from Whatman. Ferreredoxin-NADP reductase was purified

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from frozen spinach leaves (18); the final crystallization step was omitted.

Unless otherwise stated, proteins were concentrated by ultrafiltration using PM 10 membranes or Centricon 10 units, both of which were supplied by Amicon. Buffer exchanges were performed using a Sephadex G-25 column.

Growth of Synechocystis—Synechocystis PCC 6714 (ATCC 27178) was grown at 30 °C in BG-11 medium (19) supplemented with 0.9 g/liter KHCO₃ in an 18-liter fermenter at pH 7.5–8.5. Cultures were illuminated with fluorescent tubes and gassed continuously with 5% CO₂. After 4 days of growth, typically 15 g of cell paste was harvested by centrifugation, shock-frozen, and stored at −70 °C.

Isolation of C-DES and Ferredoxin—30 g of frozen cells were thawed, resuspended in 130 ml of 50 mM Mops/NaOH, pH 8.0, containing 5 mM dithiothreitol and 0.5 mM phenylmethanesulfonyl fluoride, and disrupted by sonication. After centrifugation (10,000 g, 90 min) the extract (1500 mg of protein) was concentrated, transferred into 0.15 M Tris/Cl, pH 7.8, containing 0.3 M NaCl (final volume 80 ml), and loaded onto a DEAE-Sephadex A-25 column (20 cm × 5 cm, 10 cm/h). The column was washed with 80 ml of starting buffer; all unadsorbed protein (≥1450 mg) was collected and reduced for 30 min at 30 °C with 10 mM dithiothreitol. Then the buffer was exchanged for 50 mM Mops/NaOH, pH 7.6, and this protein preparation was used for experiments requiring dithiothreoxidized extract.

Ferredoxin was eluted from the DEAE-Sephadex column with 190 ml of 0.15 M Tris/Cl, pH 7.8, containing 0.5 M NaCl. The eluate was treated with DNase and RNase (15 mg each) in the presence of 10 mM MgCl₂ (48 h, 4 °C). Ferredoxin was purified to homogeneity (yield 7–8 mg) by ammonium sulfate fractionation and DE52 cellulose chromatography according to Ref. 20. It was converted to the apo- form by double precipitation with 0.5 N HCl under argon. For purification of C-DES, deferredoxinized extract (80 ml) was gel-filtered on Ultrogel AcA 44 (20 cm × 55 cm; 5 cm/h) in 50 mM Mops/NaOH, pH 7.6, containing 20 mM EDTA. C-DES was collected in fractions corresponding to Kᵥ values between 0.525 and 0.575. These fractions were diluted with 4 volumes of 50 mM Tris/Cl, pH 7.8, containing 1 mM dithiothreitol and 20 mM EDTA and loaded onto DE52 cellulose (20 cm × 3.5 cm; 8 cm/h) equilibrated with this buffer. A linear gradient (560 ml to 160 mM Tris/Cl, pH 7.8, containing 320 mM NaCl, 1 mM dithiothreitol, and 20 mM EDTA) was applied. C-DES was eluted in the trailing shoulder of the major protein peak. This eluate was diluted with 7 volumes of 50 mM Tris/Cl, pH 7.4, containing 1 mM dithiothreitol and 20 mM EDTA and eluted on Q-Sepharose FF (5 cm × 1.5 cm; 20 cm/h) equilibrated with this buffer. A linear gradient (100 ml to 150 mM Tris/Cl, pH 7.4, containing 300 mM NaCl, 1 mM dithiothreitol, and 20 mM EDTA) was applied. C-DES was eluted just in front of the main protein peak. This fraction was transferred into 25 ml 1-methylpyrrole/mimidodiacetic acid, pH 5.7, and applied to a Mono P HR 5/5 column (5 cm/min). Homogeneous C-DES was eluted at a pH of 4.4 using Polybuffer 74 (diluted 1:29 (v/v) and adjusted to pH 4.0 with mimidodiacetic acid) as eluant. Separation of C-DES from Polybuffer and transfer into 50 mM Mops/NaOH, pH 7.6, was achieved by gel filtration on Sephadex G-75.

All operations were carried out at 4 °C except for chromatofocusing, which was performed at room temperature. The final C-DES separation was stored at −70 °C; its activity proved to be stable for several months.

