Deacetoxycephalosporin C synthase (DAOCS) is an iron(II) and 2-oxoglutarate-dependent oxygenase that catalyzes the oxidative ring-expansion of penicillin N to deacetoxycephalosporin C. The wild-type enzyme is only able to efficiently utilize 2-oxoglutarate and 2-oxoadipate as a 2-oxoacid co-substrate. Mutation of arginine 258, the side chain of which forms an electrostatic interaction with the 5-carboxylate of the 2-oxoglutarate co-substrate, to a glutamine residue reduced activity to about 5% of the wild-type enzyme with 2-oxoglutarate. However, other aliphatic 2-oxoacids, which were not co-substrates for the wild-type enzyme, were utilized by the R258Q mutant. These 2-oxoacids "rescued" catalytic activity to the level observed for the wild-type enzyme as judged by penicillin N and G conversion. These co-substrates underwent oxidative decarboxylation as observed for 2-oxoglutarate in the normal reaction with the wild-type enzyme. Crystal structures of the iron(II)-2-oxo-3-methylbutanoate (1.5 Å), and iron(II)-2-oxo-4-methylpentanoate (1.6 Å) enzyme complexes were obtained, which reveal the molecular basis for this "chemical co-substrate rescue" and help to rationalize the co-substrate selectivity of 2-oxoglutarate-dependent oxygenases.

Deacetoxycephalosporin C synthase (DAOCS) is an iron(II) and 2-oxoglutarate-dependent oxygenase that catalyzes the conversion of penicillin N to deacetoxycephalosporin C in the biosynthesis of cephem antibiotics in Strepomycetes clavuligerus (1). The subsequent hydroxylation of DAOC to deacetylcephalosporin C (DAC) is catalyzed by a closely related enzyme, deacetylcephalosporin C synthase (DACS). In Cephalosporium acremonium a single, bifunctional protein, deacetoxy/deacetylcephalosporin C synthase (DAOCS/DACS synthase), performs both reactions (2–4). DACS, deacetylcephalosporin C synthase (DAOC/DAC synthase), performs both reactions (2–4). S. clavuligerus also contains a 7α-hydroxylase, which is involved in the biosynthesis of cephamycin C from DAC (5).

DAOCS is a member of the iron(II) and 2-oxoglutarate-dependent oxygenase family, which catalyze a wide variety of oxidative reactions (6, 7). DAOCS, DACS, and DAOCS/DACS synthase belong to a subgroup of more closely related enzymes, which have significant primary sequence homology to one another (6). Also included in this group are two enzymes which do not use 2-oxoglutarate as a co-substrate, isopenicillin N synthase (IPNS), the iron-dependent oxidase responsible for formation of the penicillin nucleus, and 1-amino-1-carboxycyclopentanone oxidase (ACCO) which catalyzes the last step during ethylene biosynthesis in plants.

Mechanistic understanding of iron(II), 2-oxoglutarate-dependent oxygenases have been significantly advanced by the recently determined crystal structures of DAOCS (1, 8, 9) and clavamic acid synthase (10). The DAOCS crystal structures revealed the presence of a number of arginine residues within the active site, with arginine 258 (part of a conserved R–X–S motif residues in Aspergillus nidulans) being involved in co-substrate binding (1, 8). The equivalent RWS motif residues in Aspergillus nidulans IPNS, arginine 281, and serine 283, bind the a-carboxylate group of the L-b-(a-aminoacidipoyl)-L-cysteinyl-D-valine substrate (11). Both of these residues have been shown to be important for substrate binding in the C. acremonium isoenzyme by site-directed mutagenesis (12, 13).

