Structural Basis for the Inhibition of the Biosynthesis of Biotin by the Antibiotic Amiclenomycin*

The antibiotic amiclenomycin blocks the biosynthesis of biotin by inhibiting the pyridoxal-phosphate-dependent enzyme diaminopelargonic acid synthase. Inactivation of the enzyme is stereoselective, i.e. the cis isomer of amiclenomycin is a potent inhibitor, whereas the trans isomer is much less reactive. The crystal structure of the complex of the holoenzyme and amiclenomycin at 1.8 Å resolution reveals that the internal aldime linkage between the cofactor and the side chain of the catalytic residue Lys-274 is broken. Instead, a covalent bond is formed between the 4-amino nitrogen of amiclenomycin and the C4 carbon atom of pyridoxal-phosphate. The electron density for the bound inhibitor suggests that aromatization of the cyclohexadiene ring has occurred upon formation of the covalent adduct. This process could be initiated by proton abstraction at the C4 carbon atom of the cyclohexadiene ring, possibly by the proximal side chain of Lys-274, leading to the tautomeric Schiff base formed by the removal of the second allylic hydrogen. The carboxyl tail of the amiclenomycin moiety forms a salt link to the conserved residue Arg-391 in the substrate-binding site. Modeling suggests steric hindrance at the active site as the determinant of the weak inhibiting potency of the trans isomer.

The biosynthesis of the vitamin biotin, a cofactor in biological carboxylation reactions, occurs in micro-organisms and plants and involves at least four different steps (Scheme 1) (1–12). The second step in this pathway, the conversion of 7-keto-8-aminopelargonic acid (KAPA) into 7,8-diaminopelargonic acid (DAPA), is catalyzed by DAPA synthase, an aminotransferase that requires PLP as a cofactor (6, 7). DAPA synthase from Escherichia coli is a homodimer with a molecular mass of 94 kDa (6, 13) and contains 429 residues per monomer (14). The enzyme is unique among aminotransferases in that it uses S-adenosyl-l-methionine (SAM) as an amino group donor. The reaction catalyzed by DAPA synthase is typical of that of an aminotransferase and follows the general mechanism seen in other PLP-dependent enzymes (15).

The crystal structure of DAPA synthase (16) shows that DAPA synthase belongs to the fold type I family of PLP-dependent enzymes (17, 18). The monomer of DAPA synthase consists of two domains, a small domain comprising the N- and C-terminal part of the polypeptide chain (residues 1–49 and 330–429) and a large domain formed by the intervening residues containing the cofactor-binding site. In the crystal, the two subunits of the homodimer of DAPA synthase are related by a 2-fold non-crystallographic axis (16). The PLP-binding site is located between the two domains of the monomer at the interface of the two subunits. In the enzyme-PLP complex, the cofactor is covalently linked to the ε-amino group of Lys-274, a residue functionally invariant in the whole family of fold-type I PLP-dependent enzymes.

Because biotin synthesis is unique to plants and microorganisms, enzymes of this pathway are potential targets for the development of antimicrobial drugs and herbicides (19). Inhibitors of each of these enzymes have been characterized as either synthetic mechanism-based inhibitors (19, 20) or natural products. Several antimicrobial compounds have been isolated from Streptomyces species that specifically inhibit DAPA synthase. The most well studied of these compounds, amiclenomycin (ACM), is particularly active against mycobacteria (21–23). This amino acid has been isolated as the free acid and as a part of di- and tripeptides, respectively, and all these compounds show antibiotic activities. There is evidence, however, that ACM acts as an enzyme inhibitor in the form of the free amino acid and that the di- and tripeptides are hydrolyzed by bacterial peptidases, which release ACM (23). Amiclenomycin exists as the cis and trans isomers (Scheme 2). A trans geometry had been tentatively proposed on the basis of the 3J coupling constant between the two allylic hydrogens (21). The second isomer, however, was not available from natural sources. Recently, both isomers were synthesized, and it was concluded on the basis of their NMR spectra that the natural product had cis rather than trans geometry (24). Inhibition studies of DAPA synthase by ACM indicated that the inhibitor binds to the KAPA/DAPA-binding site of the enzyme (25).

