Engineering a Novel Iron-Sulfur Cluster into the Catalytic Subunit of Escherichia coli Dimethyl-sulfoxide Reductase

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Dimethyl-sulfoxide reductase (DmsABC) is a complex [Fe-S] molybdoenzyme that contains four [4Fe-4S] clusters visible by electron paramagnetic resonance (EPR) spectroscopy. The enzyme contains four ferredoxin-like Cys groups in the electron transfer subunit, DmsB, and an additional group of Cys residues in the catalytic subunit, DmsA. Mutagenesis of the second Cys, Cys-38, in the DmsA group to either Ser or Ala promotes assembly of a fifth [Fe-S] cluster into the mutant enzyme. The EPR spectra, the temperature dependences, and the microwave power dependences demonstrate that the new clusters are [3Fe-4S] clusters. The [3Fe-4S] clusters in both of the C38S and C38A mutant enzymes are relatively unstable in redox titrations and have midpoint potentials of approximately 178 and 140 mV. Mutagenesis of the DmsA Cys group to resemble a sequence capable of binding an [4Fe-4S] cluster did not change the enzyme type but reduced the amount of the cluster present in this mutant enzyme. This report demonstrates that all four EPR detectable [Fe-S] clusters in the wild-type enzyme are ligated by DmsB. Wild-type DmsA does not ligate an [Fe-S] cluster that is visible by EPR spectroscopy.

DmsABC is a member of a family of molybdenum-containing oxidoreductases with highly conserved sequences (1, 6–8). These are enzymes that reduce Me2SO, trimethylamine N-oxide (TMAO) (9), nitrate (7, 10–14), biotin sulfoxide (15, 16), and polysulfide (17) or enzymes that oxidize formate (18–24). Each enzyme contains a large catalytic subunit with a noncovalently bound molybdenum cofactor. The sequence identity is located in segments throughout the polypeptide. Many of these enzymes are similar to DmsABC in prosthetic groups and subunit composition.

Ferredoxins that contain [4Fe-4S] clusters usually ligate these clusters by Cys groups consisting of four Cys residues spaced such that the first two Cys residues are separated by two amino acids, while the spacing between the second, third, and fourth Cys residues is somewhat variable. A Cys group from the thermophilic methanogen Methanococcus thermo lithotrophicus (25) has four amino acids separating the first two ligands, but we have not identified a Cys group with three intervening residues. The first three Cys residues and one distal Cys, often from a second Cys group elsewhere in the protein, provide the ligands to the cluster (26, 27). Alignment of the amino-terminal regions of the large subunits of the molybdoenzymes (Fig. 1) shows four conserved Cys residues arranged in a manner reminiscent of a [4Fe-4S] ferredoxin Cys group. The sequences can be divided into three types. Type I enzymes contain three Cys residues spaced similar to a bacterial ferredoxin Cys group and one other conserved Cys, which could provide the fourth ligand. The Type II enzymes also have four Cys residues, but the spacing is such that three amino acids instead of two separate the first and second Cys residues. DmsABC belongs to this group, as do the two membrane-bound E. coli nitrate reductases in which the first Cys is replaced by a His. His can be a ligand to a [4Fe-4S] cluster, as in the nickel-iron hydrogenase from Desulfovibrio gigas, but the first two ligands of this cluster, His and Cys, are separated by two amino acids (28). The Type II enzymes include biotin sulfoxide reductase (BiSC) and TMAO reductase (TorA) which share sequence identity with the other molybdenoenzymes but do not contain the Cys region.

The periplasmic nitrate reductase, NapAB, from Thiophae ra pantotropha has been shown to contain a [4Fe-4S] cluster (29). This is a Type I enzyme, and the Cys residues in NapA are the only candidates to ligate the [4Fe-4S] cluster (14, 29). This raises the possibility that the Cys region may ligate a [4Fe-4S] cluster in other members of this family. The subunit of E. coli formate hydrogenlyase that contains the formate dehydrogenase activity, FdhF, may contain an [Fe-S] cluster based on iron analysis (30).