Apoferredoxin to Holoferedoxin Conversion Assay—The standard assay for holoferedoxin formation, run at 30 °C under argon, contained 10 mM apoferredoxin, which was incubated with samples of deferredoxinized Synechocystis extract (or purified fractions thereof) in 55 mM Mops/KOH, pH 7.5, 0.15 mM glutathione, 0.15 mM pyridoxal phosphate, and 90 μM L-cysteine. The reaction was started by the addition of Fe(NH₄)₂(SO₄)₂ to a final concentration of 0.4 mM (final volume 30 μl to 0.35 ml). The reaction was stopped, routinely after 1 h, by the addition of 1.7 mM EDTA and 10% glycerol. The holoferedoxin content of the sample was analyzed by native PAGE (20% resolving gel, Laemmli buffer system (24) with SDS omitted); the same result was obtained whether the sample was analyzed immediately or after freezing. The gels were stained with the carbocyanine dye “stains all” (4, 5, 4’, 5’-dibeno-3, 3’-diethyl-9-methylthiacarbocyanine bromide) (25); the amount of holoferredoxin (0.1–1 μg/lane) was estimated by comparison with known standards of ferredoxin.

Exact quantities of holoferedoxin were determined (after transfer into 50 mM Mops/NaOH, pH 7.6) by activity measurements made according to Ref. 26; the rate of cytochrome c reduction by NADPH via ferredoxin, which is mediated by ferredoxin-NADPH reductase, was measured, and 1 μg of ferredoxin in 0.5 ml gave a ΔA₅₅₀ of 0.2 min⁻¹ ml⁻¹ under the conditions employed.

Where specified, substrates concentrations in the standard assay were varied as follows. Free sulfide (up to 0.4 mM), mercaptopyruvate (up to 5 mM), coenzyme A (0.5 mM), or thiosulfate (0.5 mM) was used instead of cysteine; the concentration of cysteine employed was varied from 20 μM to 2 mM, and the concentration of Fe(NH₄)₂(SO₄)₂ was varied from 20 μM to 0.5 mM. ATP and NADPH (up to 5 mM each) were added to some reactions.

Quantification of Pyruvate Formation from Cysteine—The standard assay composition was modified as follows. 45 μM [¹⁴C]cysteine (adjusted to 11,200 dpm/μmol), reduced in situ prior to the addition of apopferredoxin by a 100-fold excess of glutathione to yield [¹⁴C]cysteine (5600 dpm/μmol) was employed. [¹⁴C]Pyruvate formed during the reaction was quantified both by Dowex 50 W×85 passage and phenylhydrazine derivatization. Following elution by addition of 2 N HCl to a final concentration of 285 mM, a 30-μl aliquot (13,000 dpm) of the assay mix was diluted to 1 ml with H₂O and applied to a 1-ml column of Dowex 50 W×85 (H⁻ form), equilibrated with 5 mM HCl. The [¹⁴C] content of the total eluate, including a 1.5-ml wash with 5 mM HCl, was determined and valued as pyruvate. A further 30-μl aliquot was mixed with 10 μmol of unlabeled pyruvate; after the addition of HClO₄ (final concentration 75 mM), 10 μmol of phenylhydrazine were added to give a final volume of 1 ml. The precipitated pyruvylphenylhydrazone was collected, and its specific radioactivity was determined. Each nmol of [¹⁴C]pyruvate (in the sample) raised the value obtained by 560 dpm/μmol.

Measurement of the Glutathione Content of Synechocystis Cells—About 50 mg (wet weight) of freshly grown Synechocystis cells were suspended in 0.5 ml of 0.1 M HClO₄, containing 0.1 M EDTA and disrupted by vigorous shaking (7 min) with 500 mg of glass beads (125–200 μm). The suspension was neutralized with 0.1 M KOH and centrifuged. Glutathione contained in the supernatant was determined using the kinetic glutathione reductase assay coupled to the reduction of 5,5’-dithio-bis(2-nitrobenzoate) (27).

Electrophoresis—Native separations at pH 7–9 were performed using 7% polyacrylamide gel and the buffer systems of Wu and Reisfeld (28). After electrophoresis (1.3 watts, 3 h) the gel was sliced in horizontal strips of 2 mm × 7 cm, and proteins were eluted with gentle shaking overnight in 2 ml of 50 mM Mops/NaOH, pH 7.6, containing 1 mM dithiothreitol and 20 mM EDTA.

SDS-PAGE according to Laemmli (24) was routinely performed with 12% acrylamide gels; the buffer systems of Wu and Reisfeld (28). After electrophoresis (1.3 watts, 3 h) the gel was sliced in horizontal strips of 2 mm × 7 cm, and proteins were eluted with gentle shaking overnight in 2 ml of 50 mM Mops/NaOH, pH 7.6, containing 1 mM dithiothreitol and 20 mM EDTA.

