Volume 326, number 1,2,3, 145–148 FEBS 12676 July 1993

Crystal structure of transketolase in complex with thiamine thiazolone diphosphate, an analogue of the reaction intermediate, at 2.3 Å resolution

Ulrika Nilsson[a], Ylva Lindqvist[a], Ronald Kluger[b] and Gunter Schneider[a]

[a]*Department of Molecular Biology, Swedish University of Agricultural Sciences, Biomedical Center, Box 590, S-751 24 Uppsala, Sweden* and [b]*Department of Chemistry, University of Toronto, Toronto, Ontario M5S 1A1, Canada*

Received 20 May 1993

The crystal structure of the complex of transketolase and thiamine thiazolone diphosphate has been determined at 2.3 Å resolution. The complex has a structure which closely resembles that of this enzyme with the cofactor ThDP. This is consistent with the observation that the binding of the analogue to transketolase involves ground state rather than transition state interactions. Since thiamine thiazolone diphosphate resembles an expected intermediate in the catalytic pathway, the structure of the intermediate was modelled from the crystal structure. Based on this model, enzymic groups responsible for binding of the intermediate and proton transfer during catalysis are suggested.

Protein crystallography; Thiamine diphosphate; Transketolase; Transition state analogue

1. INTRODUCTION

Enzymatic thiamine catalysis proceeds through two carbanion intermediates, the ylide of thiamine diphosphate (ThDP), formed after abstraction of the C2 proton of the thiazolium ring and the 2-α carbanion, formed after nucleophilic attack of the C2 carbon on the first substrate [1,2]. The 2-α carbanion is stabilized through its neutral resonance structure, the enamine (Scheme I). This intermediate is of central importance in thiamin catalysis, since dependent on the nature of its substituent at the C2 carbon atom of the cofactor, different enzymatic functions can be derived. In the case of pyruvate decarboxylase, the intermediate will be protonated at the α-carbon position prior to the expulsion of the product, acetaldehyde. In the transketolase reaction, the carbanion will react with an aldosugar and the product, a ketosugar with its carbon chain extended by two carbon atoms will be released.

Gutowsky and Lienhard [3] found that thiamine thiazolone disphosphate (TTPP) (Scheme I) binds very tightly to pyruvate dehydrogenase and proposed that it was functioning as an analogue of the transition state for decarboxylation of the adduct of ThDP and pyruvate. Similar conclusions were derived from studies of TTPP binding to pyruvate oxidase [4]. However, in pyruvate decarboxylase [5] and transketolase [6], TTPP binds no more tightly than does the cofactor, so that its interactions within the complex with the enzyme need to be defined specifically to find the source of this difference.

We have determined the three-dimensional structure of the complex of apotransketolase with TTPP as one step in our studies on structure–function relationships in enzymatic thiamin catalysis. The structure determination reveals the molecular interactions of TTPP with groups at the active site of transketolase and provides a structural basis for hypotheses regarding the mode of action of TTPP.

Correspondence address: G. Schneider, Department of Molecular Biology, Swedish University of Agricultural Sciences, Biomedical Center, Box 590, S-751 24 Uppsala, Sweden. Fax: (46) (18) 53-6971.

2. MATERIALS AND METHODS

Transketolase from *Saccharomyces cerevisiae* was obtained from Sigma. The commercially available enzyme is, as a result of the preparation procedure, free of ThDP as shown by activity measurements (< 4% of the holoenzyme) and a crystal structure analysis of the apoenzyme [7]. Enzyme activity measurements were carried out as described by De la Haba et al. [8]. TTPP was synthesized as described by Kluger et al. [5].

The complex of apotransketolase with TTPP was crystallized under similar conditions as used for the holoenzyme [9], except that ThDP was substituted by TTPP. The best crystals were obtained with 13–16% (w/w) of PEG 6000 in 50 mM glycyl-glycine buffer, containing 5 mM $CaCl_2$ and 5 mM TTPP at pH 7.9 as mother liquid. 7.5 μl of a 12 mg/ml protein solution were mixed with the same amount of the mother liquid and the droplets were left to equilibrate with 1 ml of the mother solution. The complex crystallizes isomorphously to holotransketolase in spacegroup $P2_12_12_1$ with cell dimensions $a = 76.3$ Å, $b = 113.3$ Å and $c = 160.9$ Å.