The [Fe-S] clusters of DmsABC have been characterized by electron paramagnetic resonance (EPR) spectroscopy. The enzyme contains four [4Fe-4S] clusters with midpoint potentials, E_m = -50, -120, -240, and -330 mV (4). These clusters are...
believed to be ligated by the four ferredoxin-like Cys groups (I-IV) in DmsB (1, 4), although the possibility exists that the DmsA residues that were examined in this study are underlined.

The DmsA Cys region has previously been examined through site-directed mutagenesis of DmsB groups I-IV (31) and has demonstrated that these Cys groups provide ligands for two [4Fe-4S] clusters.

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**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids**—E. coli strain F36 is a mutant of E. coli HB101 (supE44 lac-proAB lacIq X1, lacIq14, lacZM15) and harbors the pBR322 (Pharmacia Biotech Inc.), pDMS160, which contains the dmsABC operon cloned into pBR322 (31). The plasmids used are derivatives of pDMS160 containing point mutations with substitutions in residues Asn-37 and Cys-38 was generated through PCR mutagenesis of pC38S using the following mutagenic primers: 5'-CGAC-3' and 5'-CGAC-3'. The double mutant of dmsA with substitutions in residues Asn-37 and Cys-38 was obtained from commercial sources.

**Site-directed Mutagenesis**—Manipulations of strains and plasmids were carried out as described in Sambrook et al. (34). The double mutant of dmsA with substitutions in residues Asn-37 and Cys-38 was obtained from commercial sources.

**SITE-DIRECTED MUTAGENESIS**

**Type I**

| Cys Region | Mutations | Notes |
|------------|-----------|-------|
| Cys-38     | N37C      |       |
| Cys-42     | C38S      |       |

**Type II**

| Cys Region | Mutations | Notes |
|------------|-----------|-------|
| Cys-38     | N37C      |       |
| Cys-42     | C38S      |       |

**Type III**

| Cys Region | Mutations | Notes |
|------------|-----------|-------|
| Cys-38     | N37C      |       |
| Cys-42     | C38S      |       |

**SERVICE**

**EPR Spectroscopy**—Samples were prepared as described by Cammak and Weiner (4) for either whole cells or washed membranes. Redox titrations were performed using the following mediators: quinhydrone, 2,6-dichlorophenolindophenol, 1,2-naphthoquinone, toluidine blue, phenazine methosulfate, thionine, duroquinone, methylene blue, and resorufin. Spectra were recorded using a Bruker ESR 300 EPR spectrometer equipped with an Oxford Instruments ESR-900 flowing helium cryostat. Instrument conditions and temperatures are described in

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2. R. A. Rothery and J. H. Weiner, unpublished results.
TABLE I

| Plasmid     | Growth on Me₂SO a | Specific activity b | Expression c (Relative % DmsA) |
|-------------|-------------------|--------------------|--------------------------------|
| pBR322      | –                 | 6                  | 2                              |
| pDMS160     | +                 | 38                 | 15                             |
| pC38A       | –                 | 15                 | 10                             |
| pC38S       | –                 | 19                 | 13                             |
| pC38S,N37C  | –                 | 19                 | 12                             |

a The ability of the mutant enzymes to support growth on glycerol- Me₂SO media in the dmsABC deletion strain, DSS301 (Table I). Only the wild-type enzyme was able to support growth. DSS301 was not used for further characterization of the mutant enzymes for reasons previously mentioned (31, 32). The specific activities of HB101 membrane preparations were assayed using TMAO as the electron acceptor and the artificial electron donor, BV⁺. (Table I). Both mutant enzymes showed decreased specific activities when compared with the wild-type enzyme, as shown previously (32). The relative amounts of enzyme present in the F36 membrane preparations used for the EPR studies were determined by densitometry (Table I). The relative percentage of DmsA in the F36/pC38A membranes is lower than in the wild-type or C38S preparations.

