Examination of Substrate Binding in Thiamin Diphosphate-dependent Transketolase by Protein Crystallography and Site-directed Mutagenesis*

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The thiamin diphosphate (ThDP)-dependent enzyme transketolase (EC 2.2.1.1) catalyzes the cleavage of a carbon-carbon bond adjacent to a carbonyl group in keto sugars and transfers a ketol moiety to aldosugars (Fig. 1). Catalysis is initiated by deprotonation of the C-2 carbon of the thiazolium ring of the coenzyme (1). The carbocation then attacks the carbonyl carbon atom of the donor substrate and the carbon-carbon bond between the C-2 and C-3 carbon atoms of the keto sugar is cleaved. The first product, an aldosugar, is released, whereas the ketol group remains covalently linked to the C-2 carbon atom of the thiazolium ring of the coenzyme. This intermediate, α,β-dihydroxyethyl thiamin diphosphate (2), then reacts with the acceptor sugar, and the second product is released upon bond cleavage between the C-2 carbon of the thiazolium ring and the ketose. A large variety of phosphorylated and nonphosphorylated monosaccharides can act as donor and acceptor substrates (3, 4), a property that makes the enzyme a useful tool for stereospecific organic synthesis of carbohydrates.

Transketolase from Saccharomyces cerevisiae is composed of two identical subunits with a molecular mass of 74.2 kDa per monomer (5). The crystal structure analysis of holotransketolase from Saccharomyces cerevisiae to high resolution (6, 7) revealed the general fold for a thiamin-dependent enzyme and gave a detailed view of the interactions of the cofactor with the protein. The thiamin phosphate site is located in a deep cleft at the interface between the subunits and residues from both subunits interact with the cofactor. Bound ThDP is, except for the C-2 atom of the thiazolium ring, totally inaccessible from the outer solution. No large conformational changes such as domain rotations occur upon binding of ThDP to the apoenzyme. Instead, two flexible loops at the active site make access of ThDP to its binding site possible and then take up a “closed” conformation upon binding of the cofactor (8).

So far, no crystal structure of a ThDP-dependent enzyme with bound substrate was available. In this article, we report the results of a structure analysis of transketolase with bound acceptor substrate, erythrose-4-P. Erythrose-4-P was generated by enzymatic cleavage of the donor substrate, fructose-6-P. The structure analysis identifies amino acids at the active site that are involved in substrate binding. Based on the crystal structure of this quaternary complex, we have probed the function of amino acids located in the substrate channel by site-directed mutagenesis. Implications of these results for substrate binding, stereoselectivity, and catalysis are discussed.

EXPERIMENTAL PROCEDURES

Materials and Strains—ThDP, ribose 5-P, xylulose-5-P and glyceraldehyde-3-P dehydrogenase were obtained from Sigma; glyceral-3-P dehydrogenase and triosephosphate isomerase were purchased from Roche, and premixed deoxyribonucleotides were obtained from Pharmacia Biotech Inc. Oligonucleotides used for mutagenesis were obtained from Pharmacia Biotech Inc.

* This work was supported by a grant from the Swedish Natural Science Research Council and the National Board for Industrial and Technical Development. 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 abbreviations used are: ThDP, thiamin diphosphate. P, phosphate.

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The atomic coordinates and structure factors (code 1NGS) have been deposited in the Protein Data Bank, Brookhaven National Laboratory, Upton, NY.
The expression plasmid pTKL1 and the transketolase-deficient yeast strain H402 have been described earlier (5, 9). All preparations of single- or double-stranded phagemid/plasmid DNA were made with the Wizard kits (Promega). DNA sequencing was performed with the T7 Sequencing Kit (Pharmacia), Redivue \(^{35}\)S-dATP (Amersham Corp.), and Sequagel RAPID (National Diagnostics).

Expression and Purification of Recombinant Transketolase—H402xpTKL1 yeast cells carrying plasmids with wild-type or mutant transketolase genes were cultured in leucine-deficient medium to obtain a high copy number of the plasmid. Culture conditions and the purification procedure were as described (9).

Crystallization—Crystals of the quaternary complex of the enzyme were obtained by co-crystallization of apotransketolase with 5 mM ThDP, 5 mM CaCl\(_2\), and 50 mM fructose-6-P. The complex crystallized under similar conditions as the holoenzyme (10) with 13–16% (w/w) of PEG 6000 in 50 mM glycyl-glycine buffer at pH 7.6. 7.5 in a Parr apparatus (Parr Instrument Co.) at 25°C. Crystals grow to a maximum size of 0.3 × 0.3 × 0.5 mm in 21 days. The crystals belong to space group P2\(_1\)2\(_1\)2\(_1\), with cell dimensions identical to those for holotransketolase: \(a = 78.5\) Å, \(b = 113.3\) Å, and \(c = 160.9\) Å.