RESULTS

Studies with Crude Extract: Resolving the Holoferedoxin Formation Assay from the Complete Protein Set—To perform the apo- to holoferedoxin conversion assay, it was necessary to resolve the crude extract of Synechocystis wild type cells into two fractions: one containing specifically the abundant endogenous (holo)ferredoxin and the other comprising all remaining proteins. The strategy pursued is outlined in Fig. 1. The extract proteins were transferred into a high salt buffer (0.43 M chloride) and then applied to a DEAE-Sephadex A-25 column. The stringent buffer conditions and the small pore size of the gel matrix (exclusion limit 50 kDa) permitted essentially all proteins to pass through the column (Table I, Fig. 2). However, a very small amount of protein was retained, namely all of the ferredoxin and a few other polypeptides that could be detected by SDS-PAGE after elution from the column with 0.63 M chloride (Fig. 2, lane C). The ferredoxin in this fraction was purified to homogeneity and converted to the apo-form via acidic pre-

1 The abbreviations used are: Mops, 3-(N-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
Purification of C-DES from Deferredoxinized Extract—The purification procedure is outlined in Fig. 1 and detailed in Table I; included in the table is the dependence of holoferredoxin formation, mediated by the various protein fractions, on the presence of L-cysteine.

An initial approach to estimate the number and size of proteins involved in conversion was to perform a frontal analysis experiment; deferredoxinized extract was continuously applied to an Ultrogel AcA 44 column. Effluent fractions were assayed; holoferredoxin formation started from a Kav value of 0.5. About the same Kav value was determined from a zonal gel filtration run (Fig. 4); only protein(s) with a molecular mass of 40 ± 5 kDa should therefore be involved.

Further purification employed ion-exchange chromatography on DE 52 cellulose. However, recovery of conversion activity was rather poor (about 10%) without an extra sulfur compound added. Therefore, again the assay mixture was supplemented with the various sulfur compounds (see above). Only L-cysteine was found to restore holoferredoxin formation (recovery 60–70%), whereby a concentration of 90 μM proved to be sufficient; pyridoxal phosphate stimulated slightly, whereas ATP did not exert any effect. These properties were retained after chromatography on Q-Sepharose FF. Thus, by using ion exchange purified C-DES preparations, the standard assay conditions with 90 μM L-cysteine, 0.15 mM pyridoxal phosphate, and 0.4 mM Fe(NH4)2(SO4)2 could be established. Nevertheless, the active fractions were still rather impure.

Therefore, Q-Sepharose fractions were carried through native PAGE or chromatofocusing. Proteins eluted from slices of native gels were tested for conversion and analyzed by SDS-PAGE, which revealed a 43-kDa polypeptide contained in the active eluates (data not shown). Detection of the same single polypeptide correlated with conversion activity recovered after separation of Q-Sepharose fractions by chromatofocusing on a Mono P column (Fig. 2, lane D; Table I). Since both strategies independently identified the same 43-kDa polypeptide, this protein was considered as the active component.

Stoichiometry of the Apo- to Holoferredoxin Conversion Reaction Catalyzed by C-DES—With homogenous C-DES, L-cysteine was an absolute requirement for holoferredoxin forma-

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**TABLE I**

**Purification of C-DES**—With homogenous C-DES, L-cysteine was an absolute requirement for holoferredoxin formation.
Protein migration. The position taken by the fraction containing ferredoxin (amount corresponding to 3450 mg of extract proteins in 55 mM Mops/KOH, pH 7.5, 9 mM glutathione) indicated by an arrow. It displays reduced mobility as typical of acidic proteins.

To determine the specificity of C-DES for L-cysteine in supporting holoferredoxin formation, several related substances were tested as potential substitutes; neither D-cysteine (90 mM), cysteinylglycine (0.2–1 mM), N-acetyl-L-cysteine (90 mM), nor L-homocysteine (90 mM to 0.5 mM) was effective.

Reactivity of C-DES with L-Cysteine and L-Cystine as Alternative Substrates—Having established the stoichiometry of the complete reaction system (Reaction 1), we investigated whether cysteine was processed by C-DES in the absence of apoferredoxin. In fact, pyruvate formation occurred at the same rate as before (28 nmol min⁻¹ mg⁻¹ with 90 mM cysteine; see Figs. 5 and 6). In this context we also examined reactions with L-cysteine included instead of cysteine (glutathione omitted; Fig. 6). Surprisingly, pyruvate formation occurred readily; with 45 mM cystine instead of 90 mM cysteine an approximately 15-fold increase was observed.