EPR Characteristics of Dithionite-reduced F36 Membranes Containing Overexpressed Wild-type and Mutant DmsABC—In F36 the Mo-MGD cofactor is not inserted into DmsABC and cannot interfere with the [Fe-S] signals in the redox titrations as occurs when DmsABC is expressed in HB101 (4). Although the enzyme produced in F36 is inactive, it assembles the [Fe-S] clusters normally. Fig. 2 shows the EPR spectra of reduced membranes at 12 K. F36/pBR322 membranes contained only a little DmsABC, expressed from the chromosomal copy of the operon. The spectrum from these membranes shows a peak at g = 2.02 and a trough at about g = 1.94. These features are characteristic of the reduced [2Fe-2S] cluster of fumarate reductase, FR1 (40, 41). F36/pDMS160 membranes contained a high level of DmsABC, and the spectrum of the dithionite-reduced samples (Fig. 2b) is very similar to spectra obtained from both purified and membrane-bound DmsABC (4, 31). The spectrum contains peaks located at g = 2.06, g = 2.02, g = 1.99, and g = 1.95. Two troughs are observed at g = 1.92 and g = 1.87. The peak at g = 2.02 is most likely due to fumarate reductase, which would also contribute to the peak/trough at g = 1.95 to g = 1.92 (31). The peaks at g = 2.06 and g = 1.99 and the peak/trough at g = 1.95 to g = 1.92 arise from DmsABC. Repeating the dithionite reduction at pH 9, to decrease the redox potential, did not change the spectrum observed.

Fig. 2, c and d, shows the spectra of dithionite-reduced F36 membranes containing the C38A and C38S mutant enzymes, respectively. The features of these spectra are very similar to that of F36/pDMS160 membranes, and all of the DmsABC features are present. The slight difference in the size of some of the features in the reduced EPR spectra of the C38A mutant enzyme is due to the lower amount of enzyme present. Reduction by dithionite at pH 9 did not highlight any difference between the spectra of the wild-type and mutant enzymes (data not shown).

EPR Characteristics of Oxidized F36 Membranes Containing Amplified Wild-type and Mutant DmsABC—Fig. 3 shows EPR spectra recorded at 12 K of F36 membranes oxidized with ferrocyanide. The spectrum of F36/pBR322 membranes (Fig. 3a) has a small peak at g = 2.02 with a broad trough immediately upfield, characteristic of the oxidized [3Fe-4S] cluster of fumarate reductase, FR3 (40, 41). The spectrum of F36/pDMS160 membranes (Fig. 3b) shows major new features. The spectrum from the mutant enzymes show major new features. The spectrum of F36/pC38S membranes (Fig. 3d) is comprised of a sharp peak at g = 2.03 and a peak/trough centered at g = 2.00. The F36/pC38S spectrum (Fig. 3c) is similar, but the signal is broader than in F36/pC38S membranes. The EPR spectra of the oxidized mutant enzymes can be attributed to centers having axial symmetry with approximate g values of gₓ = 2.03 and gₕ = 2.00. Oxidized spectra of HB101 membranes containing the C425 and C755 mutant enzymes were similar to that of FR3, indicating that these mutants do not assemble significant amounts of a [3Fe-4S] cluster ligated in DmsA (data not shown).

To identify the nature of the paramagnetic species present in the oxidized Cys-38 mutants, we studied the microwave power saturation properties and the temperature dependences of the new signals. Fig. 4 shows the effect of increasing microwave power on the mutant center signals at 12 K. Microwave power
saturation data obtained from F36/pC38A membranes were fitted to an empirical equation to obtain the microwave power required for half saturation of the signal, the $P_{1/2}$ (42). A two-component model was required to fit the data giving $P_{1/2}$ values of 1 (40%) and 185 milliwatts (60%). The presence of two components suggests that the protein conformation around the cluster is not homogeneous. Microwave power saturation data from the F36/pC38S membranes were fitted to one component with a $P_{1/2}$ of 9 milliwatts.

