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Three-dimensional structure of apotransketolase

Flexible loops at the active site enable cofactor binding

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The structure determination of apotransketolase and the comparison of its three-dimensional structure with that of the holoenzyme has revealed that no large conformational changes are associated with cofactor binding. Two loops at the active site are flexible in the apoenzyme which enables ThDP to reach its binding site. Binding of the cofactor induces defined conformations for these two loops at the active site. One of these loops is directly involving in binding of the cofactors, Ca^{2+} and ThDP. This loop acts like a flap which closes off the diphosphate binding site. After binding of the cofactor, residues of this loop form interactions to residues of loop 383–398 from the second subunit. These interactions stabilize the conformation of the two loops from a flexible to a 'closed' conformation.

Protein crystallography; Thiamine diphosphate; Transketolase; Coenzyme binding

1. INTRODUCTION

The pentose phosphate pathway is a major metabolic pathway in all cells. It consists of a dehydrogenase–decarbocylating system that converts glucose-6 phosphate to ribulose-5 phosphate, generating NADPH for use in reductive biosynthesis, an isomerizing system that interconverts ribulose-5 phosphate to xylulose-5 phosphate and ribose-5 phosphate, and a sugar rearrangement system that converts ribose-5 phosphate and xylulose-5 phosphate to the glycolytic intermediates, fructose-6 phosphate and glyceraldehyde-3 phosphate. Transketolase (EC 2.2.1.1) plays an important part in the rearrangement system since it creates, together with transaldolase, a reversible link between the pentose phosphate pathway and glycolysis.

Transketolase catalyzes the cleavage of a carbon–carbon bond adjacent to a carbonyl group in keto sugars and transfers a two-carbon unit to aldosugars. The catalytic activity of transketolase is dependent on thiamine diphosphate (ThDP) and divalent cations such as Mg^{2+} or Ca^{2+}. The enzyme from baker's yeast, *Saccharomyces cerevisiae* is composed of two identical subunits with a molecular weight of 74.2 kDa per monomer [1], Sundström et al., unpublished results).

We have recently determined the crystal structure of holotransketolase from *Saccharomyces cerevisiae* to 2.5 Å resolution [2]. The structure analysis revealed the general fold for thiamine-dependent enzymes and gave a detailed view of the interactions of the cofactor with the protein. A striking observation was that the coenzyme binding 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 C2 atom of the thiazolium ring, totally inaccessible from the outer solution. In this report, we present the crystal structure of apotransketolase, depleted of its cofactors, ThDP and Ca^{2+}, and discuss the infuence of the cofactor on the conformation of loop regions at the active site of the enzyme.

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2. MATERIALS AND METHODS

Commercially available transketolase from *Saccharomyces cerevisiae* is, as a result of the preparation procedure, largely free of ThDP. In order to remove metal ions and also most of the ThDP which still might be bound to the enzyme, dissolved transketolase was dialysed extensively against a solution of 2.5 mM EDTA in water in a procedure similar to that described by Shreve et al. [3]. Enzyme activity measurements were carried out as described by De la Haba et al. [4]. Removal of ThDP was confirmed by the demonstration that apotransketolase has very little activity (<4% of the holoenzyme) left. However, enzyme activity could be fully recovered by the addition of ThDP.

Apotransketolase readily crystallized under similar conditions as used for the holoenzyme [5]. The best crystals were obtained with 14% (w/w) of PEG 6000 in 50 mM glycyl–glycine buffer containing 2.5 mM EDTA at pH 7.6 as mother liquid. 7.5 μl of a 20 mg/ml protein solution was mixed with the same amount of the mother liquid and the droplets were left to equilibrate with 1 ml of the mother solution. The largest crystals had the dimensions 0.8 × 0.8 × 1.2 mm. Apotransketolase crystallizes in spacegroup $P2_12_12_1$ with cell dimensions a = 76.4, b = 113.6 and c = 161.6 Å.

A data set to 2.8 Å resolution using one crystal (87% complete, R-merge=6.4%) was collected on a Xentronics Multiwire Area Detector [6] mounted on a Rigaku rotating anode. Data frames were proc-

essed with the program BUDDHA [7], and data scaling was carried out with the PROTEIN program [8].

