We present a 1.59-Å resolution crystal structure of reduced Paracoccus pantotrophus cytochrome cd_{1} with cyanide bound to the d_{1} heme and His/Met coordination of the c heme. Fe–C–N bond angles are 146° for the A subunit and 164° for the B subunit of the dimer. The nitrogen atom of bound cyanide is within hydrogen bonding distance of His^{342} and His^{388} and either a water molecule in subunit A or Tyr^{25} in subunit B. The ferrous heme-cyanide complex is unusually stable (K_d \sim 10^{-6} \text{ M}); we propose that this reflects both the design of the specialized d_{1} heme ring and a general feature of anion reductases with active site heme. Oxidation of crystals of reduced, cyanide-bound, cytochrome cd_{1} results in loss of cyanide and return to the native structure with Tyr^{25} as a ligand to the d_{1} heme iron and switching to His/His coordination at the c-type heme. No reason for unusually weak binding of cyanide to the ferric state can be identified; rather it is argued that the protein is designed such that a chelate-based effect drives displacement by tyrosine of cyanide or a weaker ligand, like reaction product nitric oxide, from the ferric d_{1} heme.

Cytochrome cd_{1}, nitrite reductase is a soluble dimeric enzyme located in the bacterial periplasm, and each monomer contains both a c-type cytochrome and a noncovalently bound d_{1}-type heme (1). The latter is unique to this class of enzyme and distinguished by partial saturation of the porphyrin ring and His/Met coordination at the heme (1). The latter is unique to this class of enzyme and involves nitrite binding to the ferrous heme (1). From the study of the binding of cyanide to the enzyme. Cyanide usually involves nitrite binding to the ferrous heme (1). However, ferrous d_{1} heme binds nitrite (NO_{2}^{−}), and thus we have sought to elucidate the factors that stabilize the binding of anions to cytochrome cd_{1}. On the basis of the results of our study we have made correlations between the properties of several types of anion reductase that have active site heme. This work was supported by a European Union Biotechnology Structural Biology project (BIO4 CP96-0281) and by the Biotechnology and Biological Sciences and Research Council (Grant B05860). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The atomic coordinates and structure factors (code 1e2r) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/). These authors contributed equally to this work.

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Cyanide-Bound Cytochrome cd_{1} Nitrite Reductase

### Table I

**Data collection and refinement statistics**

| Parameter | Value |
|-----------|-------|
| Resolution (Å) | 25–1.59 |
| Observations | 599,187 |
| Unique reflections | 156,509 |
| \( R_{	ext{cryst}} \) | 0.081 |
| Completeness (%) | 97.8 |

| Refinement | Value |
|------------|-------|
| Nonhydrogen atoms | 9,905 (including 1,348 waters) |
| \( R_{	ext{cryst}} \) (2σ) | 0.192 |
| Reflections used | 149,556 |
| \( R_{	ext{cryst}} \) (2σ) | 0.208 |
| Reflections used | 6273 |
| Mean temperature factor (Å²) | 16.1 |
| Refined from ideal values | 84.6 |
| Bonds (*) | 0.007 |
| Angles (Å) | 1.6 |
| Mean coordinate error (Å)* | 0.14 |

\* \( R_{	ext{cryst}} = \sum_{hkl} |I_{hkl}| - <I_{hkl}> \sum_{hkl} <I_{hkl}> ; \) where \( I_{hkl} \) is the jth observation of reflection h, and \( <I_{hkl}> \) is the mean intensity of that reflection.

\( k_{	ext{obs}} = \sum_{hkl} F_{hkl} - F_{hkl}/2S_{hkl} \) where \( F_{hkl} \) and \( F_{hkl} \) are the observed and calculated structure factor amplitudes, respectively.

\* \( R_{	ext{cryst}} \) equivalent to \( R_{	ext{cryst}} \) for a 4% subset of reflections not used in the refinement (43).

\* Determined by the SIGMAA method (40).