Redox Titrations of the C38A and C38S Mutant Enzymes—Redox titrations were carried out to determine the midpoint potentials for the [4Fe-4S] clusters and the new [3Fe-4S] clusters in the DmsA mutant enzymes (Table II). In the mutant enzymes, four [4Fe-4S] clusters were detected with midpoint potentials close to those of the wild-type enzyme. The amount of each cluster present was very similar in the wild-type and mutant enzymes. To determine the midpoint potential of the [3Fe-4S] cluster of C38A, F36/pC38A membranes were oxidized, reduced, and reoxidized (Fig. 6). Upon reoxidation of the membranes, most of the [3Fe-4S] clusters were destroyed. Data obtained from the titration were fitted to a two-component model of the Nernst equation with $E_{m,7} = 75$ mV (31%). The F36/pC38S redox titration data were fitted to one component, but the cluster routinely exhibited hysteresis. The $E_{m,7}$ in the oxidizing direction was 190 mV, and the $E_{m,7}$ in the reducing direction was 165 mV. The ratio of reduced [Fe-S] clusters to that of oxidized [Fe-S] clusters was determined from double integrations of reduced and oxidized samples. In the oxidized spectrum of membranes containing wild-type DmsABC, there is only a small amount of [3Fe-4S] cluster visible, so the ratio of reduced [Fe-S] clusters to that of oxidized [Fe-S] clusters was determined from double integrations of reduced and oxidized samples. In the oxidized spectrum of membranes containing wild-type DmsABC, there is only a small amount of [3Fe-4S] cluster visible, so the ratio of reduced [Fe-S] clusters to that of oxidized [Fe-S] clusters was determined from double integrations of reduced and oxidized samples.

Mutagenesis of the DmsA Cys Group to a Consensus Ferredoxin Cys Group—Sequences known to ligate [4Fe-4S] clusters usually contain four Cys residues (26, 27). The first and second Cys residues are separated by two amino acids, an exception being M. thermolithotrophicus ferredoxin, which has four intervening residues (25). We altered the sequence of the DmsA
Cys group so that the first two Cys residues would only be separated by two amino acids. The plasmid, pC38S, was further mutated to produce pC38S,N37C, which contains the sequence, CTVCSGSN.

This spacing of Cys residues occurs in Cys group II of DmsB and other electron transfer subunits of enzymes belonging to this family (1) and in Azotobacter vinelandii ferredoxin I (45, 46). Expression and specific activity of the double mutant, C38S,N37C, are similar to that of C38S, and this enzyme is also unable to support growth on Me2SO in DSS301 (Table I).

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**DISCUSSION**

Wild-type DmsABC has a complex EPR spectrum (Fig. 2b) that has been analyzed as two pairs of interacting [4Fe-4S] clusters (1, 4). Our research has been aimed at identifying which residues ligate the [Fe-S] clusters in DmsABC through the use of site-directed mutagenesis and EPR. Cys groups III (31) and I2 of DmsB each ligate a [4Fe-4S] cluster. In this report, the Cys region of DmsA was mutated so that it is unlikely to bind a [4Fe-4S] cluster, but the EPR spectra of reduced membranes containing DmsA mutant enzymes was essentially identical to the wild-type enzyme (Fig. 2). All four previously characterized [4Fe-4S] clusters are present in the mutant enzymes in the correct amounts and with midpoint potentials similar to those of the wild-type enzyme (Table II). We conclude that the four EPR visible [4Fe-4S] clusters are all ligated by the Cys groups of DmsB.

### TABLE II

| Enzyme       | Midpoint potentials | Redoxa |
|--------------|---------------------|--------|
|              | I      | II     | III    | IV     | V     |
| DmsABC       | -50    | -120   | -240   | -340   | 33.6  |
| C38S         | -40    | -95    | -220   | -350   | 75/140 |
| C38S,N37C    | -50    | -115   | -255   | -370   | 165, 190 |

a The ratio of reduced to oxidized [Fe-S] clusters was calculated from double integration of reduced and oxidized spectra from the redox titrations.

b Data from the redox titrations were fitted to a two-component model.

c Data from the redox titrations were fitted to two independent one-component models.