A data set to 2.3 Å resolution using one crystal (76% complete, R-merge 10.7%) was collected on a R-axis II imaging plate mounted on a Rigaku rotating anode. Images were processed with the MSC [10] software and the data were scaled [11] with Rotavata-Agrovata written by J.M. Smith and A.J. Wonnacott in the CCP4 program package (Daresbury, UK). Initial phase information was derived from the model of holotransketolase, refined at 2.5 Å resolution [12]. Crystallographic refinement was carried out with the program XPLOR [13]. The final model of the complex of transketolase with TTPP has a

crystallographic R-factor of 21.7% in the resolution interval 7.0–2.3 Å with overall RMS deviations from bond lengths of 0.020 Å and bond angles of 3.7° (no solvent molecules included). Inspection of the electron density maps, model building and comparison with holotransketolase was done using the graphics program *O* [14]. For the comparison with holotransketolase, a refined model (R-factor 15.8%) of holotransketolase at 2.0 Å resolution (Nikkola et al., unpublished results) was used.

3. RESULTS

3.1. *Electron density maps and quality of the model*

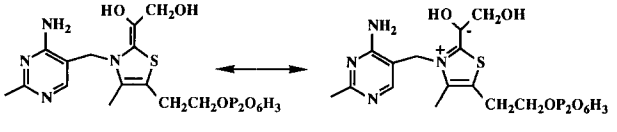
Except for some side chains at the surface of the protein, the electron density for the polypeptide chain is well defined. The density for the Ca^{2+} ion and the cofactor analogue is also well defined. The final model of the transketolase–TTPP complex consists of residues 3 to 680, one Ca^{2+} ion and one TTPP molecule per subunit. No solvent molecules were included in the refinement. The quality of the model is good as judged by conventional criteria such as R-factor (21.7%, and RMS deviations of bond lengths. The Ramachandran plot shows no outlier outside the defined regions except glycine residues. From a Luzzatti plot [15], the mean coordinate error can be estimated to 0.30 Å.

Since the crystal asymmetric unit contains the dimer, independent results are obtained for both subunits. Within the error limits of the X-ray analysis, the structure of both subunits of the complex is highly similar, with an RMS deviation for all C-α atoms of 0.41 Å between the subunits. The results described in the following are therefore valid for both subunits.

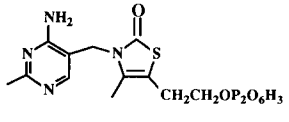
3.2. *Overall structure*

The overall structure of transketolase in the complex with TTPP is very similar to the structure of the holoenzyme. Fig. 1 shows the RMS deviations for all C-α atoms in one of the subunits, when superimposing the TTPP complex onto the holostructure. As can be seen,

a:



b:



Scheme I. (a) Two resonance forms of the reaction intermediate, 2-(α,β-dihydroxyethyl)thiamine diphosphate. (b) Thiamine thiazolone diphosphate.

