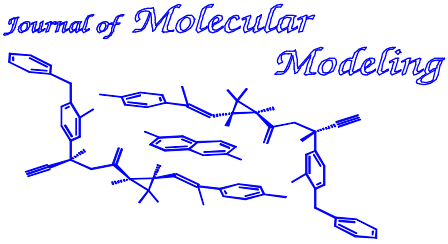
J. Mol. Model. **1996**, *2*, 312 – 318



Molecular Dynamics Simulations on the Coenzyme Thiamin Diphosphate in Apoenzyme Environment

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Abstract

Thiamin diphosphate (ThDP) is an essential cofactor for a number of enzymes, and especially involved in the nonoxidative decarboxylation of α-keto acids by pyruvate decarboxylase (PDC). Recently the crystal structure of PDC bound ThDP has been determined. Based on these X-ray data MD simulations of the isolated coenzyme as well as of ThDP in its enzymatic environment were performed, using the GROMOS87 software package. For the ThDP-apoenzyme modelling all significant amino acid residues with a cut-off radius less than 8.5 Å from the cofactor were taken into account.

Because the activity of the coenzyme mainly depends on the formation of a specific structure, the conformational behavior of ThDP and enzyme bound ThDP were investigated within the MD simulations in more detail. Therefore, trajectories of significant structural parameters such as the ring torsion angles Φ_T and Φ_P as well as essential hydrogen bonds were analyzed by our graphics tool. Moreover, Ramachandran-like plots with respect to the torsion angles Φ_T and Φ_P were used for the illustration of preferred orientations of the two aromatic rings in ThDP.

Finally, MD simulations on ThDP analogs with less or none catalytic activity and apoenzyme mutants were included, in order to get hints of conformational effects and significant interactions in relation to cofactor-apoenzyme binding and the catalytic mechanism.

Keywords: Molecular dynamics, catalytic mechanism, enzymes

Introduction

Thiamin diphosphate (ThDP), the biological active form of vitamin B1, is an essential cofactor for a number of enzymes in the carbohydrate metabolism, where it is mainly involved in the decarboxylation of α-keto acids.

In the last years we have studied the conformational and structural properties of isolated ThDP-systems [1,2]. But recently the crystal structures of three ThDP dependent enzymes have been determined [3,4,5], which also opens new possibilities for molecular modelling.

Using the X-ray data of pyruvate decarboxylase (PDC) - the simplest of the three enzymes - we examined the influ-

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ence of the apoenzyme environment on the conformational behavior and the catalytic activity of the cofactor.

ThDP consists of two aromatic rings and a diphosphate side chain (Fig.1). The orientation of the pyrimidine and the thiazolium ring is described by the torsion angles Φ_T and Φ_P defined by:

Φ_T = C2-N3-C_{br}-C5' ,

Φ_P = N3-C_{br}-C5'-C4´

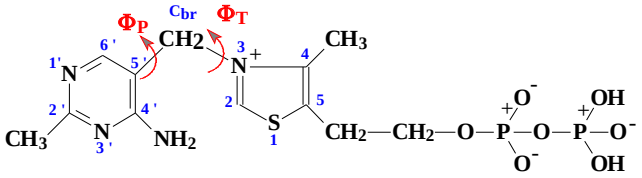


Figure 1. *ThDP system with the torsion angles Φ_T and Φ_P*

Based on this torsion angles three basic conformations were introduced in literature [6] (Fig.2).

F (Φ_T = 0°,Φ_P = ±90°) and S conformers (Φ_T = ±100°, Φ_P = ±150°) are found in the crystal structures of isolated ThDP systems, while the V-conformation (Φ_T= ±90°, Φ_P = ∓90°) was assumed to be the biological active form of the cofactor, which was verified by the X-ray structure of enzyme bound ThDP. In apoenzyme bound ThDP torsion angles of Φ_T = 95,5° and Φ_P= -69,9° were found [5].

The enzyme catalyzes the decarboxylation of pyruvate to acetaldehyde and CO_2. For the activity of the cofactor Mg^{2+} ions are also required. The holoenzyme is a tetramer, consisting of four identical subunits, whereby each subunit contains a cofactor. A subunit is formed by three domains α, β, γ (Fig.3).