The aim of this study was to reveal the structural basis of inhibition of DAPA synthase by ACM. Toward this end, we have determined the crystal structures of the complexes formed between the holoenzyme and cis and trans ACM to 1.8 and 2.2 Å resolution, respectively. The structure analysis reveals that the inhibitor forms a covalent adduct with the cofactor PLP, which mimics the external aldime. Formation of the covalent adduct is accompanied by aromatization of the cyclohexadiene ring of ACM. Inhibition studies, absorption spectra, and the crystallographic analysis provide evidence that only the cis isomer is a potent inhibitor of DAPA synthase.

* This work was supported by grants from the Foundation for Strategic Research through the Structural Biology Network and the Science Research Council and by grants from the Ministère de l’Education Nationale, de la Recherche et de la Technologie. 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 1mlz and 1mly) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

† To whom correspondence should be addressed: Dept. of Medical Biochemistry and Biophysics, Scheeles väg 2, Karolinska Institutet, S-171 77 Stockholm, Sweden; Tel.: 46-8-7287675; Fax: 46-8-327626; E-mail: gunter@alfa.mbb.ki.se.

‡ The abbreviations used are: KAPA, 7-keto-8-aminopelargonic acid; DAPA, 7,8-diaminopelargonic acid; PLP, pyridoxal-5’-phosphate; SAM, S-adenosyl-l-methionine; ACM, amiclenomycin; DTB, dethiobiotin.

Jenny Sandmark‡, Stéphane Mann§, Andrée Marquet§, and Gunter Schneider¶

From the ‡Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-171 77 Stockholm, Sweden and the §Laboratoire de Chimie Organique Biologique, Université Paris VI, UMR CNRS 7613, 75252 Paris Cedex 05, France

Received for publication, July 19, 2002, and in revised form, September 3, 2002
Published, JBC Papers in Press, September 5, 2002, DOI 10.1074/jbc.M207239200

© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.
Case of the studies were carried out at 37 °C. The expression plasmid pET24(−)/H11001−Synthesis of the cis and trans isomers of ACM was carried out as previously described (24). Aliquots of the reaction mixture were transferred to a minimal medium plate (2% agar) containing E. coli strain C268, which is deficient in biotin biosynthesis due to a lack of DAPA synthase. This strain proved to be more sensitive to DTB than to DAPA, which prompted us to use the coupled assay. Absorption spectra of DAPA synthase were recorded using a Hitachi U-3000 spectrophotometer.

Crystallization of DAPA Synthase and Data Collection—DAPA synthase was crystallized as described (13). Crystals were incubated at room temperature with a solution of 15 mM cis ACM for three hours or 5 mM trans ACM for 13 h before data collection. X-ray data from single crystals were collected to 1.8 and 2.2 Å resolution, respectively, on a macCDA detector at a temperature of 100 K at the synchrotron beam line I711 (MAX Laboratory, Lund University, Lund, Sweden). The x-ray data sets were processed with MOSFLM (27) and scaled using the CCP4 suite of programs (28). The statistics of the data sets are given in Table I. The crystals belong to the monoclinic space group C2, with the following cell dimensions: a = 127.9 Å, b = 55.9 Å, c = 116.2 Å, β = 110.1° (cis ACM) and a = 128.0 Å, b = 56.0 Å, c = 116.0 Å, and β = 109.7° (trans ACM), respectively.

Molecular Replacement and Crystallographic Refinement—The structures of the complexes were solved by molecular replacement using the program AMORE (29) with the dimer of wild type enzyme (space group P21) as search model. Refinement was performed using Refmac5 (30) and 5% of the reflections were excluded to monitor Rfree. Manual rebuilding of the model was carried out with the program O (31) based on σa-weighted 2Fo−Fc and Fc−Fo electron density maps. In both complexes, positive difference electron density was found in the active sites of the enzyme, which allowed modeling of the bound inhibitor. Water molecules were added to the model and inspected manually during refinement.