Here we report studies on the 2-oxoglutarate co-substrate-binding function of arginine 258 in DAOCS by site-directed mutagenesis. Activity of the R258Q mutant was reduced in the presence of 2-oxoglutarate, but could be fully restored using aliphatic 2-oxoacids as alternative co-substrates. The process is called "chemical co-substrate rescue." The molecular basis for this phenomenon has been investigated by crystallographic analyses of the R258Q mutant complexed with iron (II) and alternative 2-oxoacids. The results have implications for clinically observed mutations to phytanoyl-CoA 2-hydroxylase that result in Refsum’s disease.2

2 M. Mukherji, N. J. Kershaw, C. H. MacKinnon, I. J. Clifton, C. J. Schofield, A. S. Wierzbicki, and M. D. Lloyd, (2001) J. Chem. Soc. Chem. Commun., in press.
ALTERING CO-SUBSTRATE SELECTIVITY OF DAOCS

EXPERIMENTAL PROCEDURES

Materials—All chemicals were obtained from the Sigma-Aldrich Chemical Co. or E. Merck and were at least analytical grade or higher. Oligonucleotides were synthesized by V. Cooper (Dyson Perrins Laboratory, Oxford) using Applied Biosystem DNA synthesizers (Models 380B and 394). Reagents were supplied by Roche Molecular Biochemicals (ATP); MBI (1 kilobase and 100 base pair DNA gel markers); Bio-Rad (mutagenesis reagents, competent cells, Bradford Reagent); New England Bio-Labs (enzymes for molecular biology); Promega (Wizard Plus miniprep DNA purification system, Wizard Plus SV miniprep DNA purification system); and Qiagen Ltd. (RNase A).

Site-directed Mutagenesis—The primer (5′-ACTGGAGGTCTGGCTGCTGC-3′) was annealed to the DAOCS gene in the pET24a vector (1) by heating to 70 °C and cooled over 30 min to 35 °C, and mutagenesis was performed by the method of Kunkel et al. (14, 15). The presence of the R258Q mutation was confirmed by automated sequencing, using an ABI Prism sequencer.

Protein Expression, Purification, and Activity Measurements—Wild-type and R258Q mutant enzymes were purified as described (1), except that a 80–320 mM NaCl gradient over 800 ml was used to elute the Q-Sepharose anion-exchange column. HPLC assays were carried out as described in Ref. 1 with 5 ml of the appropriate 2-oxoacid substituted for 2-oxoglutarate when assaying the R258Q mutant. The products resulting from 2-oxoacid oxidation were characterized by larger scale incubation (2 ml) with the required 2-oxoacid (10 μmol) in the presence and absence of penicillin G (20 μmol) (1). 2-Oxoglutarate conversion was assayed using [14C]2-oxoglutarate as reported (16). Apparent kinetic parameters (± S.D.) were determined by HPLC assay (1) and the spectrophotometric method of Dubus et al.3 At least six concentrations of the variable substrate was used (in triplicate) at a single defined concentration of the second substrate (Table II). Data from the HPLC (1) and spectrophotometric assays were derived as described.

Crystalization, Data Collection, and Analysis of the R258Q Mutant—DAOCS was crystallized by vapor diffusion using the hanging drop method at 14 °C, using protein (2 μl) and precipitating solution (2 μl). The precipitating solution contained 100 mM HEPES-NaOH, pH 7.0, 1.5–1.7 M ammonium sulfate, 3–6% (w/v) glycerol, and 5 mM 2-oxoglutarate, 2-oxo-4-methylpentanoate (α-ketoisocaproate) or 2-oxo-3-methylbutanolate (α-ketoisovalerate). Crystals in the R3 space group resembling those of the wild-type enzyme (1) appeared after about 1 week. Prior to data collection crystals were soaked under anaerobic conditions in mother liquor supplemented with 5 mM iron(II) sulfate, 7.0, 1.5–1.7 M ammonium sulfate, 3–6% (w/v) glycerol, and 5 mM 2-oxo-3-ketoisovalerate for 2-oxo-3-ketoisovalerate. Crystals in the R3 space group resembling those of the wild-type enzyme (1) appeared after about 1 week. Following data collection crystals were soaked under anaerobic conditions in mother liquor supplemented with 5 mM iron(II) sulfate, 7.0, 1.5–1.7 M ammonium sulfate, 3–6% (w/v) glycerol, and 5 mM 2-oxo-3-ketoisovalerate, 2-oxo-3-ketoisovalerate. Crystals in the R3 space group resembling those of the wild-type enzyme (1) appeared after about 1 week. Prior to data collection crystals were soaked under anaerobic conditions in mother liquor supplemented with 5 mM iron(II) sulfate, 7.0, 1.5–1.7 M ammonium sulfate, 3–6% (w/v) glycerol, and 5 mM 2-oxo-3-ketoisovalerate, 2-oxo-3-ketoisovalerate. Crystals in the R3 space group resembling those of the wild-type enzyme (1) appeared after about 1 week.