There are no reports in the literature describing the formation of complexes of cyanide with cytochrome cd_{1} from either *P. pantotrophus* or its close relative *P. denitrificans*. Incubation of oxidized enzyme solution with 10 mM cyanide at pH 7.0 resulted in no detectable change in the visible absorption spectrum, strongly indicating that cyanide was unable to bind either heme center of *P. pantotrophus* cytochrome cd_{1}. In contrast, when cyanide was added to a solution of reduced enzyme, there were significant changes in the visible absorption spectrum (Fig. 1A). These changes were most pronounced above 600 nm, where only the \( d_1 \) heme absorbs (13), and there were virtually no changes to those regions of the spectrum previously assigned to the \( c \) heme. It can therefore be concluded that cyanide binds specifically to the \( d_1 \) heme of the reduced enzyme under these conditions. The reason for the failure of cyanide to bind to the oxidized enzyme might be that it is kinetically unable to displace Tyr^{25} from the \( d_1 \) heme. Thus, an attempt was made to oxidize the reduced enzyme-cyanide complex. When this complex was treated with ferricyanide, the visible absorption spectrum changed but did not exactly return to the spectrum of the fully oxidized enzyme (Fig. 1B). However, it is similar and there are bands characteristic of His/Tyr-ligated \( d_1 \) heme (434 nm (shoulder), 640 nm, 702 nm) (6, 21). This suggests that reoxidation of the solution enzyme-cytochrome complex produces a mixed species consisting mostly of enzyme in the “as isolated” conformation with a minor component, possibly incorporating ferric heme \( d_1 \)-CN. If so, the implication is that Tyr^{25} is able to displace CN⁻ from ferric heme \( d_1 \).

The dissociation constant (\( K_{d} \)) for cyanide binding to reduced *P. pantotrophus* cytochrome cd_{1} at 25 °C was determined by titration of cyanide into a solution of reduced enzyme. Clearly no data could be obtained for either the ferric, “as isolated” enzyme, to which cyanide does not bind, nor for the ferricyanide-reoxidized enzyme-CN complex. \( K_{d} \) was found to be 0.7 ± 0.2 × 10⁻⁸ M at pH 7.0. This value was essentially independent of pH in the range 7.0–9.0, suggesting that proton uptake did not accompany cyanide binding. Isobestic points during the titration indicated the presence of a two-state mixture of reduced enzyme and enzyme-CN complex. A Hill plot gave a value for the Hill coefficient of 0.85 ± 0.06 (data not shown).

Following these observations, we determined the structure of the reduced enzyme with cyanide bound, by the following procedure. Cytochrome cd_{1} was crystallized in the oxidized state and reduced with dithionite; then cyanide was diffused into the crystals as described under “Experimental Procedures.” Typical crystals obtained this way diffracted x-rays beyond 2-Å resolution, and the structure was refined using the coordinates of the fully reduced enzyme as a starting model (6). The resultant structure, obtained at 1.59 Å, was essentially identical to that seen previously except that cyanide was clearly visible, bound to both \( d_1 \) heme iron atoms of the dimeric protein. The differences in structure between the two monomers of the \( d_1 \) subunits A and B, were preserved with cyanide bound. These differences include more disorder in the \( c \) heme domain in subunit A than in subunit B. Figs. 2A and 2B show the details of the cyanide coordinated \( d_1 \) heme for the A and B subunits, respectively. We assume that the carbon atom of cyanide is bound to the heme iron, as is the case for many other heme proteins (e.g. sulfite reductase (22) and horseradish peroxidase (23)). This assumption is supported by the B-factor.
values. In the model with a carbon to iron bond, the B-factors of C and N atoms were refined to similar values, 9.1 and 10.6 Å² in subunit A, and 7.7 and 8.2 Å² in subunit B, respectively. When the cyanide was modeled to bind to the heme iron via its nitrogen atom, i.e. when the C and N atomic-scattering factors were swapped, the B-factors of the N and C atoms differed more, 10.5 and 8.0 Å² (in subunit A) and 9.6 and 5.9 Å² (in subunit B), respectively. The results of this analysis clearly favored Fe–C bond formation for two reasons. First, atoms in the same chemical groups should show similar B-factors, because of their similar chemical environment. Second, smaller B values (thus less mobility) are associated with tighter binding. The nitrogen atom of cyanide is within hydrogen bonding distance of the Ne2 atoms of both histidines (His6345 and His6388, 2.96 Å and 2.78 Å for subunit A; 2.75 Å and 3.10 Å for subunit B), which have previously been proposed as proton donors to the oxygen atom of the substrate nitrite that is destined to become water (8). This hydrogen bonding pattern contrasts with that seen when another diatomic species, NO, is bound to the d1 heme (Fig. 2C (6)). In the latter case there are no such hydrogen bonds formed (Fig. 2, A and B). Unlike nitric oxide, the cyanide ligand has a negative charge formally localized on the nitrogen atom, which may account for the hydrogen bonding patterns seen in Fig. 2. The cyanide is bound to the B subunit d1 heme iron with an Fe–C–N angle of 164°. A striking feature is the presence of Tyr25, which if protonated could be hydrogen-bonded to the nitrogen atom of the cyanide ligand (distance, 3.10 Å). Similar to the unliganded-reduced structure (6), the d1 heme ring adopts a saddle shape and is not distorted further by the cyanide binding. The c heme domains have His/Met coordination to the heme iron, as seen in the fully reduced unliganded structure. The A subunit shows the same features at the d1 heme site except that no electron density for Tyr25 could be identified, just as was found for the reduced unliganded structure (6). Instead, there is a water molecule in a slightly different position, but still within hydrogen bonding distance (3.18 Å) of the cyanide ligand of this A subunit; hence the Fe–C–N angle in subunit A is 146°. Recent high resolution structures of sulfite reductase (22) and horseradish peroxidase (23) show similar cyanide binding geometry with the heme groups, where cyanide ligands are also hydrogen-bonded to basic Arg, His, and Lys residues.