Statistics of the refinement and the resulting model are given in Table I. Two stretches of disordered residues are observed for the subunits in both models (158–167 and 180–189, respectively). These residues are situated in two loop regions at the surface of the protein and are also poorly defined in the wild type structure. The refined models consists of residues 1–182 and 184–428 for each of the two subunits, two molecules of the covalent PLP-ACM adduct (one in the trans ACM-derived complex), two sodium ions, and 537 (324 trans ACM) water molecules.

The stereochemistry of the models was analyzed using PROCHECK (32). The coordinates and structure factors have been deposited in the Protein Data Bank, accession numbers 1myl (cis ACM) and 1mlx (trans ACM). Structural comparisons were carried out using the program O (31) with default parameters. Figures were made with MOLSCRIPT (33), BOBSCRIPT (34), and RASTER3D (35).

RESULTS

Cis Amiclenomycin Is a Strong Inhibitor of DAPA Synthase—Addition of a 5-fold excess of cis ACM to holo-DAPA synthase results in complete inactivation of the enzyme in a time-dependent fashion (Fig. 1). The trans isomer, although given in 20-fold excess over DAPA synthase, only inhibits the enzyme to about 50% (Fig. 1). The weaker inhibition by the trans isomer could reflect an inherent difference in chemical reactivity between the two isomers and/or weaker binding of the trans isomer to the enzyme.

The near UV-visible absorption spectrum of the holoenzyme exhibits two absorption maxima at wavelengths 335 and 425 nm, respectively, the latter being characteristic of a protonated aldimine (36). Addition of cis ACM results in an increase of the absorption at 335 nm and the disappearance of the longer wavelength band at 425 nm (Fig. 2), indicating that the aldi-
mine structure in the active site is lost in the enzyme-PLP-ACM complex. The time frame of these spectroscopic changes is of the same order of magnitude as the appearance of strong inhibition of the enzyme by cis ACM. Incubation of DAPA synthase with the trans isomer results in similar but far less pronounced spectroscopic changes (Fig. 2). While exhibiting the same trend, increase of the absorption maximum at 335 nm and decrease at 425 nm, these changes occur at a lower rate and are smaller than those observed with cis ACM.

Crystal Structures of the DAPA Synthase-PLP-ACM Complexes—The structures of the complexes were solved using molecular replacement and refined. The resulting electron density maps were of good quality, and the refinement statistics are as expected for models at the given resolution. The overall structure of the polypeptide chain in the complexes is very similar to the structure of holo-DAPA synthase. Superposition of 427 Cα atoms of the DAPA synthase subunit results in root mean square deviations of 0.26 Å for both complexes, which indicates that no large-scale conformational transitions occur upon binding of the inhibitor.

One significant feature in the initial 2F_0 − F_c and F_0 − F_c difference electron density maps for the ACM complexes was a negative difference electron density between the ε-amino nitrogen atom of Lys-274 and the C4 carbon atom of PLP (Fig. 3A), revealing that the cofactor binding between these atoms observed in the holoenzyme was broken. Furthermore, strong positive electron density extended from the C4 carbon atom of PLP into the substrate-binding cleft, indicating a covalent adduct involving this carbon atom. A model of ACM fits well into this difference electron density, and the maps clearly show a covalent linkage of the 4-amino group of the inhibitor with the C4 carbon atom of the cofactor (Fig. 3B). After refinement, the B-factors for the atoms of the ACM moiety (31.6 Å²) in the enzyme-PLP-cis ACM complex are similar to the overall B-factor for all protein atoms (25.8 Å²), indicating high occupancies for ACM in the active sites.

The electron density for the bound inhibitor is not consistent with a cyclohexadiene ring but fits best to a structure where the carbon atoms of the ring and the substituents at the C1 and C4 carbon position are lying in one plane. A change in hybridization from sp² to sp³ for the C1 and C4 carbon atoms must have occurred, resulting in the formation of an aromatic ring system.