X-ray Analysis—The x-ray data set to 2.4 Å resolution was collected on a R-AXIS II imaging plate mounted on a Rigaku rotating anode. The data set was processed with the MSC software (11). Details of the data collection statistics are given in Table I. Most crystallographic computing was carried out with the CCP4 suite (12). Initial 2\(F_o\) – \(F_c\) and \(F_o\) – [Fo] electron density maps were calculated with phase angles derived from the model of holotransketolase refined at 2.0 Å. Protein Data Bank accession code 1trk (7).

Crystalline refinement was carried out with the program package XPLOR (13) using the force field parameters as described by Engh and Huber (14). For calculation of the free \(R\) value (15), a randomly selected subset of 7% of the data were omitted in the refinement. The crystal asymmetric unit contains a transketolase dimer. Due to the limited resolution of the data, tight crystallographic symmetry restraints were maintained in the refinement. For the same reason, the B-factor model from the refined model of native holotransketolase was used without any further refinement. After an initial round of positional refinement, bound substrate was included in the model and a few more cycles of positional refinement, followed by manual intervention were carried out. At the end of the refinement procedure, solvent molecules were introduced in the model. The inclusion of 468 solvent molecules resulted in a drop of \(R_m\) from 26.4 to 23.9%. The results of the crystallographic refinement and the details of the model are described in Table I.

Inspection of the electron density maps, model building, and structural comparisons were carried out using the graphics program O (16). The final model was analyzed with PROCHECK (17). Atomic coordinates for the transketolase-substrate complex have been deposited with the Protein Data Bank, Brookhaven, accession number 1NGS.

Site-directed Mutagenesis—Standard molecular biology procedures were used (18). Site-directed mutagenesis was performed directly on the expression plasmid pTKL1 by the unique site elimination technique (19). Details of the mutagenesis procedure have been described previously (9, 20). The gene for every mutant was sequenced over its entire coding region to verify that no unintended mutations had been introduced.

Activity Assay—The specific activity for wild-type and mutant transketolase was measured spectrophotometrically at 25°C. In this assay, the reaction is followed by the rate of NAD\(^+\) reduction in a coupled system with glyceraldehyde-3-P dehydrogenase, where 1 unit is defined as the formation of 1 \(\mu\)mol of glyceraldehyde-3-P per minute (21).

Kinetic Analysis—Steady state kinetic parameters for the mutant enzymes were determined by measuring initial rates at different thiamin diphosphate or substrate concentrations with xylulose-5-P as donor and ribose-5-P as acceptor substrate at 25°C. All measurements were carried out in triplicate. The \(K_m\) and \(V_{max}\) values were calculated using the program ULTRAFIT (Biosoft). Protein concentrations were determined using the extinction coefficient \(E^{280}_{\text{cm}} = 14.5\) (22).

Circular Dichroism Measurements—Near ultraviolet CD spectra were obtained using a protein concentration of 0.5–0.7 mg/ml of transketolase in 50 mM glycylglycine buffer at 25°C using an Aviv 62DS circular dichroism spectrometer.

RESULTS

Crystal Structure of the Quaternary Complex

Electron Density Map and Quality of the Model—The model of the quaternary complex has been refined to a crystallographic \(R\) value of 20.5\% (\(R_m\) 23.9\%) with good stereochemistry (Table I). Except glycine residues, all amino acids are within the allowed regions of the Ramachandran plot. The final 2\(F_o\) – \(F_c\) electron density map shows well defined electron density for the protein, the cofactors ThDP, Ca\(^{2+}\), and the substrate.

Overall Structure—Comparison of the refined model of the quaternary complex with the structure of holotransketolase (7) showed that the overall structure of the enzyme is very similar in the two cases. The two structures superimpose well with an overall root mean square deviation of 0.19 Å for 678 Ca positions in the subunit. These root mean square deviations are distributed along the polypeptide chain, and only at a few places in the structure do we observe deviations of Ca atoms larger than 0.8 Å from the structure of holotransketolase. Superposition of the Ca atoms of the dimer gives a root mean square deviation of 0.19 Å. These observations indicate that binding of the acceptor substrate to transketolase does not induce any large conformational change such as domain-domain rotations or changes in the packing of the two subunits.