The cofactors are located in clefts between the α and γ domains of two different subunits (Fig.4), whereby the α domain is mainly involved in binding the pyrimidine ring, while the γ domain interacts with the diphosphate side chain.

The diphosphate side chain, is tightly bound to the enzyme by hydrogen bonds and the octahedral coordinated Mg^{2+}-ion, which assists in anchoring the diphosphate to the protein. The binding of the pyrimidine ring by hydrogen bonds to the amino acid residues GLU 51, ILE 476 and GLY 413 is important for the mechanism [5] (Fig.5).

Beside ThDP a number of ThDP analogs (Fig.6) with different catalytic activity synthesized by Schellenberger et al. [7] were taken into account.

The N1'- and N3'-ThDP analogs differ from ThDP by the substitution of the nitrogen atoms N1' and N3' with a C-H group respectively, and show less or none activity.

A substitution of the 6'-H atom by a methyl group results in the 6'-Me-ThDP-analog which is also inactive.

These experimental findings are hints, that the N1'atom plays an important role in the catalytic mechanism of decarboxylation [8].

The mechanism of decarboxylation of α-keto acids by ThDP is a subject of intensive studies since a long time.

In the mechanism suggested in 1958 by Breslow [9] (Fig.7) the formation of an ylide (**1**) by deprotonation of the C2 atom of the thiazolium ring is assumed. The addition of the ylide to the substrate gives the 2-lactyl-ThDP intermediate (**2**). Its decarboxylation leads to a α-carbanion structure (**3**) and finally to the elimination of acetaldehyde.

Especially from the recently solved X-ray structure of PDC a refined mechanism for the deprotonation of the C2 atom was supported [5]. The N1'atom is protonated by the amino acid residue GLU 51. This protonation increases the acidity of the 4'-NH_2 group and favours the formation of the imino structure. In the active V-like conformation the 4'-N position and the C2 atom are neighbouring. Therefore the

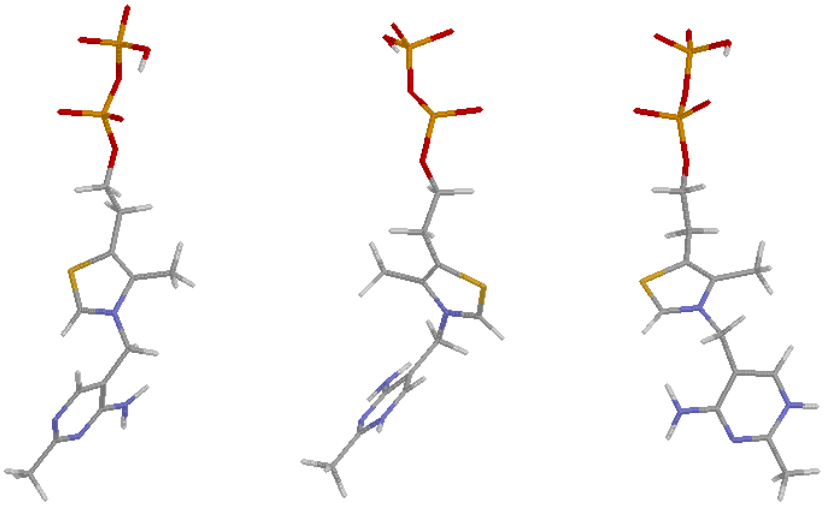


Figure 2. *Basic conformations of ThDP from left to right: F-conformation S-conformation V-conformation*

Table1 *Occurence of the hydrogen bonds HN1'--- GLU 51 in %*

System	N1'-H --- OE1	N1'-H --- OE2	OE1 --- N1'-H --- OE2
N1'-H -ThDP	84,5	43	19,6
N1'-H-pyridyl-ThDP	65,9	62,8	18,5
N1'-H-6'-MeThDP	551	48,7	15,2

deprotonation of the C2 atom by interaction with the imino group should be possible.

For that reason we have performed MD-simulations on isolated and enzyme bound ThDP and its analogs, in order to investigate the conformational behavior and to get hints about the catalytic activity of the cofactors as well as mechanistic aspects .