The difference electron densities for the bound inhibitor in the active sites of the enzyme-PLP-trans ACM complex were in general weaker than for the cis isomer. Although the electron density maps did allow fitting of the inhibitor in subunit A (Fig. 3C), the electron density for the B-subunit was too weak to reliably model bound amiclenomycin. Increase of the concentration of trans ACM during soaking did not improve the electron density maps. The lower occupancy of the active site with the trans compound is also indicated by the B-factor (51.7 Å²) for the ACM moiety in the A-subunit. The mean B-factor for all protein atoms is significantly lower (36.0 Å²). The binding of trans ACM to the A-subunit of DAPA synthase is, within the error limits of the electron density maps at the present resolution, identical to the binding of the cis isomer. Superposition of the cis and trans ACM complexes shows that the bound cofactor adducts fall on top of each other and that the same side chain rearrangements occur in the two active sites. In the following, we describe the binding of amiclenomycin as observed in the enzyme-PLP-cis ACM complex due to the higher resolution and occupancy.

Amiclenomycin-binding Site—The inhibitor binds in the substrate-binding cleft of the enzyme with a number of interactions to groups on the enzyme and to PLP (Fig. 4A). The 4-amino nitrogen of ACM, in addition to being covalently linked to the C4 carbon atom of PLP, also forms a hydrogen bond to the side chain of the catalytic residue Lys-274. The C-terminal end of the ACM moiety extends toward the entrance of the active site cleft, and the carboxyl group forms a salt linkage to Arg-391 and a hydrogen bond to the nitrogen atom of the side chain of Trp-52. Arg-391 is invariant in DAPA synthases (16), and Trp52 is a highly conserved residue. The ε-amino group of ACM points toward the bulk solution and only interacts with surrounding water molecules. The hydrophobic part of ACM is bound in the substrate channel, which is lined by predominantly hydrophobic residues, Tyr-17, Trp-52, Trp-53, Tyr-144, Ala-217, and Phe-393. The aromatic ring of the cofactor pack against the aromatic side chains of Trp-52, Trp-53, and Tyr-144, but at angles that prevent favorable π-π stacking interactions (the latter would require parallel or perpendicular ring stacking). The interactions of the PLP cofactor with the enzyme are the same as observed in the 3D structure of the holoenzyme, with the exception of the aldimine linkage to the catalytic lysine residue and a 17° rotation of the pyridine ring of PLP relative to its position in the holoenzyme.

In the cis ACM complex, the inhibitor binds somewhat differently in the two active sites. In the A-subunit a few minor

| Data collection and refinement statistics | cis ACM | trans ACM |
|-----------------------------------------|---------|-----------|
| Wavelength (Å)                          | 0.968   | 0.991     |
| Resolution (Å)                          | 20.0–1.81 (1.90–1.81) | 20.0–2.15 (2.27–2.15) |
| R_{work} (%)                            | 6.2 (25.9) | 6.6 (29.1) |
| R/I (I)                                 | 17.4 (3.4) | 13.9 (2.9) |
| Completeness (%)                        | 99.0 (94.1) | 98.6 (96.0) |
| No. of reflections                      | 276229  | 112172    |
| Unique reflections                      | 69507   | 38070     |
| R refinement (%)                        | 19.6    | 20.5      |
| R_{free} (%)                            | 22.0    | 24.0      |
| B-factor (Å²)                           | 25.8    | 36.0      |
| protein atoms                           | 34.3    | 44.5      |
| water                                   | 13.0    | 21.2      |
| sodium ions                             | 4.010   | 0.016     |
| Ramachandran plot, residues in most favorable regions (%) | 1.376 | 1.583 |
| Additional allowed regions (%)           | 90.0    | 90.1      |
| Disallowed regions (%)                   | 9.3     | 9.3       |
| Disallowed regions (%)                   | 0.7     | 0.6       |

*a* Values in parenthesis are data for the highest resolution shell.
Conformational changes are seen, which involve movements of side chains in response to the binding of the inhibitor. For example, the side chain of Arg-391 moves a few Å toward the carboxyl group of the inhibitor. The largest movement is a 90° rotation of the indole side chain of Trp-53 that packs against the hydrophobic part of the ACM moiety. The situation in this subunit is similar to that of the enzyme-PLP-KAPA complex (16) where the same local conformational changes have been found upon binding of the substrate KAPA. In the B-subunit, the binding of the hydrophobic part of the inhibitor to the enzyme is slightly different (Fig. 4B). While the covalent linkage with PLP is formed and the interactions of the hydrophilic tail with the conserved Arg-391 are retained, the benzene ring of the adduct is shifted from its position in the A-subunit toward residue Trp-53. The side chain of this residue has not changed its position from that in the holoenzyme, which might be the reason why the benzene ring of the inhibitor can approach this residue more closely than in the A-subunit.