Cofactor Binding—In each subunit, well defined electron density for the cofactors ThDP and Ca\(^{2+}\) was found. The positions of the cofactor molecules are similar to that in the holoen-

**FIG. 1. Scheme of the transketolase reaction.** Only the first half of the reaction is shown. In the second half of the catalytic cycle, the order of the chemical steps is reversed.
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enzyme and observed differences are within the error limit of the x-ray analysis. In one of the subunits, residual electron density in the vicinity of the C-2 carbon atom of the thiazolium ring was observed. Its distance to the C-2 carbon is too close for a solvent molecule, and we interpret this density as being caused by low occupancy at this site of the intermediate, α,β-dihydroxyethyl thiamin diphosphate (see below). Attempts to model this intermediate in this electron density were ambiguous with respect to the positions of the α- and β-hydroxyl groups, probably due to too low occupancy.

Substrate Binding—In a cleft at the active site, electron density was observed representing bound substrate. However, attempts to fit the donor substrate, fructose-6-P, which was used in the crystallization experiments, into this density were not successful. The position of the phosphate group could be determined unambiguously due to the strong electron density at the entrance of this substrate channel. The residual density would not fit a substrate with a six-carbon chain; however, modeling the acceptor substrate erythrose-4-P into this density was straightforward and in the refined electron density map, all hydroxyl groups of this substrate are well defined (Fig. 2). In this model, no electron density at the active site is left unassigned in terms of an atomic model. The holoenzyme-donor substrate complex is not stable for longer time periods, and during the crystallization experiment (which requires several weeks at 4°C) the donor substrate is cleaved into the corresponding aldehyde, i.e. erythrose-4-P and the intermediate, α,β-dihydroxyethyl thiamin diphosphate. This intermediate is however only stable for a few hours in solution and slowly decomposes into ThDP and glycoaldehyde (23). We therefore conclude that the quaternary complex described here represents the enzyme-ThDP-Ca²⁺-erythrose-4-P complex. This conclusion is consistent with biochemical data and the observed electron density map, but it should be kept in mind that an unambiguous identification of the bound ligand at 2.4 Å resolution is not possible.

The substrate molecule extends from the surface of the protein into the cleft with the C-1 carbon atom of the sugar phosphate within 3.8 Å distance to the C-2 atom of the thiazolium ring of ThDP. Fig. 3 gives a schematic view of the interactions of the substrate with residues at the active site of transketolase. The phosphate group is bound at the entrance of the substrate cleft accessible to solvent and interacts with the conserved residues Arg359, Ser386, Arg528, and His469. The side chains of Arg359, His469, and Ser386 are within hydrogen bonding distance to phosphate oxygen atoms, and the side chain of R359 forms a salt bridge to the phosphate group. Another polar interaction between the substrate and the protein is made between the C-2 hydroxyl group of the substrate and the side chain of Asp477. The aldehyde oxygen atom is within hydrogen bonding distance to the side chains of His30* and His263*.

These residues are conserved in all transketolase sequences known so far. The binding of substrate is accompanied by minor adjustments of the side chains of a few residues in the active site that might or might not be significant at the present resolution.

Site-directed Mutagenesis

Kinetic Properties of Mutant Enzymes—Site-directed mutagenesis was used to investigate some of the invariant amino acids close to the acceptor substrate in the quaternary complex. Amino acids Arg359, Arg528, His469, and Asp477 were replaced by alanine, and the steady state parameters of the mutant enzymes were determined.

Replacement of the amino acid side chains interacting with

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TABLE I
Details of crystallographic data collection and refinement

| Description                              | Value          |
|------------------------------------------|----------------|
| Resolution interval (Å)                  | 10–2.4         |
| Number of measured reflections           | 156468         |
| Unique reflections                       | 46990          |
| Completeness of x-ray data (%) overall   | 81             |
| Highest resolution bin (2.4–2.5 Å)       | 58             |
| R<sub>merge</sub>                         | 0.062          |
| No. of amino acid residues in the model  | 2 × 678        |
| No. of nonhydrogen protein atoms in the final model | 10396 |
| No. of cofactor and substrate atoms      | 78             |
| No. of solvent molecules                 | 468            |
| Root mean square deviation of Ca atoms between subunits related by non-crystallographic symmetry (Å) | 0.06 |
| R factor                                 | 20.6%          |
| R<sub>free</sub>                          | 23.9%          |
| Mean temperature factor (Å<sup>2</sup>)  | 17.7           |
| Root mean square bond deviations (Å)     | 0.011          |
| Root mean square angle deviations (°)    | 1.789          |
| Dihedral (°)                             | 24.251         |
| Improper (%)                             | 1.812          |
| Ramachandran plot, % of residues in the most favorable region | 88 |
| No. of nonglycine residues in unfavourable (disallowed) regions | 0 (0) |