Methods

The calculations were performed on a SGI workstation. For the MD simulations we used the GROMOS 87 software package [10]. Atomic net charges of the cofactor were calculated by PM3 [11] and adapted to the GROMOS 87 charges. During the calculations the N1' was protonated and for the isolated ThDP-systems Mg^{2+} were taken into account as well. Classical MD studies with NVP ensemble were performed at a temperature of 300 K and simulation periods of 500 ps for the isolated systems and 150 ps for the enzyme bound systems were used. Time steps of 1 fs were regarded. The starting structures are based on the pdb-file of the X-ray structure of the enzyme.

For the MD simulations of the cofactor in the apoenzyme environment we considered all amino acid residues located in a cut off radius of 8.5 Å from the ThDP molecule. Due to the long calculation times required by this model, amino acids described in the literature as significant were taken into account, only [5] (Fig.8). In order to simulate the rather rigid structure of a complete protein environment the C and N atoms of the protein backbone have been localized with position restraining while the calculations.

Results and Discussion

In order to get information about the conformational dynamics of the isolated and enzyme bound ThDP-systems the behavior of the torsion angles Φ_T and Φ_P during the MD run

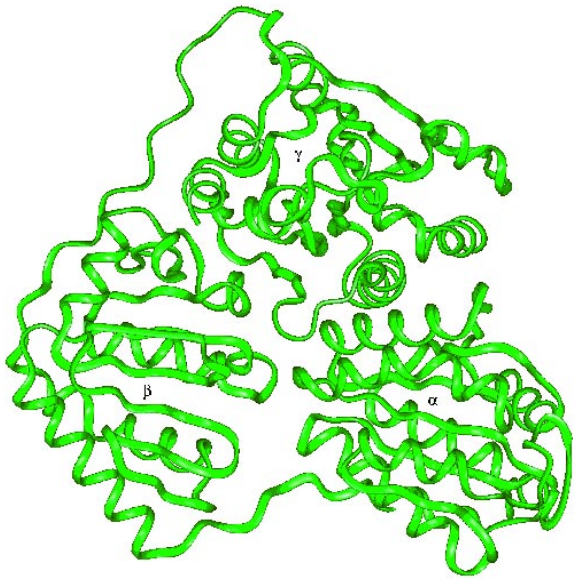


Figure 3. *Domains of the apoenzyme monomer (image available as PDB and inventor file)*

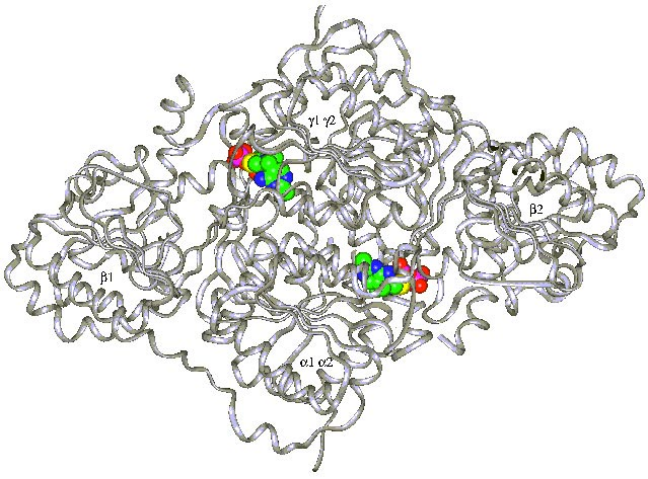


Figure 4. *Ribbon drawing of the PDC-Dimer (image available as PDB and inventor file)*

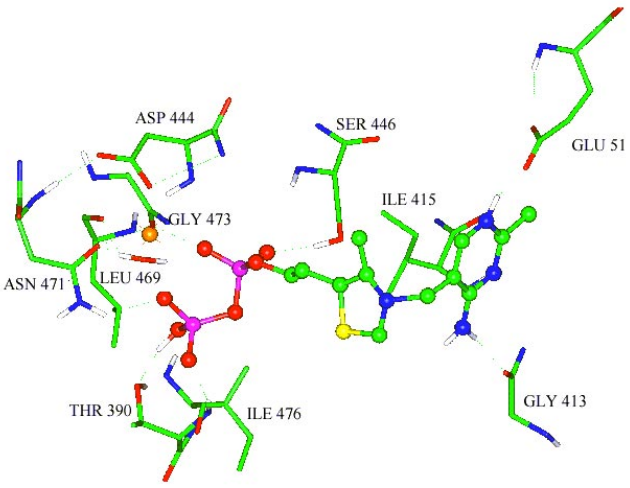
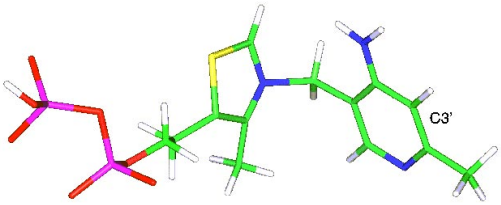