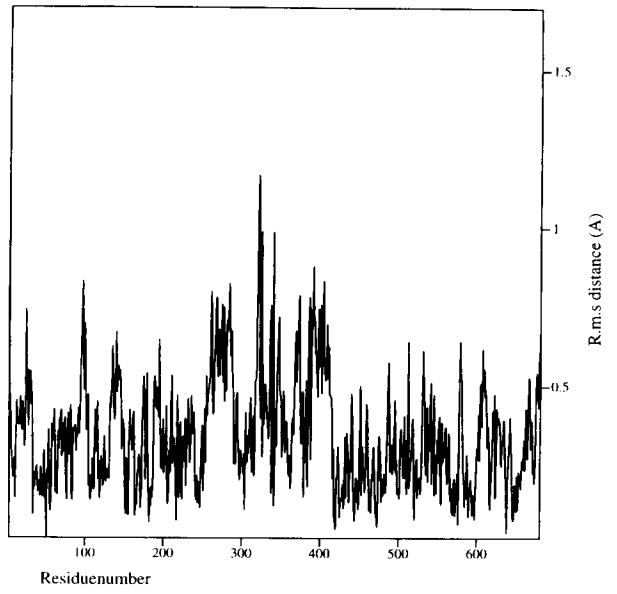


Fig. 1. Distance between corresponding C-α atoms of transketolase in complex with ThDP or TTPP, respectively, as a function of residue number after superposition of the two structures.

the two structures superimpose well with an overall RMS deviation of 0.39 Å for 678 C-α positions in the subunit (0.43 for both subunits).

3.3. *TTPP binding*

In the initial $|F_o|-|F_c|$ electron density map, calculated with phase angles derived from the model of holotransketolase, strong electron density (4.5 times the standard deviation of the electron density map) was found very close to the C-2 carbon atom of the cofactor analogue, corresponding to the C-2 carbonyl oxygen of TTPP (Fig. 2). Well-defined density for the cofactor analogue and the Ca^{2+} ion was observed in the $2|F_o|-|F_c|$ electron density map.

TTPP is bound in an identical fashion to the enzyme as ThDP and the interactions of the diphosphate group with the enzyme and the metal ion are identical to those observed in the structure of holotransketolase. The C-2 oxygen points away from the enzyme into the substrate channel and does not form any hydrogen bonds to protein atoms. Instead, the 4-NH_2 group of the pyrimidine ring of TTPP is within hydrogen bonding distance to this atom. The closest protein atom to the C-2 oxygen is Nε2 from His^{481} at a distance of 3.8 Å (Fig. 2).

Superposition of the binary complex of transketolase–TTPP with the holoenzyme shows that there are no significant changes in side chain positions at the coenzyme binding site.

3.4. *Model building of 2-(α,β-dihydroxyethyl) thiamine disphosphate*

This reaction intermediate was modelled from the TTPP molecule assuming the enamine resonance form,

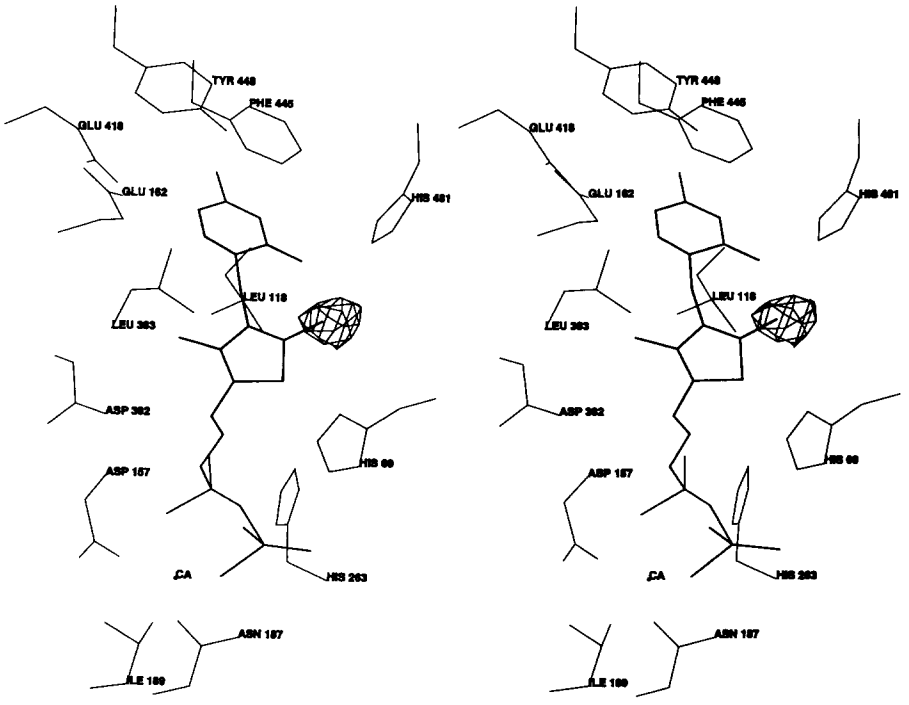


Fig. 2. Stereo view of the surroundings of the bound analogue of the reaction intermediate, TTPP at the coenzyme binding site of transketolase. Part of the initial $|F_o|-|F_c|$ electron density is shown at contour level 2.5 times the standard deviation of the electron density map.