Fig. 2b. Redox titration curves showing the change in signal amplitude of the g = 2.03 signal of F36/pC38A (a) and F36/pC38S membranes (b). Spectra were recorded under the conditions outlined in Fig. 2, and (□) represents data obtained during the addition of dithionite. C38A data were fitted to two components with Em, values of 75 and 140 mV. C38S data were fitted in the oxidizing direction to an Em, of 190 mV and in the reducing direction to an Em, of 165 mV.

Cys group so that the first two Cys residues would only be separated by two amino acids. The plasmid, pC38S, was further mutated to produce pC38S,N37C, which contains the sequence, CTVCSGSN.

This spacing of Cys residues occurs in Cys group II of DmsB and other electron transfer subunits of enzymes belonging to this family (1) and in Azotobacter vinelandii ferredoxin I (45, 46). Expression and specific activity of the double mutant, C38S,N37C, are similar to that of C38S, and this enzyme is also unable to support growth on Me2SO in DSS301 (Table I).

Fig. 7 shows spectra of the ferricyanide-oxidized membranes from the DmsA mutants. The double mutant ligated a [3Fe-4S] cluster, but the amount of cluster was reduced to approximately 25% of the amount of cluster ligated by C38S, estimated by double integration. The line shape is similar to C38S, but the signal is broader. Spectra of the mutants in whole cells are identical to that of the membrane preparations, indicating that the clusters in all three mutant enzymes are [3Fe-4S] clusters in vivo and are not [4Fe-4S] clusters altered upon oxidation during cell breakage (data not shown). The signal intensity of the double mutant appeared larger in whole cells than in the membrane samples. Redox titrations of the double mutant identify four [4Fe-4S] and one [3Fe-4S] cluster (Table II) with the ratio of reduced to oxidized [Fe-S] clusters being approximately 13:1. The high ratio is likely due to the reduced amount of [3Fe-4S] cluster present in these membrane preparations.
DmsA mutants with a peak at g = 2.03 and a peak/ trough at g = 2.00 (Fig. 3). The line shapes of the new signals are distinct from the spectrum of fumarate reductase center FR3 (40, 41). The temperature and power dependences shown in Figs. 4 and 5 are similar to those of the artificial [3Fe-4S] clusters formed by site-directed mutagenesis of Cys groups (31, 47). The multiple components, hysteresis, and fragility displayed by the DmsA mutant clusters demonstrate cluster instability in this environment. It is unlikely that the [3Fe-4S] signal is due to oxidative damage of the DmsB clusters, as the levels of the [4Fe-4S] clusters are the same in the wild-type and mutant enzymes, and the C42S and C75S mutant enzymes do not show this new [3Fe-4S] cluster signal.

The possibility exists that the [3Fe-4S] cluster in the DmsA mutants could be generated from a [4Fe-4S] cluster present in the wild-type enzyme. Conversion of Fe-S (31, 47) to ligate an [3Fe-4S] cluster. Another possible reason for the loss of which may destroy function in the Cys-38 and 39. Substitution of Ser or Ala for Cys should cause little perturbation of the protein structure, indicating that the sulfhydryl of the Cys-38 in DmsA mutants is not conserved in the Type II enzymes (Fig. 1). A cluster may act as an "insulating cluster" (28, 53) between the [4Fe-4S] clusters and Mo-MGD, where the substrate is reduced (32). The high E mø of the [3Fe-4S] cluster in C38 relative to the potentials of the Mo(VI)/(IV) and Mo(V)/(IV) couples (−75 and −90 mV, (4)) suggests that the [3Fe-4S] cluster may act as an "insulating cluster" (28, 53) between the [4Fe-4S] clusters and the Mo-MGD to decrease the rate of electron transfer.

We have divided the enzymes into three classes. The Type I enzymes such as NapAB, FdhF, and perhaps the other members of this type are likely to have a [4Fe-4S] cluster located in their amino terminus. The Type II enzymes, DmsABC, and the two E. coli nitrate reductases have a Cys region, but they are not likely to ligate an [Fe-S] cluster. The Type III enzymes lack this region altogether, and neither TorA or BisC have been demonstrated to contain [Fe-S] clusters. This region in DmsA is likely a degenerate Cys group that has lost [Fe-S] binding capability upon evolution of the enzyme, although in DmsA the Cys group retains an essential role in electron transfer, perhaps interacting with the Mo-MGD.