Crystallographic Details—Diffraction data were collected on an X8-1A automated rotating anode generator at a wavelength of 1.5418 Å, with a crystal size of approximately 0.12 × 0.12 × 0.1 mm. The data were processed with the program MOSFLM (17) and data were reduced with the CCP4 suite (18). The approximate R factors and the R values against the crystals were assessed for 

RESULTS

Biochemical and Kinetic Analysis of the R258Q Mutant—The R258Q mutant was obtained via site-directed mutagenesis, and the enzyme purified to >99% homogeneity (as judged by SDS-polyacrylamide gel electrophoresis) (1). Analyses by ESI MS (observed 34,532 ± 6 Da; calculated 34,525 Da) and DNA sequencing indicated that no undesired mutations were present, while CD analyses suggested there were no gross changes

3 A. Dubus, M. D. Lloyd, H.-J. Lee, C. J. Schofield, J. E. Baldwin, and J.-M. Frere, Cell. Mol. Life Sci., in press.

4 I. J. Clifton, unpublished results.

Relative penicillin N and penicillin G conversions (% of wild-type) by the R258Q mutant (17 μg/assay) in the presence of alternative 2-oxoacids

| 2-oxoacid          | Penicillin N<sup>3</sup> (% WT activity) | Penicillin G<sup>3</sup> (% WT activity) | Penicillin G<sup>4</sup> (% WT activity) |
|--------------------|----------------------------------------|----------------------------------------|----------------------------------------|
| 2-oxoglutarate     | 16                                     | 4                                      | 5                                      |
| 2-oxo-4-methylpentanoate | 4                                    | <2                                     | N/D                                    |
| Pynurate           | 10                                     | 8                                      | 2                                      |
| 2-oxo-2-butanoate  | N/D                                    | N/D                                    | 30                                     |
| R<sub>4</sub> = CH<sub>3</sub>OH | 101                                   | 84                                     | 82                                     |
| 2-oxo-3-methylbutanoate | 105                                   | 75                                     | 35                                     |
| R<sub>4</sub> = CH<sub>3</sub>OH | 65                                     | 8                                      | 4                                      |

<sup>3</sup> Activities measured with HPLC assay. Activities are normalized to wild-type (WT) enzyme (24 μg/assay) with penicillin N (92 nmol/min/mg). The activity of wild-type enzyme with 2-oxoglutarate and penicillin G was 46 nmol/min/mg. Wild-type enzyme showed no significant activity with any other 2-oxoacid by HPLC assays except with 2-oxo-adipate (64%) and pyruvate (5%) with penicillin N as substrate.

<sup>4</sup> Activities measured with the spectrophotometric assay. Activities are calculated at the optimum 2-oxoacid concentration and compared to wild-type enzyme at 2-oxoglutarate (100 μM). Standard deviations for these data are estimated to be 10–15% with both assays. No activity with either penicillin was observed for the wild-type or R258Q mutant in the presence of oxaloacetate, ketomalonate, phenylpyruvate, mercaptopyruvate, or indole-3-pyruvate.

ND, not determined.
least in part) for the poor efficiency of penicillin oxidation by the R258Q mutant. The possibility of penicillin oxidation being uncoupled from 2-oxoglutarate oxidation should be taken into consideration when comparing the apparent kinetic parameters in Tables I, II, and Table III.