i.e. the substituents are in the plane of the thiazolium ring (Fig. 3). In this conformation there is only space for the $-CH_2OH$ group in *cis*-position to the sulfur atom. In the *trans*-position severe steric interference occurs with either His^{481}, Phe^{442} or the pyrimidine part of the cofactor. In the model structure, the α-hydroxyl group is able to make hydrogen bonds to His^{481} and to the 4-NH_2-group of the cofactor. The β-hydroxyl group could be modelled in several possible ways making a hydrogen bond with either His^{30}, His^{69} or His^{103}, respectively.

4. DISCUSSION

The observed binding mode of TTPP in the complex with transketolase shows clearly that there are no addi-

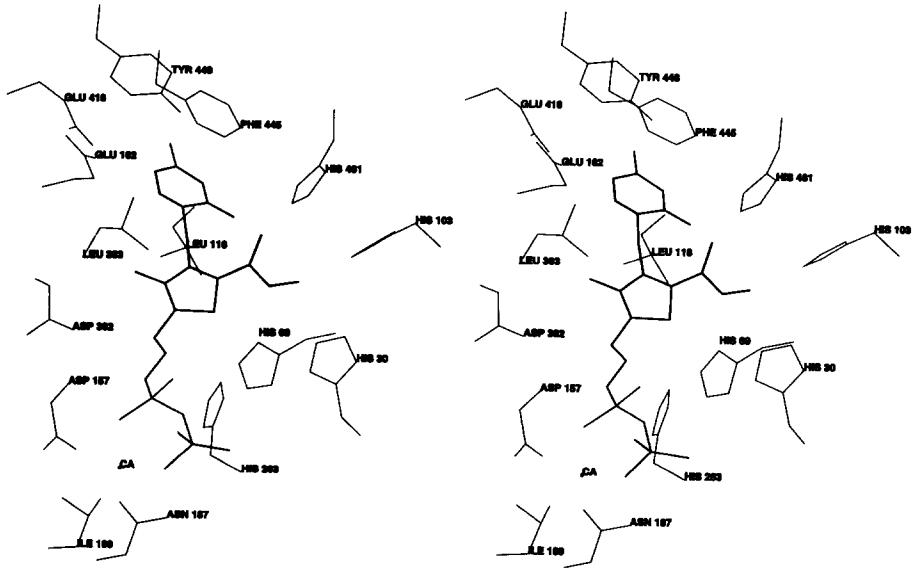


Fig. 3. Model of the bound reaction intermediate, 2-(α,β-dihydroxyethyl)thiamine diphosphate at the active site of transketolase (for details of model building see text).

tional specific interactions of the cofactor analogue with the protein when compared to the binding of ThDP to the enzyme. This makes it unlikely that TTPP acts as an analogue of the transition state, since in that case one would expect a number of additional specific interactions of the analogue with the protein responsible for stabilization of the transition state. The three-dimensional structure of the complex of TTPP with transketolase suggests that TTPP merely acts as a competitive inhibitor of the enzyme. This view is also supported by the fact that TTPP binds to the enzyme with about the same affinity as the coenzyme, ThDP, which led Sable and coworkers to suggest that TTPP is not a transition state analogue for transketolase [6]. The observed stronger binding of TTPP to pyruvate dehydrogenase and pyruvate oxidase might then reflect a larger contribution of non-polar interactions to the binding of TTPP to these enzymes than in the case of transketolase, as has been suggested by others [5].