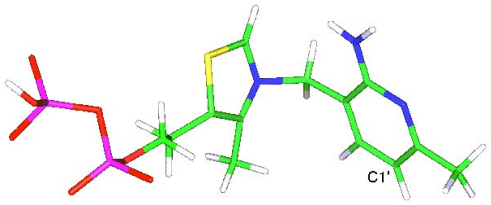
Figure 5. *Significant coenzyme-apoenzyme interactions (image available as PDB file or VRML scene)*

is considered in more detail. The preferred orientations of the two aromatic rings are indicated by fluctuations of these angles resulting from the corresponding trajectories, which are illustrated by Ramachandran-like plots. The position of the V-conformation is specified in the plots as a reference value.

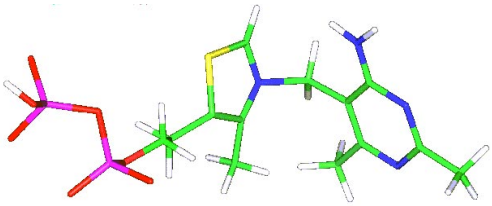
By these Ramachandran-like plots we wanted to investigate if there are significant differences in the conformational behavior of ThDP and its analogs and consequently hints for a different catalytic activity by conformational reasons.



N1'-pyridyl-ThDP



N3'-pyridyl-ThDP



6'-Me-ThDP

Figure 6. *ThDP analogs*

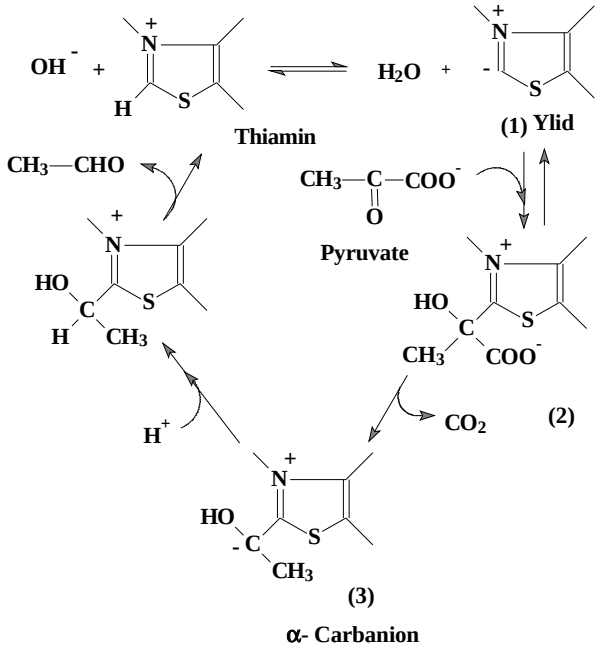


Figure 7. *Breslow mechanism of thiamin catalysis*

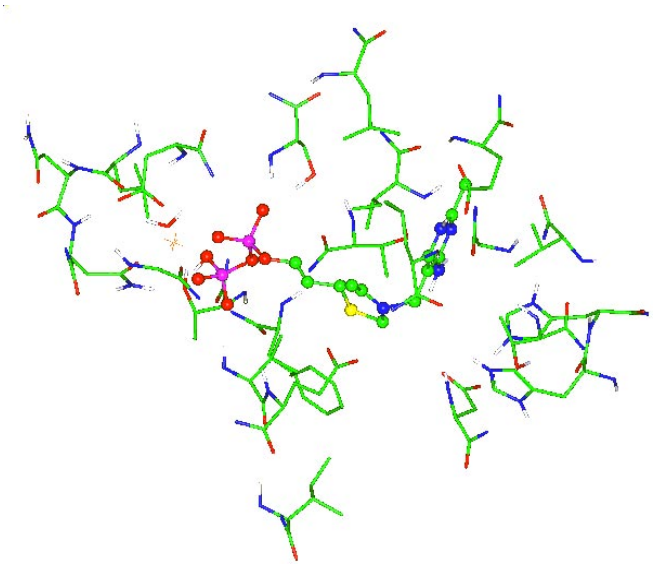


Figure 8. *Apoenzyme environment used for MD simulations (image available as PDB file and VRML scene)*