REFERENCES
1. Weiner, J. H., Rothey, R. A., Sambasivarao, D., and Triever, J. H. (1992) Biochim. Biophys. Acta 1102, 1–18
2. Sambasivarao, D., Scraba, D. G., Triever, C., and Weiner, J. H. (1990) J. Biol. Chem. 265, 5813–5812
3. Sambasivarao, D., Simala Grant, J. L., Johnson, J. L., Rajagopalan, K. V., and Weiner, J. H. (1995) J. Biol. Chem. 270, 23720–23727
4. Blasco, F., Iobbi, C., Giordano, G., Chippaux, M., and Bonnefoy, V. (1990) Biochim. Biophys. Acta 1057, 157–185
5. Mujean, V., Iobbi-Nicol, C., Lepetitier, M., Giordano, G., Chippaux, M., and Bonnefoy, V. (1994) Mol. Microbiol. 13, 1169–1179
6. Blasco, F., Iobbi, C., Ratouchniak, J., Bonnefoy, V., and Chippaux, M. (1990) Mol. Gen. Genet. 225, 104–111
7. Andrieuëse, X., and Bakker, H. (1993) EMBL database, accession number X74597
8. Lin, J. T., Goldman, B. S., and Stewart, V. (1993) J. Bacteriol. 175, 2370–2378
9. Siddiqui, R. A., Warnecke-eberz, U., Hengsberger, A., Schneider, B., Kostka, S., and Friedrich, B. (1993) J. Bacteriol. 175, 5967–5976
10. Blasco, F., Iobbi, C., Giordano, G., Chippaux, M., and Bonnefoy, V. (1990) J. Bacteriol. 172, 2194–2198
11. Pollock, V. V., and Barber, M. J. (1995) Arch. Biochem. Biophys. 318, 322–332
12. Lin, J. T., Goldman, B. S., and Stewart, V. (1993) J. Bacteriol. 175, 2370–2378
13. Siddiqui, R. A., Warnecke-eberz, U., Hengsberger, A., Schneider, B., Kostka, S., and Friedrich, B. (1993) J. Bacteriol. 175, 5967–5976
14. Berks, B. C., Richardson, D. J., Reilly, A., Willis, A. C., and Ferguson, S. J. (1995) Biochim. Biophys. Acta 1233, 193–203
15. Berks, B. C., Richardson, D. J., Reilly, A., Willis, A. C., and Ferguson, S. J. (1995) Biochim. Biophys. Acta 1233, 193–203
16. Piers, D. E., and Campbell, A. (1990) J. Bacteriol. 172, 2194–2198
17. Pollock, V. V., and Barber, M. J. (1995) Arch. Biochem. Biophys. 318, 322–332
18. Kraft, T., Bokranz, M., Klimmek, O., Schröder, I., Fahrenholz, R., Kojro, E., and Kröger, A. (1992) Eur. J. Biochem. 206, 503–510
19. Bokranz, M., Guttman, M., Kortnor, C., Kojro, E., Fahrenholz, R., Lauterbach, W. A., and Bray, R. C. (1991) Arch. Microbiol. 156, 119–128
20. Plunkett, G. I., Burland, B. D., Daniels, D. L., and Blattner, F. R. (1993) Nucleic Acids Res. 21, 3391–3398
21. Plunkett, G. I., Burland, B. D., Daniels, D. L., and Blattner, F. R. (1993) Nucleic Acids Res. 21, 3391–3398
22. Shubert, A. P., Orr, E. C., Reemy, M. A., Schendel, P. F., May, H. D., Schauer, N. L., and Ferry, J. G. (1986) J. Bacteriol. 126, 12942–12947
23. Sauter, M., Böhm, R., and Bück, A. (1992) Mol. Microbiol. 6, 1523–1532
24. White, W. B., and Ferry, J. G. (1993) J. Bacteriol. 175, 4997–5004
25. Zinoni, F., Birkmann, A., Stadtman, T. C., and Böck, P. (1996) Proc. Natl. Acad. Sci. U.S.A. 93, 6560–6564