Apoferredoxin + 2 L-cysteine + 2 Fe²⁺ + 2 O₂ → [2Fe-2S] ferredoxin + 2 pyruvate + 2 NH₃

Reaction 1
higher rate was observed. When the reaction was run until substrate depletion, finally 2 mol of pyruvate were yielded per mol of cysteine employed. The sulfur-containing product, possibly H₂S₂, was not characterized. In light of these findings, C-DES should more properly be considered as a cysteine/cysteine C-S-lyase.

The question of whether cystine is also a better substrate in the holoferrredoxin formation assay could not be investigated, since in the presence of excess glutathione (required for apoferrredoxin protection) cystine is predominantly reduced to cysteine.

**Structural Properties of C-DES**—The molecular mass of C-DES was determined as 40 ± 5 kDa by gel filtration (Fig. 4) and as 43 ± 2 kDa by SDS-PAGE (Fig. 2). These data identified the enzyme as a monomer. Stimulation of C-DES activity by the addition of pyridoxal phosphate in context with the β-elimination reaction observed with either cysteine or cystine as substrate was taken as evidence for C-DES being a pyridoxal phosphate-dependent enzyme whereby about 50% of the molecules retain their coenzyme after purification. Given the small amount of protein available, this assumption could not be directly tested by chemical or spectroscopic analyses. However, NaBH₄ treatment of C-DES (10 mM NaBH₄ for 1 h at 30 °C, pH 7.5–7.2) resulted in complete inactivation of the pyridoxal phosphate-loaded enzyme, indicating susceptibility to Schiff base reduction (32).

NiF₅ protein (producing sulfur and alanine from cysteine) has been reported to be particularly sensitive to inactivation by various thiol-alkylating agents including unsaturated amino acid derivatives (8, 33). We therefore tested the susceptibility of C-DES toward N-ethylmaleimide (0.1 mM), iodoacetamide (1 mM), L-allylglycine (5 mM), and L-vinylglycine (5 mM). After an incubation period of 30 min at 30 °C, C-DES was separated from excess reagent and assayed for apo- to holoferrredoxin conversion. Its activity proved to be virtually unimpaired. A further experiment was conducted employing 5 mM L-propargylglycine, which is an irreversible inhibitor of γ-cystathionase (34). Again C-DES activity remained stable.

**DISCUSSION**

Based on a self-contained assay system derived from the cyanobacterium *Synechocystis* we pursued a systematic search for a protein capable of directing [2Fe-2S] cluster assembly of ferredoxin. Purification identified a monomeric, 43-kDa, pyridoxal phosphate containing lyase named C-DES, which specifically and efficiently used l-cysteine as precursor for cluster sulfide.

From our purification results, the cellular concentration of ferredoxin is estimated to be 60–120 μM, and the concentration of C-DES is estimated to be 0.15–1 μM. The resulting molar ratio of about 150:1 is of the same order as that employed in our assays (e.g. 120:1 for the experiment outlined in Fig. 5). Taking into account the doubling time of about 10 h under the growth conditions employed, *Synechocystis* cells synthesize approximately 1 mg of ferredoxin/30 g of wet cells/h. The endogenous ferredoxin [2Fe-2S] cluster-forming activity detected by our assay was more than sufficient to meet the cells’ requirements (Table 1).

In the initial purification stages, the addition of l-cysteine was not required for *in vitro* cluster formation. Obviously, some enzyme component(s) of the crude extract supplied the sulfur source, probably by utilizing glutathione. Free sulfide did not exert any effect in our system at any stage of purification, which is in contrast to the chloroplast system (15). A further difference is the lack of any requirement for ATP.