Initial 2|Fo|−|Fc| and |Fo|−|Fc| electron density maps were calculated with phase angles derived from the model of holotransketolase [2], where the contributions of the cofactor atoms to the calculated structure factors had been excluded. Crystallographic refinement was carried out with the program XPLOR [9]. The protocol used consisted of a molecular dynamics simulation where the temperature was raised to 4,000 K and then slowly cooled to 300 K in steps of 50 K, followed by energy minimization. This protocol decreased the initial crystallographic R-factor from 34.2% to 22.2%. A second molecular dynamics simulation from 2,000 K to 300 K with subsequent energy minimization resulted in an R-factor for the final model of 21.8% with overall rms deviations from bond lengths of 0.016 Å and bond angles of 3.5°. Inspection of the electron density maps, model building and comparison of holo- and apotransketolase was done using the graphics progam O [10].

3. RESULTS AND DISCUSSION

3.1. *Electron density map*

The 2|Fo|−|Fc| electron density map for the refined model of apotransketolase shows well-defined electron density except at a few protein regions close to the active site. Electron density for loops comprising residues 187–198 and 383–393 is totally absent, which reflects disorder of these two regions in the structure of the apoenzyme. Furthermore, residues 259 and 260 are less well defined in the electron density, which probably reflects an inceased mobility for these regions as compared to holotransketolase. At a cutoff level of the standard deviation of the electron density map, no electron density for ThDP and the Ca^{2+} ion is observed, which clearly shows that the crystals do not contain any of the cofactors bound and therefore indeed represent the apoform of the enzyme.

3.2. *Overall structure*

The crystal asymmetric unit contains the transketolase dimer and the structure determination gives, therefore, independent results for the two subunits. Within the error limits of the X-ray analysis, the structure of both subunits of apotransketolase is highly similar, with an rms deviation for all Cα atoms of 0.36 Å between the subunits. The results described in the following are therefore valid for both subunits.

The refined model of apotransketolase calculated at 2.8 Å resolution showed that the general folding of the enzyme is very similar to holotransketolase. Fig. 1 shows the rms deviations for all Cα atoms in one of the subunits, when superimposing the apo- onto the holostructure. As can be seen, with only two localized exceptions, the two structures superimpose well, with an overall rms deviation of 0.53 Å for 656 Cα positions (the flexible loops were not included) in the subunit. Cofactor binding to transketolase does not induce any large conformational change, such as domain–domain rotation or a difference in the packing of the two subunits.

3.3. *Flexible loops*

In the apostructure, two loops regions are flexible. One of these loops, the cofactor binding loop, comprises region 187–198 and contains a number of conserved amino acids. In the holoenzyme, this loop is involved in the binding of the Ca^{2+} ion and provides two ligands to the metal, the side chain of Asn^{187} and the main chain oxygen of Ile^{189}. Residues Asp^{192} and Ile^{191} are in contact with atoms of the cofactor [2]. These interactions with the metal ion and the cofactor keep the loop in a 'closed' position and it acts like a flap closing off the metal and the diphosphate binding site from the outer solution (Fig. 2). In the apostructure, these interactions are lost and the loop has a high mobility, which enables access of the coenzyme to its binding site.