Regardless whether or not TTPP can be considered a transition state analogue, it is the simplest chemical analogue of the enamine form of the reaction intermediate, the carbanion of 2-(α,β-dihydroxyethyl)thiamine diphosphate. As such, it is a more reliable starting point to model the structure of this intermediate into the active site of transketolase. Furthermore, since the C-2 oxygen atom only makes van der Waals interactions with protein atoms (as would be expected for the C-α carbon of the intermediate), it seems unlikely that the position of this atom is shifted very much from the position of the C-α atom of the intermediate.

The three-dimensional model of the reaction intermediate immediately suggests amino acid residues responsible for specificity and catalysis. A characteristic difference in the structure of the reaction intermediate in the transketolase reaction, compared to other ThDP-dependent enzymes is the β-hydroxyl group. In the reaction of pyruvate decarboxylase, pyruvate dehydrogenase and pyruvate oxidase, this group is a methyl group. The hydrogen bond between the β-hydroxyl group and a side chain of the protein must therefore be one of the interactions responsible for the difference in specificity between these enzymes. From the model presented above, we suggest that one of the differences at the active sites of these enzymes will include at least one of the histidine residues at positions 30, 69 and 103, respectively.

One step in enzymatic thiamin catalysis is the nucleophilic attack of the C-2 carbanion of ThDP on the carbonyl group of the substrate. The developing negative charge on the carbonyl oxygen will be stabilized by the transfer of a proton from an enzymic or cofactor group acting as a general acid. From the model of the reaction intermediate, only two such groups are within hydrogen bonding distance to the carbonyl oxygen, the 4-NH_2 group of the cofactor and the side chain of His^{481}. At first sight, the 4-NH_2 group as proton donor seems not very likely, but for reasons described elsewhere [12,16] this group might be protonated at this step in catalysis and could therefore in principle act as proton donor. With the availability of an expression system for yeast transketolase (Wikner et al., unpublished results), the hypotheses suggested above can be tested using site-directed mutagenesis.

Acknowledgements: This work was supported by a grant from the Swedish Natural Science Research Council.

REFERENCES

[1] Breslow, R. (1958) J. Am. Chem. Soc. 80, 3719–3725.
[2] Kluger, R. (1987) Chem. Rev. 87, 863–876.
[3] Gutowsky, J.A. and Lienhard, G.E. (1976) J. Biol. Chem. 251, 2863–2866.
[4] O'Brien, T.A. and Gennis, R.B. (1980) J. Biol. Chem. 255, 3302–3307.
[5] Kluger, R., Gish, G. and Kauffmann, G. (1984) J. Biol. Chem. 259, 8960–8965.
[6] Shreve, D.S., Holloway, M.P., Haggerty III., J.C. and Sable, H.Z. (1983) J. Biol. Chem. 258, 12405–12408.
[7] Sundström, M., Lindqvist, Y. and Schneider, G. (1992) FEBS Lett. 313, 229–231.
[8] De la Haba, G., Leder, I.G. and Racker, E. (1955) J. Biol. Chem. 214, 409–426.
[9] Schneider, G., Sundström, M. and Lindqvist, Y. (1989) J. Biol. Chem. 264, 21619–21620.
[10] Sato, M., Yamamoto, M., Imada, K. and Katsube, Y.J. (1992) Appl. Crystallogr. 25, 348–357.
[11] Fox, G.C. and Holmes, K.C. (1966) Acta Crystallogr. 20, 886–891.
[12] Lindqvist, Y., Schneider, G., Ermler, U. and Sundström, M. (1992) EMBO J. 11, 2373–2379.
[13] Brünger, A.T. (1989) Acta Crystallogr. Sect. A 45, 50–61.
[14] Jones, T.A., Zou, J.-Y. and Cowan, S.W. (1991) Acta Crystallogr. A47, 110–119.
[15] Luzzati, V. (1952) Acta Crystallogr. 5, 802–810.
[16] Schneider, G. and Lindqvist, Y. (1993) Bioorg. Chem., 21, 109–117.