The product pattern resulting from l-cysteine is identical to that of cysteine desulphhydrase of *Salmonella typhimurium* (31). However, in contrast to C-DES the *S. typhimurium* protein is tetrameric and is a more typical catabolic enzyme, with its high specific activity (450 μmol min⁻¹ mg⁻¹ with 2 mM cysteine as substrate; Ref. 35); furthermore, this protein was reported not to attack cysteine (31). From a functional point of view, isolation of a NiF₅-like protein might have been expected, especially when considering the widespread occurrence of NiF₅-type sequences (8, 12), which has recently been extended to *Synechocystis*: analysis of the total genome sequence of strain PCC 6803 identified three homologues (36). Indeed, C-DES and NiF₅ are both pyridoxal phosphate-containing enzymes, degrading l-cysteine with similar rates (89 nmol min⁻¹ mg⁻¹ for NiF₅ with 0.5 mM cysteine (8), 28 nmol min⁻¹ mg⁻¹ for C-DES with 90 μM cysteine). Both catalyze cysteine cleavage in the absence as well as in the presence of apoprotein; therefore, the release of sulfur from cysteine and its insertion into the Fe-S cluster do not seem to be necessarily coupled processes with these enzymes. However, it should be emphasized that cysteine sulfur released by C-DES was quantitatively found assembled in the [2Fe-2S] cluster of ferredoxin (see Fig. 5), indicating interaction between C-DES and the apoprotein substrate. Distinct from NiF₅, C-DES is monomeric, produces pyruvate, ammonia, and sulfide instead of alanine and sulfur, and cannot be inhibited by thiol-alkylating reagents.

A striking feature of C-DES is its reactivity with cystine, which finally yields two equivalents of pyruvate. This reaction is known as a secondary activity of certain β- and γ-cystathionases (37, 38), which are all homologous enzymes. Including cystine in the presence of excess glutathione in our reaction medium, we initially thought of this reduced compound as the primary sulfur donor species for [2Fe-2S] ferredoxin formation. By the increased reactivity of C-DES with cystine, we became aware of the possibility that the equilibrium concentration of cystine during conversion might be crucial. β-Elimination starting with cystine should yield cysteine persulfide as substrate-derived sulfane carrier compound. Whether this persulfidic intermediate is formed during catalysis by C-DES and is involved in sulfur transfer to apoferrredoxin awaits further studies. Work on such mechanistic problems will be greatly aided by the availability of substantial amounts of purified C-DES. Cloning, sequencing of the gene, and overexpression of C-DES in *E. coli* are in progress. Based on the sequence information, targeted inactivation should reveal the physiological consequences of a defective C-DES gene in *Synechocystis*. Us-
ing this approach we hope to examine the in vivo role of C-DES in ferredoxin Fe-S cluster formation.

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REFERENCES
1. Beinert, H. (1990) FEBS Lett. 382, 218–219
2. Beinert, H., and Kiley, P. (1996) FEBS Lett. 382, 218–219
3. Malkin, R., and Rabinowitz, J. C. (1966) Biochem. Biophys. Res. Commun. 23, 822–827
4. Cerletti, P. (1986) Trends Biochem. Sci. 11, 369–372
5. Taniguchi, T., and Kimura, T. (1974) Biochim. Biophys. Acta 364, 284–295
6. Nobrega, F. G., Nobrega, M. P., and Tzagoloff, A. (1992) EMBO J. 11, 3821–3829
7. Jacobson, M. R., Cash, V. L., Weiss, M. C., Laird, N. F., Newton, W. E., and Dean, D. R. (1989) Mol. Gen. Genet. 219, 49–57
8. Zheng, L., White, R. H., Cash, V. L., Jack, R. F., and Dean, D. R. (1994) J. Biol. Chem. 269, 18723–18726
9. Shin, M. (1971) Methods Enzymol. 23, 440–447
10. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) J. Gen. Microbiol. 111, 1–61
11. Khoroshilova, N., Beinert, H., and Kiley, P. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2499–2503
12. Hidalgo, E., and Demple, B. (1996) J. Biol. Chem. 271, 7269–7272
13. Flatin, D. H. (1996) J. Biol. Chem. 271, 16968–16974
14. Takahashi, Y., Mitsu, A., Hase, T., and Matsubara, H. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2434–2437
15. Meyer, J., Moulis, J.-M., and Lutz, M. (1986) Biochim. Biophys. Acta 871, 243–249
16. Pilen, M., de Kruijff, B., and Weisbeek, P. J. (1992) J. Biol. Chem. 267, 2548–2556
17. Shin, M. (1971) Methods Enzymol. 23, 440–447
18. Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., and Stanier, R. Y. (1979) J. Gen. Microbiol. 111, 1–61
19. Hidalgo, E., and Demple, B. (1996) J. Biol. Chem. 271, 7269–7272
20. Canek, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hiroswa, M., Sugura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsu, A., Muraki, A., Nakazaki, N., Naru, K., Okumura, S., Shimo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M., and Tabata, S. (1996) DNA Res. 3, 109–136
21. Delavier-Klutchko, C., and Flavin, M. (1965) Biochim. Biophys. Acta 99, 375–377
22. Flavin, M. (1962) J. Biol. Chem. 237, 768–777
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