The R258Q mutant was able to utilize several other 2-oxoacids as co-substrates when penicillin N was a substrate, most notably 2-oxo-3-methylbutanoate, 2-oxo-4-methylpentanoate, and 2-oxo-5-thiahexanoic acid. The alternative 2-oxoacids were also utilized when penicillin N was the prime substrate although the activity was somewhat lower. As with the reaction with penicillin N, the two best co-substrates were 2-oxo-3-methylbutanoate and 2-oxo-4-methylpentanoate. The lower activity for penicillin G compared with penicillin N is probably due to poorer coupling of penicillin oxidation to co-substrate conversion for the former. The increased tolerance for alternative co-substrates by the single R258Q mutation to the wild-type enzyme is noteworthy.

\(^1\)H NMR and ESI MS analyses were used to characterize products of the incubation of 2-oxo-4-methylpentanoate and 2-oxo-3-methylbutanoate with the R258Q mutant in the presence and absence of penicillin G. No evidence for their modification, other than oxidative decarboxylation to give 3-methylbutanoate and 2-methylpropanoate, respectively, was obtained. This implies that 2-oxo-4-methylpentanoate and 2-oxo-3-methylbutanoate fulfill the same role as 2-oxoglutarate in the wild-type enzyme.

Apparent steady-state kinetic parameters for the R258Q mutant with the “unnatural” 2-oxoacids were determined at a single concentration of the second substrate. Analysis of the R258Q mutant showed that 2-oxo-4-methylpentanoate and 2-oxo-3-methylbutanoate are utilized with a efficiency similar (as judged by \(K_m/K_n\)) to that of 2-oxoglutarate with the wild-type enzyme (Tables II and III). Detailed kinetic analyses have revealed that substrate inhibition of the wild-type enzyme occurred at high concentrations of 2-oxoglutarate.\(^3\) A similar although less pronounced phenomenon was observed for the R258Q mutant with 2-oxo-4-methylpentanoate and 2-oxo-3-methylbutanoate as co-substrates with penicillin G as substrate. Due to the low levels of activity of the R258Q mutant with 2-oxoglutarate in the presence of penicillin G it was not possible to obtain accurate values for the kinetic parameters, but the apparent \(K_m\) for 2-oxoglutarate was increased by >1000-fold compared with the wild-type enzyme.\(^3\)

**Altering Co-substrate Selectivity of DAOCS**

| Fixed substrate | Variable substrate | \(K_m\) \(s^{-1}\) | \(k_{cat}\) \(s^{-1}\) | \(k_{cat}/K_m\) \(s^{-1}\) |
|-----------------|-------------------|-----------------|-----------------|-----------------|
| HPLC assay (1)  | Penicillin N (100 \(\mu\)M) | 2-Oxoglutarate | 0.038 ± 0.01 | 0.03 ± 0.0050 | 789 |
|                 | Penicillin N (100 \(\mu\)M) | 2-Oxoadipate    | 0.05 ± 0.002  | 0.005 ± 0.0003 | 100  |
|                 | 2-Oxoglutarate (10,000 \(\mu\)M) | 2-Oxoglutarate | 0.13 ± 0.030 | 0.008 ± 0.0015 | 61   |
|                 | 2-Oxoglutarate (2,000 \(\mu\)M) | Penicillin N (1) | 6.6 ± 0.5 \(\times\) 10^{-3} | 0.42 ± 0.05 | 6.36 \(\times\) 10^{6} |
|                 | 2-Oxoglutarate (2,000 \(\mu\)M) | Penicillin G (1) | 2.13 ± 0.12 | 0.062 ± 0.003 | 29.1  |
| Spectrophotometric assay (fn. 3) | 2-Oxoglutarate (100 \(\mu\)M) | Penicillin G | 0.95 ± 0.027 | 0.085 ± 0.0010 | 89   |