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2 The cleavage of the donor substrate by the enzyme even in the absence of the acceptor substrate can also be followed spectrophotometrically using the standard assay mixture devoid of acceptor substrate. In this case, formation of the aldose is slow and occurs at rates about 2–5% of the overall reaction.

3 In order to emphasize the fact that every active site in the dimer is built up from residues of the two subunits, residues from one of the subunits are distinguished from those of the other subunit by an asterisk.

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Fig. 2. Stereo view of a part of the 2Fo – Fc electron density map for the quaternary complex of holotransketolase with erythrose-4-P after crystallographic refinement, contoured at 1.0 o. Electron density within a 1.4 Å sphere of substrate atoms is shown. Superimposed is the model of the quaternary complex. Residues in the substrate channel contributed from the second subunit are indicated by asterisks.
the phosphate group has no large influence on catalytic rates. These mutants have considerable residual specific activities (R359A, 31%; R538A, 17%; and H469A, 77% activity of the wild-type enzyme). However, substitution of Asp477 by alanine resulted in a mutant enzyme severely impaired in specific activity, 1.7% of wild-type enzyme (Table II).

The $K_m$ values for the cofactor thiamin diphosphate were not or only moderately affected by the mutations. The largest effect was observed for the R538A mutant transketolase, where the $K_m$ for ThDP increases 4-fold. In the mutant enzymes significant changes were found for the $K_m$ values for donor and especially acceptor substrates (Table II). In particular the $K_m$ values for the acceptor substrate ribose-5-P in the mutants at the phosphate binding site are increased by up to 50-fold.

Circular Dichroism Measurements—Near UV CD spectra of the apo- and holoforms of the mutant enzymes are very similar to the corresponding spectra of wild-type transketolase (Fig. 4; data only shown for R528A). The CD spectra of the mutant enzymes show the same response as wild-type enzyme to the addition of donor and acceptor substrate, respectively. The presence of the donor substrate hydroxypyruvate leads to inversion of the trough at 320 nm, and this spectral change can be reversed by the addition of the acceptor substrate (Fig. 4).

The acceptor substrate erythrose-4-P is bound with the phosphate group at the entrance of this channel and reaches in an extended conformation into the active site. However, the C-1 carbon atom does not approach the C-2 carbon of the thiazolium ring of ThDP closer than 4 Å. There is sufficient space in the structure of the complex to allow for the presence of the $\alpha,\beta$-dihydroxyethyl group of the catalytic intermediate, $\alpha,\beta$-dihydroxyethyl thiamin diphosphate, in a catalytic competent complex. Modelling of this intermediate at the active site, based on the structure of transketolase complexed with a simple analogue of this intermediate, thiamin thiazolone diphosphate (25), gives a distance of 3.2 Å between the $\alpha$-carbon atom of the intermediate and the C-1 carbon atom of the acceptor substrate, the two bond forming atoms. Thus, the acceptor substrate has to move only very slightly to bring the C-1 carbon atom within bond-forming distance, and such a movement is possible without rearrangement of residues at the active site except a few side chain rotations. Large conformational
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changes seem therefore not required to bring atoms participating in catalysis in close proximity. This indicates that the binding mode for the acceptor substrate observed in the crystals is similar to the binding mode in the catalytic competent complex.

Transketolase can use nonphosphorylated keto- and aldosugars such as fructose, ribose, etc., as substrates. Usmanov and Kochetov (3) have shown that these substrates are, however, much less efficient; the $K_m$ values for these sugars are orders of magnitude larger than the $K_m$ values for their phosphorylated counterparts. These observations can be understood in view of the tight ionic and polar interactions of the phosphate group of the substrate with the protein in the quaternary complex that contribute to the binding affinity of the substrate. The site-directed mutagenesis experiments at the phosphate binding site highlight the importance of these residues for substrate binding. The increase in $K_m$ values in the R359A, R528A, and H469A mutants is consistent with the structural data that show tight interactions of the phosphate group of the acceptor substrate with the side chains of these residues. It is of interest to note that the effect is most pronounced for the acceptor substrate ribose-5-P and less evident for the donor substrate xylulose-5-P. This can be understood from the fact that the donor substrate has to bind much deeper in this channel, with its C-2 carbon atom close to the thiazolium ring of ThDP. This means that the phosphate group of xylulose-5-P will be located deeper in the channel, further away from the two arginine residues than the phosphate group of the acceptor ribose-5-P. Consequently, the interactions between these residues and the phosphate group of this particular donor are weaker compared with the acceptor, ribose-5-P. His$^{469}$ is located more in the interior of the substrate channel, closer to the phosphate group of xylulose-5-P and therefore is still able to form a hydrogen bond to the phosphate group. This is reflected in a stronger effect of this residue on the $K_m$ value for the donor substrate compared with the two arginine residues.