The cofactor binding loop is also involved in subunit–

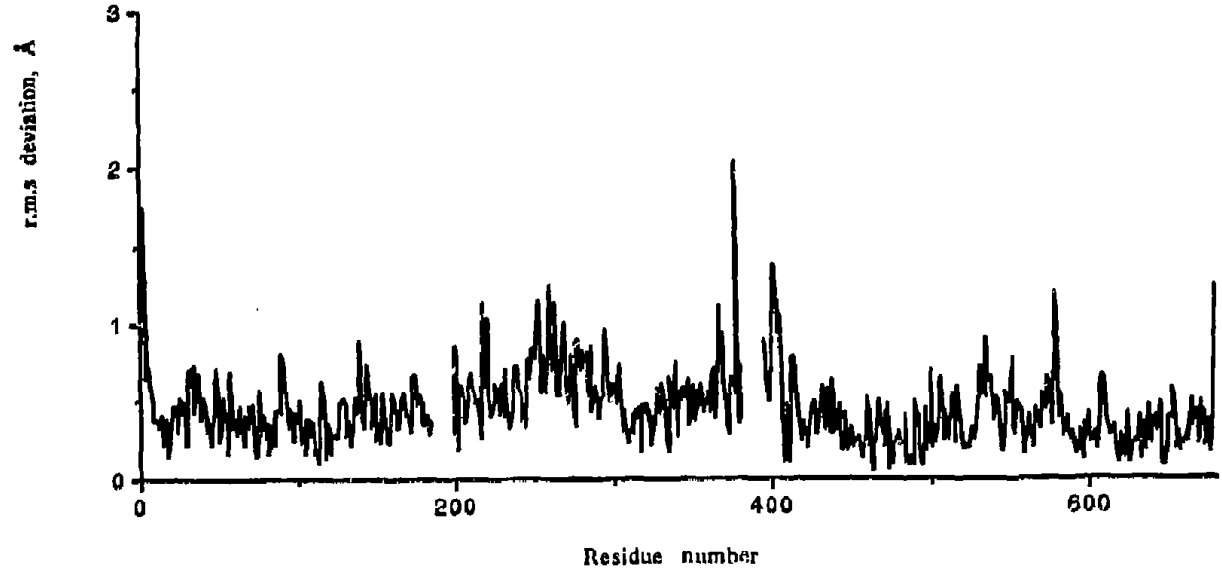

Fig. 1. Distance between corresponding Cα atoms of holo- and apotransketolase as a function of residue number after superposition of the two structures.

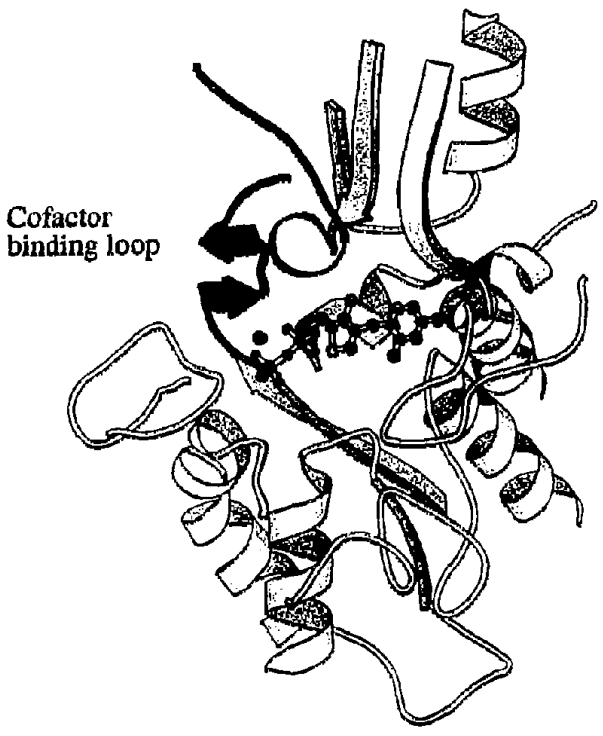

Fig. 2. Schematic view of the ThDP binding site in transketolase. The cofactor binding loop and loop 383–393 in the holoenzyme are shown in black. β-strands (indicated by arrows) in the top belong to the sheet in the middle domain of one subunit, β-strands below to the sheet in the N-terminal domain of the other subunit.

subunit interactions. Thr^{190} makes a hudrogen bond to the side chain of Asp^{382} in the second subunit. This side chain in turn forms hydrogen bonds to main chain nitrogens of residues 191 and 192. Another subunit–subunit interaction is formed through the hydrogen bond between Asp^{192} and the side chain oxygen and main chain nitrogen of Thr^{384}. There are also hydrophobic interactions between the side chains of residues Ile^{191} and Leu^{383}. These extensive interactions probably stabilize the conformation of loop region 383–393 in the holoenzyme. The loss of these interactions due to the mobility of loop 187–198 in the apostructure will then destabilize the conformation of loop 383–393 observed in the holoenzyme and it also becomes flexible. The absence of these subunit–subunit interactions might also partially be responsible for the observed decrease in dimer stability for the apoenzyme [1]. In the holoenzyme, the side chain of Leu^{383} makes a hydrophobic contact to the C4 methyl group of the thiazolium ring. the absence of this interaction might also contribute to the destabilization of this loop.

At the surface of the protein, residues 259 and 260 are found in a less well-defined area of electron density, reflecting increased mobility for part of the polypeptide chain. Residues 259 and 260 are, however, not in direct contact to the cofactor and there is no obvious connection between ThDP removal and increase in flexibility at this part of the structure.

3.4. *Active site*

A detailed comparison of the coenzyme binding site in the apo- and holoform of the enzyme shows that the position of most of the residues which interact with ThDP in the holoenzyme are very similar in both structures. Except for loops 187–198 and 383–393, structural changes upon removal of the Ca^{2+} ion and ThDP only cause very minor rearrangements for some of the side chains.

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