**DISCUSSION**

The R258Q mutant was chosen for analysis as it was hoped that the glutamine side chain would allow for an active site with an intact conformation, while allowing other 2-oxoacids with hydrophobic side chains to bind. The utilization of alternative co-substrates has been previously investigated for several oxygenases related to DAOCS. 2-Oxoadipate, which possesses an additional methylene group compared with 2-oxoglutarate, has been shown to be a co-substrate for clavaminic acid synthase (25), taurine dioxygenase (26), DAO/ DAC synthase (27), and DAOCS from *N. lactamdurans* (28). γ-Butyrobetaine hydroxylase is also able to utilize both 2-oxoadipate and oxaloacetate, the latter of which is one methylene group shorter than 2-oxoglutarate, albeit with greatly increased \(K_m\) values (29). In contrast, 2,4-dichlorophenoxycetate dioxygenase is reported to be able to utilize a number of alternative 2-oxoacids, albeit relatively inefficiently (30). Other than IPNS and ACCO, the only known non-heme-dependent oxygenase that does not utilize 2-oxoglutarate as its preferred “co-substrate” is 4-hydroxyphenylpyruvate dioxygenase. However, the structure of 4-hydroxyphenylpyruvate dioxygenase places it in the family of catechol dioxygenases, (31) and it carries out a four-electron oxidation and decarboxylation of the same 2-oxoacid, 4-hydroxyphenylpyruvate. It is related to the 2-oxoglutarate-dependent oxygenases by mechanism and can utilize 3-thiophenylpyruvate (32), \(\alpha\)-ketoisocaproate (2-oxo-4-methylpentanoate) (33), and 2-oxo-5-thiahexanoic acid (34) as (co)substrates. In contrast, \(\alpha\)-ketoisovalerate (2-oxo-3-methylbutanoate) and \(\alpha\)-keto-β-methyl-n-valerate (2-oxo-3-methylpentanoate), derived from valine and isoleucine, respectively, were not converted to their corresponding products (32).

The results show that the side chain of arginine 258 is a major determinant of the 2-oxoacid co-substrate selectivity of DAOCS and, by implication, related oxygenases. The decrease in activity of the R258Q DAOCS mutant observed with 2-oxo-
glutamate is probably due to the loss of a favorable ionic interaction between the 5-carboxyl group of 2-oxoglutarate and the guanidino group of arginine 258. Thus, it appears that 2-oxo-3-methylbutanoate and 2-oxo-4-methylpentanoate are utilized as efficient co-substrates by the R258Q mutant because they possess hydrophobic side chains similar to that of 2-oxoglutarate. 

The authors proposed that His-501 directed binding of the 2-oxoacid moiety of 2-oxoglutarate and accelerated the breakdown of the ferryl intermediate. In the light of the results presented in this paper it may be that His-501 is ligating to the iron(II)-dependent oxygenase, isopenicillin N synthase has been recently obtained (38).

The kinetic results further support the idea that cooperative binding of the 2-oxoacid and penicillin sites is required for efficient catalysis. The relatively large amount of uncoupled conversion of 2-oxoglutarate in the presence of penicillin G for the R258Q DAOCS mutant may be a reflection of an "editing" mechanism used by the enzyme to select for the correct or "correctly bound" prime substrate (1).

Studies on human prolyl-4-hydroxylase (39) have shown that mutation of His-501 lead to increases in the level of uncoupled conversion of 2-oxoglutarate by about 12-fold. This appears to be due to a relative reduction in the rate of prolyl-residue hydroxylation compared with that of 2-oxoglutarate conversion of 2-oxoglutarate by about 12-fold. The authors proposed that His-501 directed binding of the 2-oxoacid moity of 2-oxoglutarate and accelerated the breakdown of the ferryl intermediate. In the light of the results presented in this paper it may be that His-501 is ligating to the 5-carboxylate of the 2-oxoglutarate co-substrate in addition to ligation by Lys-493, and that mutation led to a decrease in coupling of 2-oxoglutarate and prime substrate oxidation.