Despite the broad substrate specificity of transketolase, there are a number of constraints with regard to the stereoconfiguration of the hydroxyl groups of the sugar. For example, Usmanov and Kochetov (3) and Kobori et al. (4) noted that only sugar phosphates with $\alpha$-threo configuration at the C-3 and C-4 carbon atoms can act as efficient donors in the transketolase reaction. The high stereoselectivity of the enzyme for the donor substrate can be understood from the observed pattern of hydrogen bonds that the substrate forms at the active site. The C-3 and C-4 hydroxyl groups of the ketose donor correspond to the C-1 and C-2 carbon positions of the aldose acceptor, and these two hydroxyl groups form hydrogen bonds to the side chains of Asp$^{477}$ and His$^{307}$ and His$^{263}$, respectively. Removal of one of these hydroxyl groups or inversion of the stereocenters will result in a disruption of these hydrogen bonds, decreasing the affinity for the substrate. The preference for $\alpha$-hydroxylated acceptor substrates can be explained on the same structural basis, the hydrogen bond formed by this hydroxyl group to the side chain of Asp$^{477}$, which will provide additional binding energy. This is, however, not an absolute requirement, $\alpha$-unsubstituted aldehydes have been used in organic synthesis using transketolase as catalyst, albeit at low rates (26).

In the case of transketolase, CD spectroscopy provides a convenient means to assess functional integrity of the enzyme. Individual catalytic steps such as formation of holoenzyme, binding of donor-substrate and formation of a reaction intermediate, and cleavage of this intermediate upon binding of the acceptor substrate have been correlated with characteristic features of the CD spectrum (3, 27). These optical properties of transketolase can be used to probe mutant transketolases for catalytic deficiencies and abnormalities.

We conclude from the comparison of the CD spectra of wild-type enzyme and the D477A mutant that this mutant enzyme behaves as wild-type enzyme, which suggests that the catalytic mechanism is unperturbed. Therefore the significant drop in $b_{\alpha}$ upon amino acid replacement is not due to unexpected structural disturbances of the enzyme structure but reflects the importance of this side chain for catalysis. Thus, Asp$^{477}$ functions not only in binding of substrate and selecting the proper stereoisomer but is also required for efficient catalysis. This residue is located in the middle of the substrate channel, and the interactions of the substrate hydroxyl group with Asp$^{477}$ might be important for maintaining the catalytic competent binding mode of the substrates and reaction intermediates at the active site.

The catalytic cycle of transketolase involves a series of proton transfer steps (28). One of these is the initial proton abstraction at the C-2 carbon atom of the thiazolium ring of ThDP, a step common to all ThDP-dependent enzymes. Crystallography (6, 7, 29) and site-directed mutagenesis (9) revealed the critical role of an invariant glutamic acid residue (Glu$^{418}$ in transketolase) in this cofactor-assisted proton abstraction.

In the second half of the catalytic cycle, after the first product is released, the $\alpha$-carbion intermediate performs a nucleophilic attack on the C-1 carbon atom of the acceptor substrate. This catalytic step requires at least one proton transfer step, the protonation of the aldehyde oxygen of the substrate. Furthermore, a negative charge at the oxygen atom will be generated in the transition state that has to be stabilized, either by charge compensation or direct proton transfer to the oxygen atom. The side chains of two conserved histidine residues, His$^{307}$ and His$^{263}$, are within hydrogen bonding distance to this oxygen atom in the quaternary complex, and one or both might participate in transition state stabilization and/or proton transfer.