The ACM moiety binds in a similar fashion to the binding of the substrate KAPA to the enzyme. A superposition of the substrate complex with the inhibitor complex (Fig. 5) shows...
that the interactions of the terminal carboxyl group are preserved and that the reactive ends of the molecules, the 7-keto group of KAPA and the 4-amino group of ACM, point toward or are engaged in a covalent interaction with the C4 carbon atom of PLP, respectively. The position of the aliphatic carbon chain of KAPA and the hydrophobic carbon skeleton of the ACM moiety superpose well in the two complexes, and the same hydrophobic interactions with enzyme side chains are made.

**DISCUSSION**

These studies provide evidence that the cis isomer of ACM is a potent inhibitor of DAPA synthase. The crystal structure analysis shows that the inhibitor binds in the substrate-binding site of the enzyme and forms a covalent adduct with the cofactor. Formation of the covalent adduct is accompanied by the aromatization of the cyclohexadiene ring of ACM. This adduct mimics the external aldimine (albeit in its reduced form) formed with the substrate. The crystallographic findings are consistent with the absorption spectra, which showed that the characteristic absorption at 425 nm is lost, indicating breakage of the internal aldimine linkage between the cofactor and the catalytic lysine residue.

The proposed mechanism of formation of the aromatic adduct of ACM with PLP involves as the first step the transimination of the internal aldimine between PLP and Lys-274. Aromatization of the cyclohexadiene ring of the external aldimine is then initiated by proton abstraction at the C4 carbon, most likely by Lys-274, the only base in close proximity of this atom (Fig. 6). The same residue would then be able to transfer a proton to the C4 atom of PLP. In these proton transfer steps Lys-274 acts in the same manner as in the classical mechanism for aminotransferases (37, 38). To complete the aromatization of the cyclohexadiene ring, the proton at the C1 carbon of the ring has to be removed. In the structures of the covalent complex of holoenzyme with ACM there is no base close enough to participate in proton abstraction. On the other hand, it may not be necessary to invoke acid/base chemistry for this step, and the reaction might proceed at significant rates after proton removal at the C4 position. In the vicinity of the aromatic ring of the covalent adduct, there are several water molecules, which provide a proton relay system to bulk solvent. The significant gain in free energy upon aromatization certainly favors the overall reaction, i.e. formation of the aromatic product.

It is obvious from Fig. 1 that the trans isomer of ACM is not a very efficient inhibitor compared with the cis isomer. This is also supported by crystallographic data, which demonstrated partial occupancy of the active sites when preparing the enzyme-PLP-ACM complex with the trans isomer. Nevertheless, crystallography and mass-spectrometry indicate that both compounds form the same aromatic adduct with PLP. Although the trans isomer in principle can react with DAPA synthase, there is a large difference in the kinetics and extent of formation of the covalent adduct. This certainly reflects a difference in reactivity between the two stereoisomers within the active site and possibly a weaker affinity of the trans isomer for the enzyme.