In an attempt to obtain the structure of the oxidized enzyme with cyanide bound, crystals that were reduced and exposed to cyanide, as described above, were subsequently treated with potassium ferricyanide. X-ray diffraction data collected from those crystals showed unambiguously that the native fully oxidized structure was regained; therefore, cyanide had dissociated (data not shown). It can be concluded that it is a thermodynamic rather than a kinetic factor that prevents binding of cyanide to the oxidized as isolated enzyme.

**DISCUSSION**

The structure and characterization of cyanide binding to *P. pantotrophus* cytochrome cd1 gives a number of insights into the properties of the enzyme and its catalysis of the nitrite reductase reaction. The present work shows that only the reduced form of the enzyme can bind cyanide. Oxidation of the enzyme-cyanide complex with ferricyanide clearly provides a sufficient driving force for displacement of the cyanide ligand by Tyr25 and switching from His/Met to His/His ligation at the c heme iron. In previous work it has been proposed that the return of the Tyr25 ligand to the d1 heme could be a mechanism for displacing the physiological reaction product, nitric oxide (6, 8). This has yet to be demonstrated directly, but our results show that in general terms this displacement mechanism is feasible. Cyanide generally binds very strongly to the ferric state of heme iron as a result of both sigma bond donation from cyanide and electrostatic interaction. Typical dissociation constants are $1 \times 10^{-8}$ M for human hemoglobin (11, 24), 2.5 $\times 10^{-7}$ M for mitochondrial (horse) cytochrome c (24), 2 $\times 10^{-8}$ M for horseradish peroxidase (25), and 7 $\times 10^{-7}$ M for cytochrome aa3 oxidase (26). In general, the affinity of cyanide for this oxidation state of heme can be expected to be greater than the affinities of either nitric oxide or the phenolate ion side chain of tyrosine. Cyanide binding to ferric heme is weaker the greater the deviation from linearity of the M–C–N bond (27), but our structure suggests, at least for the ferrous state, that cyanide can bind to cytochrome cd1 with only relatively small deviation from linearity. Consequently, a geometric factor is unlikely to account for failure to observe cyanide binding to the ferric form given that, apart from the dissociation of tyrosine 25, there is little change with oxidation state in the structure around the d1 heme site. Therefore, the simplest interpretation of our results is that they show that the energetics of tyrosine re-ligation are sufficiently favorable to drive off other ligands with up to the affinity exhibited by cyanide, from all ferric cytochrome cd1. The likely explanation for this is a chelate effect; if Tyr25 binds the d1 heme and His17 the c heme cooperatively, the entropy decrease is minimized. Thus, the enthalpically favorable ferric-iron-histidine and iron-tyrosine bonds can be formed with a sufficiently negative $\Delta G$ to permit displacement of cyanide.