The metal-binding sites of non-heme oxygenases containing iron are closely related to those of hydrolytic enzymes containing either zinc or iron (40). Before the concentration of dioxygen reached present day levels it is possible that iron-dependent oxygenases, isopenicillin N synthase has been recently obtained (38).

The kinetic results further support the idea that cooperative binding of the 2-oxoacid and penicillin sites is required for efficient catalysis. The relatively large amount of uncoupled conversion of 2-oxoglutarate in the presence of penicillin G for the R258Q DAOCS mutant may be a reflection of an "editing" mechanism used by the enzyme to select for the correct or "correctly bound" prime substrate (1).
enzymes were involved in many non-redox reactions, e.g. hydrolytic reactions, reflecting the bioavailability of this metal (41). Upon increases in dioxygen levels it is possible that some of these enzymes evolved to use zinc, thereby avoiding deleterious oxidative damage, while others evolved into oxygenases/oxidases. The increases in dioxygen levels probably also caused proliferation of the Kreb’s cycle of which 2-oxoglutarate is an intermediate. The parallel increases in dioxygen and 2-oxoglutarate concentrations may have also influenced the evolution of selectivity for 2-oxoglutarate over other less abundant or ubiquitous 2-oxoacids such as 2-oxoacidipate.

2-Oxoacids are common metabolites and readily form bidentate ligands with iron. The question therefore arises how and why the non-heme iron(II) and 2-oxoglutarate-dependent oxygenases appear to have evolved a (relatively strict) selectivity for 2-oxoglutarate. One reason may be that 2-oxoglutarate is a common primary metabolite, but other 2-oxoacids, e.g. pyruvate, are also ubiquitous. The proposed binding mode of 2-oxoglutarate at the active site of DAOCS rationalizes the utilization of 2-oxoglutarate and its close analogue 2-oxoadipate, and the deselection of 2-oxoacids with hydrophobic side chains. The results presented here and elsewhere (39) also demonstrate that incorrect binding of 2-oxoacids can increase the extent of prime substrate uncoupled oxidation. Furthermore, it seems that cooperativity between the 2-oxoacid and the prime substrate-binding sites is required for efficient catalysis. The exact mechanism for this cooperativity in the catalytic process during and subsequent to dioxygen binding requires further investigation. The evolution of the use of 2-oxoglutarate as a co-substrate may reflect a need for precision (including ordered binding/release) during part of the catalytic process. This may be best achieved using a relatively rigid electrostatic interaction such as that formed between Arg-258 and the 5-carboxylate rather than a hydrophobic interaction or other electrostatic/hydrogen bonding interactions. Thus, the selection of 2-oxoglutarate over pyruvate may also reflect mechanistic considerations.

We have termed the restoration of activity by the use of a modified 2-oxoacid “chemical co-substrate rescue.” The chemical rescue of mutant enzymes has been extensively investigated, although studies have focused on restoring the role of a mutated amino acid residue, e.g. by using organic amines (42, 43), ions (44, 45), or a modified residue involved in metal binding (46). The rescue of activity using modified (co)-substrates has been relatively underexplored, although ATP derivatives have been elegantly used to investigate the function of kinases (47). We have recently demonstrated chemical co-substrate rescue of several clinically observed mutants of phytanoyl-CoA 2-hydroxylase using alternative 2-oxoacids. The results in this paper give the first indications of the molecular and structural basis for this process, and suggest that many non-heme-dependent oxygenases control 2-oxoacid co-substrate selectivity in a similar manner.

Acknowledgments—We thank Dr. A. Leslie (University of Cambridge) for access to his program detwin, and Dr. R. C. Wilmouth (University of Oxford), Professors J. Hajdu (Uppsala University) and I. Andersson (Swedish University of Agricultural Sciences) for discussions. The Staff at Daresbury SRS are thanked for facilities and assistance.