The structures of the enzyme-PLP-ACM complexes suggest a structural explanation for the difference in reactivity of the two isomers. The crystal structures described here are of the covalent adduct formed between ACM and holoenzyme, which is identical for both isomers of ACM. To determine factors that might influence reactivity/binding of the inhibitor, we have manually modeled binding of the two ACM isomers into the active site of the enzyme before the reaction takes place. The cis isomer can be modeled in an orientation suitable for formation of a covalent adduct, whereas the trans isomer is less well accommodated in this orientation.
of the covalent adduct without any sterical hindrance with active site residues. The trans isomer can be modeled in two different binding modes into the active site of the enzyme. However, none of the two binding modes is ideal for formation of the covalent adduct. In one model, the 4-amino group binds in an orientation such that the proton to be abstracted points toward the catalytic base Lys-274. This, on the other hand, has as a consequence that the carboxylic acid tail of the inhibitor will point in a different direction compared with the cis isomer. Although there is sufficient space in the substrate-binding cleft for the aliphatic part of the carboxylic acid tail of the latter, in the trans configuration it will be too close to the side chain of Trp-52. This results in a few unfavorable interactions, mainly for the carbon atoms in α and β position to the benzene ring of the adduct. If, on the other hand, the trans isomer binds with its carboxyl tail avoiding too close contacts (i.e. similar to the cis isomer), the 4-amino group can still be positioned favorably for transamination. However, in this case the proton, which has to be abstracted to initiate aromatization, is located on the “wrong” side of the ring and not accessible for Lys-274. Thus both models for binding of trans ACM result in scenarios that counteract the formation of the covalent adduct. Either binding occurs in a manner suitable for reaction, but at the expense of binding energy or unfavorable interactions during binding are avoided, and then the necessary chemistry can not occur. Sterical hindrance thus appears to be the underlying determinant of the stereospecificity of inhibition of DAPA synthase by ACM.

The mode of inhibition through formation of a covalent linkage with PLP and subsequent aromatization as observed for ACM is reminiscent to that observed for gabaculine (5-amino-1,3-cyclohexadienyl carboxylic acid) (39, 40). This inhibitor targets a number of vitamin B₆-dependent enzymes of fold type I, and the structures of several of these complexes are known (41–43). The overall features of the gabaculine-enzyme interactions are preserved in these enzymes and are also seen in the DAPA synthase-PLP-ACM complex. These include the covalent linkage with PLP and aromatization of the ring system, hydrophobic interactions of the aromatic ring with enzymic groups in the substrate-binding cleft, and a salt bridge of the carboxyl group of the inhibitor with an arginine side chain.

Acknowledgments—We thank Katharine Gibson and Anthony Gatenby for providing the plasmid-producing DAPA synthase. We are grateful for access to synchrotron radiation at Beamline I711, MAX Laboratory, University of Lund, Lund, Sweden.