Given that cyanide is readily displaced from all ferric cytochrome cd1, one must also consider the possibility that the d1 heme itself binds cyanide weakly. However, in a study where d1 heme was incorporated into the heme binding pocket of apo-
FIG. 2. Stereo view of the electron density maps around the active site d heme of cytochrome cd1. A, cyanide binding in subunit A; B, cyanide binding in subunit B (this work); and C, nitric oxide binding in subunit B (drawn from Protein Data Bank entry 1aom (6)). The SIGMAA (40)-weighted $2mFo - DFo$ electron density using phases from the final model is contoured at the 1-$\sigma$ level, where $\sigma$ represents the root mean square.
myoglobin, its affinity for cyanide, in the ferric state, was $10^{-5}$ M. This value was only 50 times lower than the dissociation constant for cyanide from native myoglobin containing heme b (28). In the absence of structural data, this could reflect a poor fit of the $d_1$ heme into myoglobin, and it indicates that cyanide binding is not intrinsically weak. The $d_1$ heme is a chemically unique species, only found in cytochromes $d_1$. It is a dioxy-isobacteriochlorin (3,8-dioxy-17-acetyloxyphyrindione), the ring being reduced by 4-electrons compared with protoporphyrin IX. In addition, three further electron-withdrawing substituents are bound to the ring; two carbonyl groups and an acrylate group, conjugated with the porphyrin (2). The effects of these structural features on the chemical properties of the heme are still poorly understood. However, the $d_1$ heme ring is very acidic compared with protoporphyrin IX (28), and the reduction potential of the ring with iron bound is increased by $\sim 200$ millivolts, relative to free porphyrin or isobacteriochlorin (29). The ferric heme iron also has an unusual inverted electronic ground state ($d_{xy}^2d_{yz}^2$) compared with the more common ($d_{xz}^2d_{xy}^2$) (21), but this does not obviously correlate with weak cyanide binding. Biosynthesis of heme $d_1$ is particularly demanding (30), yet the reason for requiring it has not been convincingly explained.

In previous work (6), structures of $P$. pantotrophus cytochrome $d_1$ with either nitrite or nitric oxide bound to the $d_1$ heme showed the $c$ heme with His/His coordination. The contrast between those structures and the present ferrous enzyme-cyano complex (c heme His/Met) suggests that, in the former cases the $c$ heme must have been oxidized (6). Unlike nitrite, cyanide cannot be reduced and therefore cannot have oxidized the heme centers in the structure presented in this paper. It was not possible to definitively establish the oxidation state of the $d_1$ heme in the earlier structures (6), and if it were ferrous that may account for the failure in that work of Tyr$^{25}$. In the present structure, it is notable that Tyr$^{25}$ is seen in a well-defined position in subunit B, where it is both apparently poised ready to displace cyanide and suitably positioned to hydrogen-bond to the nitrogen atom of cyanide. Either this or other noncovalent interactions must account for its discrete position in the structure. Despite the well defined position of Tyr$^{25}$, close to its location in the fully oxidized enzyme, the $c$ heme iron has His/Met rather than His/His ligation. This suggests that the important factor in the ability of the $c$ heme to switch ligation is its oxidation state, rather than the location of the Tyr$^{25}$ amino acid residue.

The somewhat surprising failure to observe the cyanide-bound form of the ferric state of the $d_1$ heme is complemented by the unusual observation of a stable complex between the anion CN$^-$ and the ferrous heme. The present work shows that the affinity of cyanide for reduced $d_1$ heme is such that the dissociation constant is of the order of $10^{-6}$ M. This high stability seems likely to be explicable in terms of the properties of the unusual $d_1$ heme macrocycle and to the environment found inside the protein. This, we argue, is such as to promote the binding of anions, specifically nitrite, to the ferrous active site.