1. Isolated ThDP-Systems

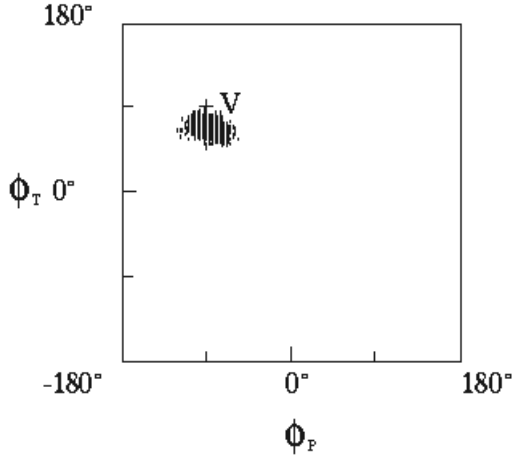
The diagramms (Fig.9) illustrate that in the isolated ThDP-Systems V-like conformations are generally energetically preferred. There are no hints for a different catalytic activity by steric effects.

2. Apoenzyme bound ThDP-Systems

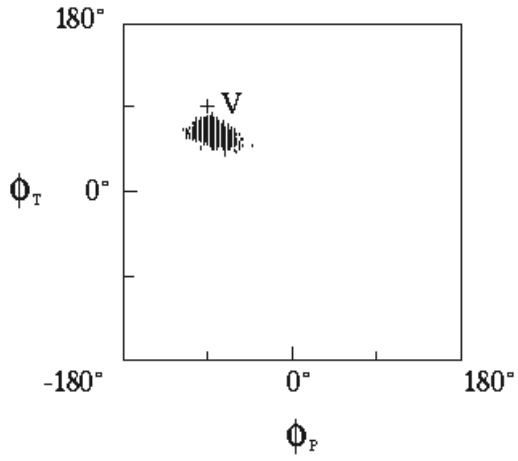
From the Ramachandran plots (Fig.10) it is obvious, that in case of the N3'-pyridyl-ThDP, which shows no catalytic activity, V-like-conformations are less stable within the MD run. The active N1'-H-pyridyl-ThDP shows similar conformational behavior in the apoenzyme environment as N1'-H-ThDP. It is remarkable that in the case of the apoenzyme bound inactive 6'-Me-N1'-H-ThDP beside V-like conformations other conformers are also found.

In our MD simulations on coenzyme-apoenzyme interactions we have assumed that the N1'atom is protonated and the formation of possible hydrogen bonds to both oxygens of the carboxylate group of GLU 51 (Fig.11) has been investigated in more detail.

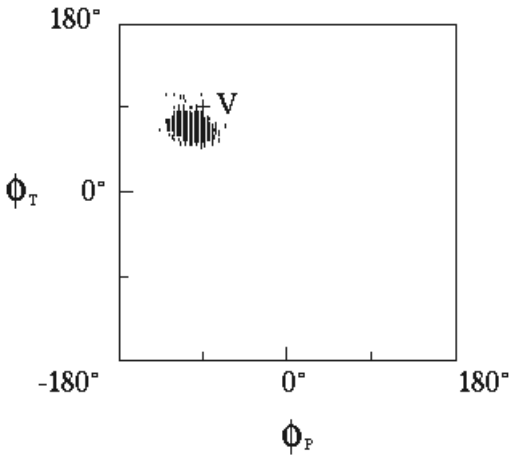
The trajectories of the N1'-H --- GLU 51 hydrogen bonds show, that these specific interactions are less important in the case of the 6'-Me-ThDP analog in comparision to the N1'-pyridyl-ThDP one as well as ThDP. Obviously, the for-