REFERENCES
1. Lloyd, M. D., Lee, H.-J., Harlos, K., Zhang, Z. H., Baldwin, J. E., Schofield, C. J., Charnock, J. M., Garner, C. D., Hara, T., Terweilseha van Schellinga, A. C., Valegård, K., Viklund, J. A. C., Hajdu, J., Andersson, I., Daniellsen, Å., and Bhikhabhai, R. (1999) J. Mol. Biol. 287, 943-960
2. Baldwin, J. E., and Abraham, E. (1988) Nat. Prod. Rep. 5, 129–145

Fig. 1. A, View of the crystal structure of the iron(II)-2-oxo-3-methylbutanoate complex of the R258Q DAOCS mutant. Hydrophobic residues, including the side chains of Met-180, Ile-192, and Leu-204 apparently bind the side chain of the 2-oxoacid ligand. In this view His-243, which would be directly behind the iron, has been omitted for clarity. The electron density map is contoured at 1.59 σ. B, Close up of the map of structure lacking 2-oxo-3-methylbutanoate ligand showing positive density (blue). In this view His-183 would be directly behind the iron, and has been omitted for clarity. The electron density map is contoured at 2.46 σ and omit map at 1.5 σ. This figure was produced using BOBSCRIPT (48, 49).
3. Baldwin, J. E., and Schofield, C. J. (1992) in The Chemistry of β-Lactams (Page, M. I., ed) Blackie Academic and Professional, London
4. Cooper, R. D. G. (1993) Bioorg. Med. Chem. 1, 1–17
5. Xiao, X., Wolfe, S., and Demain, A. L. (1991) Bioorg. Med. Chem. Lett. 1, 344–348
6. Prescott, A. G., and Lloyd, M. D. (2000) FEBS Lett. 505, 524–528
7. Engh, R. A., and Huber, R. (1993) Acta Cryst. Sect. A. 47, 392–400
8. Jones, T. A., Zou, J. Y., Cowen, S. W., and Kjeldgaard, M. (1991) Acta Crystal. Sect. A. 47, 110–119
9. Baldwin, J. E., and Crabbé, M. J. C. (1987) FEBS Lett. 214, 357–361
10. Zhou, J., Gunster, M., Bachmann, B., Townsend, C. A., and Soloman, E. I. (1998) J. Am. Chem. Soc. 120, 13539–13540
11. Kupka, J., Shen, Y. Q., Wolfe, S., and Demain, A. L. (1983) J. Biol. Chem. 258, 5433–5439
12. Lee, H.-J., Lloyd, M. D., Harlos, K., Clifton, I. J., Baldwin, J. E., and Schofield, C. J. (1998) J. Mol. Biol. 280, 471–474
13. Yeates, T. O. (1992) Methods Enzymol. 198, 367–382
14. Williams, R. J. P., and Prado de Silva, J. R. R. (1992) The Natural Selection of the Chemical Elements, Oxford University Press, Oxford
15. Rynkiewicz, M. J., and Seaton, B. A. (1996) Biochemistry 36, 14609–14615
16. Huang, S., and Tu, S.-C. (1991) Biochemistry 30, 214, 3552–3560
17. Myllyharju, J., and Kivirikko, K. I. (1997) EMBO J. 16, 1173–1180
18. Van Duyne, J., and Deisenhofer, J. (1996) Nature 387, 38–44
19. Mccoy, T. A., and Huxley, A. (1997) Nature 387, 38–44
20. Burzlaff, N. I., Rutledge, P. J., Clifton, I. J., Hensgens, C. M., Pickford, M., Adlington, R. M., Roach, P. L., and Baldwin, J. E. (1999) Nature 401, 721–724
21. Eichhorn, E., van der Plouw, J. R., Kertesz, M. A., and Liesinger, T. (1997) J. Mol. Graph. 15, 132–134
22. Crouch, N. P., Adlington, R. M., Baldwin, J. E., Lee, M.-H., and MacKinnon, C. H. (1997) Acta Crystallogr. Sect. D. 53, 774–780
23. Huang, S., and Tu, S.-C. (1997) Biochemistry 36, 14609–14615