Binding of anions is not a usual property of ferrous heme rings; indeed, CN$^-$ is the only anion known to bind to ferrous hemoglobin and the $K_1$ is $1 \mu$M (31); for horse cytochrome $c$ it is $>10 \mu$M (24) and for horseradish peroxidase 1 M (25). However, it is commonly believed that the mechanism of nitrite reductase involves the binding of nitrite ($NO_2^-$) to ferrous $d_1$ heme (6, 10). Although a structure of $c_d$ with nitrite bound to $d_1$ heme has been obtained (6), the oxidation state of the heme in that study cannot be specified with certainty. Thus the present work is important, because it demonstrates for the first time that an unusually stable complex of ferrous $P$. pantotrophus cytochrome $cd_1$ with an anion can be obtained. A contributory factor in anion binding may be a positive electrostatic potential in the $d_1$ heme cavity. This could be provided by protonation of residues His$^{145}$, His$^{388}$, and Tyr$^{25}$, which are within hydrogen bonding distance of bound CN$^-$ in the present structure, on the distal side of the heme. Furthermore, the $d_1$ heme itself is within hydrogen bonding distance of 5 arginine residues, 1 serine, 1 threonine, and another tyrosine (9). These were proposed to stabilize the $d_1$ heme in its binding pocket. However, if protonated, each could contribute to an overall positive electrostatic field close to the $d_1$ heme that would aid binding of anions by counterbalancing the relatively low electrostatic interaction between ferrous iron and anionic cyanide. During reduction of nitrite to NO and H$_2$O, the histidine residues 345 and 388 are implicated as proton donors in the formation of water (6, 8), and thus it seems likely they will be protonated when nitrite binds the heme.

It is noteworthy that one of the few other examples of a well characterized ferrous heme-cyano complex is that of assimilatory sulfite ($SO_3^{2-}$) reductase (22), which can also act as a nitrite reductase. This type of enzyme has a siroheme center, which, it can be argued, is designed to bind anions as is the $d_1$ heme.

In contrast to cytochrome $cd_1$, the siroheme-type nitrite reductase reduces nitrite to ammonia (a 6-electron reaction) via bound nitric oxide and hydroxylamine (32). A second type of nitrite reductase that will reduce nitrite to ammonia contains five c-type cytochrome centers. The special feature of the active-site c-type heme in this enzyme is that it has an axial lysine ligand provided by a CXXCH heme-binding motif within the protein (the other four hemes have the usual CXXCH motif) (33). The active-site heme is also surrounded by amino acid residues that are likely to be protonated, contributing to a positive electrostatic potential when nitrite binds (33). Strong cyanide binding to the reduced form of this type of enzyme from Wollinella succinogenum has been reported (34). We argue that the lysine in the heme binding motif is of crucial importance in promoting the binding of anions, and the environment of this heme contributes to the provision of a reduction potential low enough to reduce substrate nitrite through to ammonia.

In a nonphysiological reaction, cytochrome $cd_1$ will reduce hydroxylamine to ammonia (35), but it cannot reduce nitric oxide to hydroxylamine. Thus a rationale for the occurrence of the $d_1$ heme is that, unlike siroheme, it is designed not to reduce nitrite past the oxidation level of nitric oxide. The reduction potential of the nitric oxide/hydroxylamine couple is $-40$ millivolts (36). This is consistent with the reduction potentials (37, 38), approximately 0 millivolt or lower, of the redox centers in the siroheme or cytochrome c types of nitrite reductase, which reduce nitrite to ammonia via hydroxylamine. However, $d_1$ heme has a reduction potential of $-200$ millivolts (39), which correlates with the inability to reduce nitric oxide to hydroxylamine. If cytochrome $cd_1$ were able to reduce NO to NH$_2$OH, denitrification, and thus the nitrogen cycle, would be "short circuited." Cytochrome $cd_1$ receives electrons from donor proteins with midpoint reduction potentials of approximately $+250$ millivolts, which are in turn reduced by the electron density for the unit cell. Contours more than 1.4 Å from any of the displayed atoms have been removed for clarity. Dashed lines indicate hydrogen bonds (drawn with MolScript (41, 42)).
cytochrome bc

complex (a ubiquinol-cytochrome c oxidoreductase) (1). The latter is a proton translocator. The cytochrome c-type nitrite reductase, for which ammonia is the product, requires that electrons be provided directly from the quinol pool of the membrane, a process that does not involve the cytochrome bc

complex. Therefore, for each electron reaching cytochrome cd
, more protons will be translocated compared with each electron reaching the other type of nitrite reductase; thus the ATP/2e

ratio will be higher when cytochrome cd
 is used (1). Thus, the inability of d
 heme to reduce further nitric oxide can be correlated with an energetically favorable proton translocation process involving the bc
 complex.