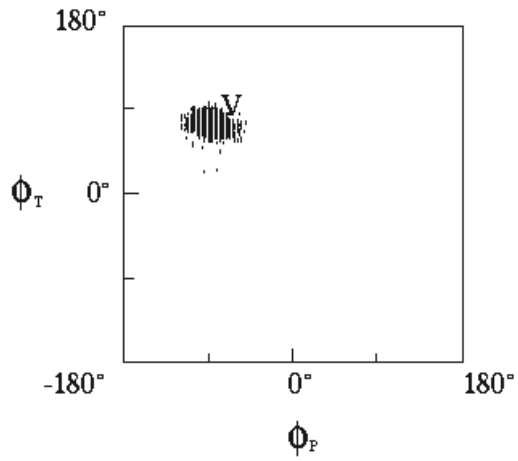
N1'-H- Mg^{2+}-ThDP



N1'-H-6'-Me-Mg^{2+}-ThDP



N1'-H-pyridyl-Mg^{2+}-ThDP



N3'-pyridyl-Mg^{2+}-ThDP

Figure 9. *Ramachandran-like plots of isolated ThDP-systems*

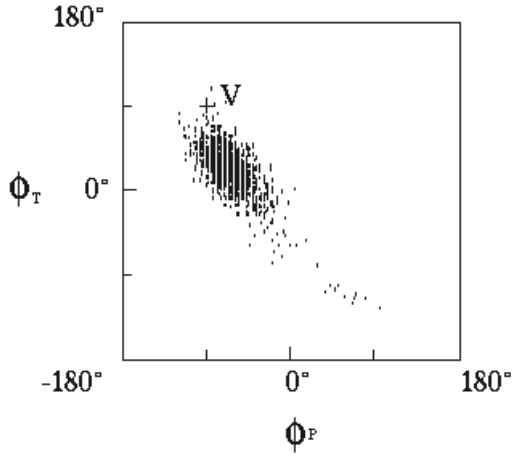
mation of the hydrogen bonds is hindered by the additional methyl group in the 6'-position by sterical reasons. For the N3'-pyridyl-ThDP analog the formation of a hydrogen bond to GLU 51 is excluded.

For a better illustration of these findings we have calculated the corresponding histograms from the trajectories of the distances N1'-H---OE1 and N1'-H---OE2 (Fig.11) with respect to the three ThDP systems. The histograms indicate the frequency distributions of the possible two hydrogen bonds as functions of the distances N1'-H---OE1 and N1'-H---OE2.

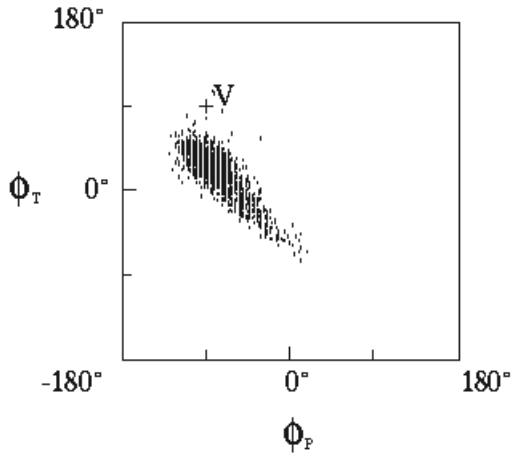
The histogram of N1'-H-ThDP shows an asymmetric frequency distribution of the two hydrogen bonds. The formation of the OE1---HN1' hydrogen bonding in comparision to the OE2---HN1' one is significantly preferred (Fig12). The frequency distributions of both hydrogen bonds in the N1'-H-pyridyl-ThDP system are comparable but show lower absolute values for the maxima related to N1'-H-ThDP. The lowest tendency to form hydrogen bonds to GLU 51 was found for the 6'-Me-N1'-H-ThDP system.

Similar results were also obtained within the special analyzing option for hydrogen bonds in the GROMOS Program. This procedure calculates the percental occurence of hydrogen bonds during the MD-run.

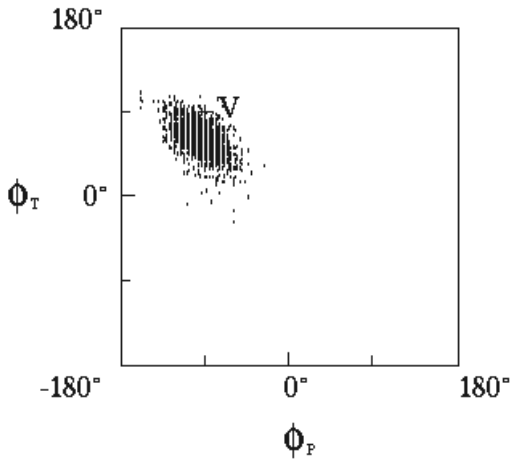
The results for the both hydrogen bonds to GLU 51 for the considered systems are illustrated in Tab.1.