REFERENCES
1. Eisenberg, M. A. (1973) Adv. Enzymol. 38, 317–371
2. Schneider, G., and Lindqvist, Y. (2001) FEBS Lett. 495, 7–11
3. Marquet, A., Bui, B. T., and Florentin, D. (2001) Vitam. Horm. 61, 51–101
4. Ploux, O., and Marquet, A. (1996) Eur. J. Biochem. 236, 301–308
5. Webster, S. P., Alexeev, D., Campopiano, D. J., Watt, B. M., Alexeeva, M., Sawyer, L., and Baxter, R. L. (2000) Biochemistry 39, 516–528
6. Stoner, G. L., and Eisenberg, M. A. (1975) J. Biol. Chem. 250, 4019–4036
7. Stoner, G. L., and Eisenberg, M. A. (1975) J. Biol. Chem. 250, 4037–4043
8. Baxter, R. L., and Baxter, H. C. (1994) J. Chem. Soc. Chem. Commun. 759–760
9. Alexeev, D., Baxter, R. L., Smekal., O., and Sawyer, L. (1995) Structure 3, 1207–1215
10. Huang, W., Jia, J., Gibson, K. J., Taylor, W. S., Rendina, A. R., Schneider, G., and Lindqvist, Y. (1995) Biochemistry 34, 10985–10995
11. Gibson, K. J., Lorimer, G. H., Rendina, A. R., Taylor, W. S., Cohen, G., Gatenby, A. A., Payne, W. G., Roe, C., Lockett, B. A., Nudelman, A., Marcovici, D., Nachum, A., Wexler, B., Marsili, E., Turner, I. M., Lowe, L. D., Kalbach, C. E., and Hongji, C. (1995) Biochemistry 34, 10976–10984
12. Kack, H., Gibson, K. J., Lindqvist, Y., and Schneider, G. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5495–5500
13. Kack, H., Gibson, K. J., Gatenby, A. A., Schneider, G., and Lindqvist, Y. (1998) Acta Crystallogr. D54, 1397–1398
14. Otsuka, A. J., Buoncristiani, M. R., Howard, P. K., Flamm, J., Johnson, C., Yamamoto, R., Uchida, K., Cook, C., Ruppert, J., and Matsuzaki, J. (1988) J. Biol. Chem. 263, 19577–19585
15. Hayashi, H. (1995) J. Biochem. 118, 463–473
16. Kack, H., Sandmark, J., Gibson, K., Schneider, G., and Lindqvist, Y. (1999) J. Mol. Biol. 291, 857–876
17. Jansonius, J. N. (1998) Curr. Opin. Struct. Biol. 8, 759–769
18. Schneider, G., Kack, H., and Lindqvist, Y. (2000) Structure 8, 1–6
19. Rendina, A. R., Taylor, W. S., Gibson, K., Lerner, G., Rayner, D., Locket, B.,
      Kranis, K., Wexler, B., Marcovici-Mizrahi, D., Nudelman, A., Nudelman, A.,
      Marsili, E., Hongji C., Wawrzak, Z., Calabrese, J., Huang, W., Jia, J.,
      Schneider, G., Lindqvist, Y., and Yang, G. (1999) J. Pestic. Sci. 55, 236–247
20. Ploux, O., Breyne, O., Carillon, S., and Marquet, A. (1999) Eur. J. Biochem.
      259, 63–70
21. Okami, Y., Kitahara, T., Hamada, M., Naganawa, H., Kondo, S., Maeda, K.,
      Takeuchi, T., and Umezawa, H. (1974) J. Antibiot. 27, 656–664
22. Kern, A., Kabatek, U., Jung, G., Werner, R. G., Poetsch, M., and Zahner, H.
      (1985) Liebigs Ann. Chem. 5, 877–892
23. Poetsch, M., Zahner, H., Werner, R. G., Kern, A., and Jung, G. (1985) J.
      Antibiot. 38, 312–320
24. Mann, S., Carillon, S., Breyne, O., and Maequet, A. (2002) Chem. Eur. J.
      8, 439–450
25. Hotta, K., Kitahara, T., and Okami, Y. (1975) J. Antibiot. 28, 222–228
26. Izumi, Y., Sato, K., Tani, and Ogata, K. (1975) Agric. Biol. Chem. 37, 2683–2684
27. Leslie, A. (1992) Joint CCP4 and ESF-EACBM Newsletter on Protein Crystal-
      lography, vol. 26, Daresbury Laboratory, Warrington, UK
28. Collaborative Computational Project, Number 4 (1994) Acta Crystallogr. D50,
      760–763
29. Navaza, J. (1994) Acta Crystallogr. A50, 157–163
30. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Acta Crystallogr.
      D53, 240–258
31. Jones, T. A., Zou, J., Cowan, S., and Kjeldgaard, M. (1991) Acta Crystallogr.
      A47, 110–119
32. Laskowski, R. A., McArthur, M. W., Moss, D. S., and Thornton, J. M. (1993)
      J. Appl. Crystallogr. 26, 282–291
33. Kraulis, P. J. (1991) J. Appl. Crystallogr. 24, 946–950
34. Esnouf, R. M. (1997) J. Mol. Graphics Modelling 15, 133–138
35. Merrit, E. A., and Bacon, D. J. (1997) Methods Enzymol. 277, 505–524
36. Metzler, C. M., Visvanath, R., and Metzler, D. E. (1991) J. Biol. Chem. 266,
      9374–9381
37. Gehring, H. (1984) Biochemistry 23, 6335–6340
38. Julin, D. A., Wiesinger, H., Tuney, M. D., and Kirsch, J. F. (1989) Biochemistry
      28, 3815–3821
39. Rando, R. R. (1977) Biochemistry 16, 4604–4610
40. Fu, M., and Silverman, R. B. (1999) Bioorg. Med. Chem. 8, 1581–1590
41. Shah, S. A., Shen, B. W., and Brugger, A. T. (1997) Structure 5 1067–1075
42. Hemmig, M., Grimm, B., Contestabile, R., John, R. A, and Jansonius J. N.
      (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 4866–4871
43. Eads, J. C., Beeby, M., Scapin, G., Yu, T.-W., and Floss, H. G. (1999) Biochem-
      istry 38, 9840–9849