Cytochrome cd
 is a dimer. The distances between heme groups are such that electron transfer is only likely to take place on the biological time scale between the two hemes on one monomer (8). It might, therefore, be expected that the two monomers function independently from one another. However, in previous studies of the crystal structure of this enzyme we have repeatedly observed reproducible differences between the two subunits of the dimer. In the experiments in which nitrite or nitrite oxide was bound to the two subunits of the dimer. In the present work we have observed similar cyanide binding to both subunits, but the structural differences between the two subunits persist.

This suggests that the two monomers may not function entirely independently, which correlates with the Hill coefficient of 0.85 ± 0.06 that we have determined for cyanide binding to the ferrous enzyme. A Hill coefficient of less than one indicates negative cooperativity, or inequivalence of binding sites, although the value of 0.85 indicates that the extent of any such cooperativity is small. The significance of any functional interaction between the two subunits is not clear.

The study of the binding of cyanide reported in this paper clearly adds to our knowledge of cytochrome cd
. It contributes to our understanding of the reasons for the occurrence of the unusual d
 heme, the possibility that the two monomers do not function independently in catalysis, and the basis for the remarkable ligand switches in the P. pantotrophus enzyme.

Acknowledgment—A. J. thanks the Christopher Welch Fund and J. W. A. A. the Engineering and Physical Sciences Research Council for the award of a studentship. We thank Philip Mountford for helpful discussion. We thank the staff of beam line BW7B of the DORIS storage ring and support under the Training and Mobility of Researchers/Large Scale Facilities program to the European Molecular Biology Laboratory Hamburg Outstation, reference number: ERBFMGECT980134.