26. Bruschi, M., Bonio, J., Hachthiakian, C., Fardelle, M. L., Belaicher, J. P., and Frey, M. (1991) Biochim. Biophys. Acta 1076, 79–85
27. Bruschi, M., and Guerelguin, F. (1988) FEMS Microbiol. Rev. 45, 155–176
28. Matsubara, J., and Saeki, K. (1992) Adv. Inorg. Chem. 38, 223–230
29. Witzot, A., Charron, F. M., and Fontecave-Camps, J. C. (1995) Nature 372, 580–587
30. Breton, J., Berks, B. C., Reilly, A., Thomson, A. J., Ferguson, S. J., and Richardson, D. J. (1994) FEMS Lett. 134, 75–80
31. Ackley, M. J., Grehan, D. A., and Stadtman, T. C. (1990) J. Bacteriol. 126, 1821–1828
32. Rothey, R. A., and Weiner, J. H. (1991) Biochemical Society 30, 8296–8305
33. Triever, C. A., Rothey, R. A., and Weiner, J. H. (1994) J. Biol. Chem. 269, 7103–7109
34. Sambasivarao, D., and Weiner, J. H. (1991) J. Bacteriol. 173, 5933–5943
39. Laemmli, U. K. (1970) Nature 227, 680–685
40. Morningstar, J. E., Johnson, M. K., Cecchini, G., Ackrell, B. A. C., and Kearney, E. B. (1985) J. Biol. Chem. 260, 13631–13638
41. Johnson, M. K., Kowal, A. T., Morningstar, J. E., Oliver, M. E., Whittaker, K., Gunsalus, R. P., Ackrell, B. A. C., and Cecchini, G. (1988) J. Biol. Chem. 263, 14732–14738
42. Rupp, H., Rao, K. K., Hall, D. O., and Cammack, R. (1978) Biochim. Biophys. Acta 537, 255–269
43. Gayda, J. P., Bertrand, P., and Theodule, F. X. (1982) J. Chem. Physiol. 77, 3397–3391
44. Bertrand, P., Guigliarelli, B., Meyer, J., and Gayda, J. P. (1984) Biochimie (Paris) 66, 77–79
45. Stout, G. H., Turley, S., Sieker, L. C., and Jensen, L. H. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 1020–1022
46. Morgan, T. V., Stephens, R. J., Burgess, B. K., and Stout, C. D. (1984) FEBS Lett. 167, 137–141
47. Warren, P. V., Smart, L. B., McIntosh, L., and Golbeck, J. H. (1993) Biochemistry 32, 4411–4419
48. Zhao, J., Li, N., Warren, P. V., Golbeck, J. H., and Bryant, D. A. (1992) Biochemistry 31, 5093–5099
49. Manodori, A., Cecchini, G., Schröder, I., Gunsalus, R. P., Werth, M. T., and Johnson, M. K. (1992) Biochemistry 31, 2703–2712
50. Guigliarelli, B., Asso, M., More, C., Augier, V., Blasco, F., Pommier, J., Giordano, G., and Bertrand, P. (1992) Eur. J. Biochem. 207, 61–68
51. Augier, V., Guigliarelli, B., Asso, M., Bertarand, P., Frixon, C., Giordano, G., Chippaux, M., and Blasco, F. (1993) Biochemistry 32, 2013–2023
52. Augier, V., Asso, M., Guigliarelli, B., More, C., Bertrand, P., Santini, C.-L., Blasco, F., Chippaux, M., and Giordano, G. (1993) Biochemistry 32, 5099–5108
53. Kowal, A., Werth, M. T., Manodori, A., Cecchini, G., Schröder, I., Gunsalus, R. P., and Johnson, M. K. (1995) Biochemistry 34, 12284–12293
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