In conclusion, the structure of the complex of holotransketolase with bound acceptor substrate identifies amino acid residues responsible for binding of substrate, suggests possible side chains involved in proton transfer during catalysis, and provides insights in the molecular basis of the stereoselectivity in the transketolase reaction. The results of site-directed mutagenesis experiments are consistent with the proposed role of the invariant residues Arg$^{429}$, Arg$^{439}$, and His$^{469}$ in promoting the binding site for the phosphate group of the substrate. Mechanistic hypotheses on residues involved in proton transfer during catalysis are presently being tested by site-directed mutagenesis.

Acknowledgments—We thank Dr Steve Gutteridge (DuPont Agricultural Products, Newark, NJ) for the syntheses of the oligonucleotides used for mutagenesis and Mona Gullmert for excellent technical assistance. We also thank Dr. Kurt Berndt (Department of Medical Biochemistry and Biophysics, Karolinska Institute) for access to the CD spectrometer.

REFERENCES
1. Breslow, R. (1958) J. Am. Chem. Soc. 80, 3719–3726
2. Krampitz, L. O. (1969) Anaerobic Biochem. 38, 213–241
3. Usmanov, R. A., and Kochetov, G. (1983) Biochemistry (Engl. Transl. Biohimiyo) 48, 478–484
4. Kobori, Y., Myler, D. C., and Whitesides, G. M. (1992) J. Org. Chem. 57, 5989–5997
5. Sundstrom, M., Lindqvist, Y., Schneider, G., Helman, U., and Ronne, H. (1993) J. Biol. Chem. 268, 24346–24352
6. Lindqvist, Y., Schneider, G., Ermler, U., and Sundstrom, M. (1992) EMBO J. 11, 2373–2379
7. Nikkola, M., Lindqvist, Y., and Schneider, G. (1994) J. Mol. Biol. 238, 378–404
8. Sundstrom, M., Lindqvist, Y., and Schneider, G. (1992) FEBS Lett. 313, 229–231
9. Wikner, C., Meshalkina, L., Nilsson, U., Nikkola, M., Lindqvist, Y., Sundstrom, M., and Schneider, G. (1994) J. Biol. Chem. 269, 32144–32150
10. Schneider, G., Sundstrom, M., and Lindqvist, Y. (1989) J. Biol. Chem. 264,
Substrate Binding in Transketolase

11. Sato, M., Yamamoto, M., Imada, K., and Katsube, Y. (1992) J. Appl. Crystallogr. 25, 348–357
12. Collaborative Computing Project, Number 4 (1994) Acta Crystallogr. Sec. D 50, 760–763
13. Brünger, A. T. (1989) Acta Crystallogr. Sec. A 45, 50–61
14. Engh, R. A., and Huber, R. (1991) Acta Crystallogr. Sec. A 47, 392–400
15. Brünger, A. T., (1992) Nature 355, 472–475
16. Jones, T. A., Zou, J.-Y., Cowan, S., and Kjellgaard, M. (1991) Acta Crystallogr. Sec. A 47, 110–119
17. Laskowski, R. A., McArhur, M. W., Moss, D. S., and Thornton, J. M. (1993) J. Appl. Crystallogr. 26, 282–291
18. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Deng, W. P., and Nickoloff, J. A. (1992) Anal. Biochem. 200, 81
20. Wikner, C., Meshalkina, L., Nilsson, U., Backstrom, S., Lindqvist, Y., and Schneider, G. (1995) Eur. J. Biochem. 233, 750–755
21. Kochetov, G. A. (1982) Methods Enzymol. 90, 209–223
22. Heinrich, P. C., Steffen, H., Janser, P., and Wiss, O. (1972) Biochem. Biophys. Res. Commun. 49, 1427–1432
23. Kochetov, G. A., Usmanov R. A., and Mevkh, A. T. (1973) Biochem. Biophys. Res. Commun. 54, 1619–1626
24. Kremer, A. R., Egan, R. M., and Sable, H. Z. (1980) J. Biol. Chem. 255, 2405–2410
25. Nilsson, U., Lindqvist, Y., Kluger, R., and Schneider, G. (1993) FEBS Lett. 326, 145–146
26. Demuyck, C., Bolte, J., Hecquet, L., and Dalmas, V. (1991) Tetrahedron Lett. 32, 5085
27. Heinrich, P. C., Steffen, H., Janser, P., and Wiss, O. (1971) Biochem. Biophys. Res. Commun. 44, 275–279
28. Schneider, G & Lindqvist, Y. (1993) Bioorg. Chem. 21, 109–117
29. König, S., Schellenberger, A., Neef, H., and Schneider, G. (1994) J. Biol. Chem. 269, 10879–10882