REFERENCES

1. Berk, B. C., Ferguson, S. J., Moir, J. W. B., and Richardson, D. J. (1995) Biochim. Biophys. Acta 1232, 97–173
2. Wu, W., and Chang, C. K. (1987) J. Am. Chem. Soc. 109, 3149–3150
3. Silvestrini, M. C., Tordi, M. G., Musci, G., Schinini, E., and Brunori, M. (1990) J. Biol. Chem. 265, 11783–11787
4. Weeg, Aarsens, E., Wu, W., Ye, R. W., Tiedje, J., and Chang, C. K. (1991) J. Biol. Chem. 266, 7496–7502
5. Yamanaka, T., and Okunuki, K. (1963) Biochem. Z. 338, 62–72
6. Williams, P. A., Fulop, V., Garman, E. F., Saunders, N. F. W., Ferguson, S. J., and Hajdu, J. (1997) J. Mol. Biol. 269, 440–455
7. Rainey, F. A., Kelly, D. P., Stackebrandt, E., Burghardt, H., Hiraishi, A., and Wood, A. P. (1999) Int. J. Syst. Bacteriol. 49, 645–651
8. Fulop, V., Moir, J. W. B., Ferguson, S. J., and Hajdu, J. (1995) Cell 81, 369–377
9. Baker, S. C., Saunders, N. F. W., Willis, A. C., Ferguson, S. J., Hajdu, J., and Fulop, V. (1997) J. Mol. Biol. 269, 440–455
10. Cutruzzola, F. (1989) Biochim. Biophys. Acta 1141, 231–249
11. Alon, R., and Brunori, M. (1971) in Hemoglobin and Myoglobin in Their Reactions with Ligands (Newberger, A. E., and Tatum, T. L., eds) North-Holland, Amsterdam
12. Moir, J. W. B., Baratta, D., Richardson, D. J., and Ferguson, S. J. (1993) Eur. J. Biochem. 212, 377–385
13. Kobayashi, K., Koppenhoefer, A., Ferguson, S. J., and Tagawa, S. (1997) Biochemistry 36, 13611–13616
14. Fulop, V., Moir, J. W. B., Ferguson, S. J., and Hajdu, J. (1993) J. Mol. Biol. 232, 1211–1212
15. Otwinowski, Z., and Minor, W. (1997) Methods Enzymol. 276, 307–326
16. Collaborative Computational Project, No 4 (1994) Acta Crystallogr. Sect. D 50, 760–763
17. Brunger, A. T. (1992) X-PLOR: Version 3.1, Yale University Press, New Haven, CT
18. Jones, T. A., Zou, J. Y., Cowan, S. W., and Kjeldgaard, M. (1991) Acta Crystallogr. Sect. A 47, 110–119
19. Vogel, A. I., and Jeffery, G. H. (1989) in Vogel’s Textbook of Quantitative Chemical Analysis, 5th Ed., pp. 309–310 and 358, Longman Scientific, Harlow, UK
20. Koppenhoefer, A., Little, R. H., Lowe, D. J., Ferguson, S. J., and Watmeough, J. (1990) Biochemistry 29, 4029–4036
21. Cheesman, M. R., Ferguson, S. J., Moir, J. W. B., Richardson, D. J., Zumft, W. G., and Thomson, A. J. (1997) Biochemistry 36, 16267–16276
22. Cramer, B. R., Siegel, L. M., and Getzoff, E. D. (1997) Biochemistry 36, 12120–12137
23. Henriksen, A., Smith, A. T., and Gajhede, M. (1999) J. Biol. Chem. 274, 35005–35011
24. Vida, F., Aime, S., Coletta, M., Desideri, A., Fasano, M., Pauletta, S., Taricone, C., and Ascenzi, P. (1996) J. Inorg. Biochem. 62, 213–222
25. Keilin, D., and Hartree, E. F. (1955) Biochem. J. 61, 153–171
26. van Buuren, K. J., Nicholls, P., and van Gelder, B. F. (1972) Biochim. Biophys. Acta 256, 258–276
27. Yu, N.-T. (1986) Methods Enzymol. 130, 350–409
28. Westphal, M., and Moeser, B. B. (1989) J. Inorg. Biochem. 37, 233–258
29. Barkigis, K. M., Chang, C. K., Fajer, J., and Renner, M. W. (1992) J. Am. Chem. Soc. 114, 1701–1707
30. Zumft, W. G. (1997) Microbiol. Mol. Biol. Rev. 61, 533–616
31. Bricogne, G., Antonini, G., Castagnola, M., and Bellelli, A. (1992) J. Biol. Chem. 267, 2538–2563
32. Vega, J. M., and Kamin, H. (1977) J. Biol. Chem. 252, 896–909
33. Einsele, O., Messenschmidt, A., Stach, P., Boureken, G. P., Bartunik, H. D., Huber, R., and Krueck, P. M. H. (1999) Nature 400, 476–480
34. Blackmore, R. G., Vadee, P. M. A., Greenwood, C., and Thomson, A. J. (1990) Biochim. Biophys. Acta 1062, 283–294
35. Inohara, K., Aoki, H., Aoki, H., and Nakamura, K. (1997) J. Biol. Chem. 272, 253–257
36. Jones, C. W. (1982) in Aspects of Microbiology (Knowles, C. J., and Cole, J. A., eds) Vol. 5, p. 41, Nelson, Walton-on-Thames, UK
37. Hino, K., Hirasawa, M., Knaff, D. B., and Shaw, R. W. (1991) Acta Crystallogr. 476, 195–199
38. Eaves, D. J., Grove, J., Staudenmann, W., James, P., Poole, R. K., White, S. A., Griffiths, I., and Cole, J. A. (1998) Mol. Microbiol. 28, 205–216
39. Besson, S., Carneiro, C., Moura, J. D. G., Moura, I., and Fauque, G. (1995) Anaerobe 1, 219–226
40. Read, R. J. (1986) Acta Crystallogr. Sect. A 42, 140–149
41. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
42. Esnouf, R. M. (1997) J. Mol. Graph. 15, 133–138
43. Brunger, A. T. (1992) Nature 355, 472–474