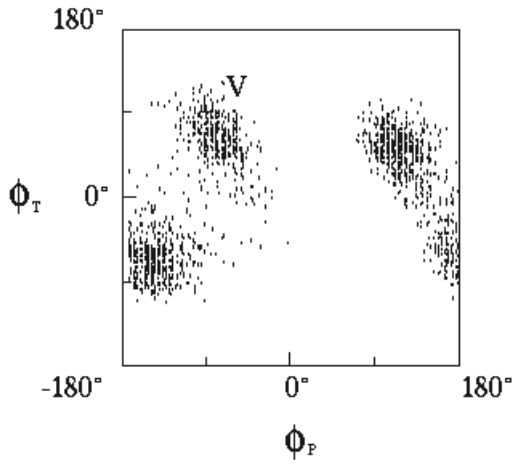
N1'-H-ThDP-Apoenzyme



N1'-H-6'-Me-ThDP-Apoenzyme

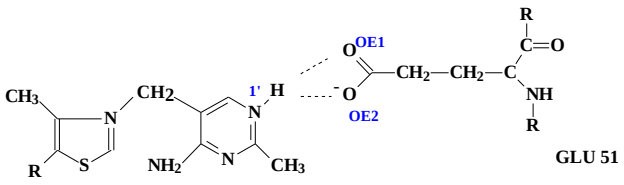


N1'-H-pyridyl-ThDP-Apoenzyme



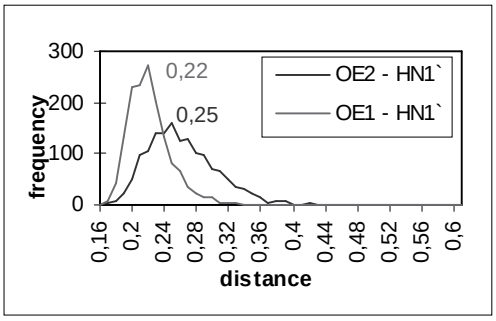
N3' -pyridyl-ThDP-Apoenzyme

Figure 10. *Ramachandran-like plots of enzyme bound ThDP-systems*

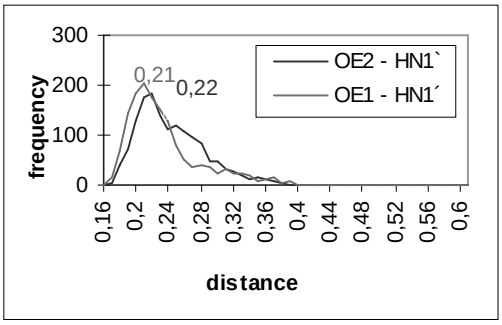


ThDP

Figure 11. *Schematic drawing of the hydrogen bond N1'-H---GLU 51*



N1'-H-ThDP



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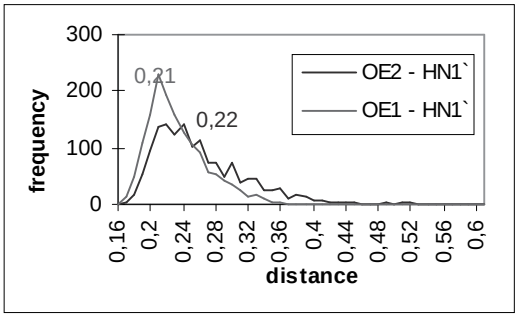

N1'-H-6'-Me-ThDP

Figure 12. *Histograms of hydrogen bonding distances HN1'--- GLU 51 for enzyme bound ThDP-systems*

A tendency between the frequency distributions of the hydrogen bonding and the catalytic activity of the ThDP systems was found. Nevertheless the inactivity of the 6'-Me-ThDP analog can not be explained with this simulation. A possible reason for this failure could be seen in the limited included amino acid residues within our calculations.

Our MD simulations on coenzyme-apoenzyme interaction support the assumption, that the hydrogen bonding of the cofactor to the amino acid residue GLU 51 has a key function in the formation of the active V-like conformation. Moreover the hydrogen bond is necessary to explain the mechanistic pathway of the ThDP catalysis with respect to the cleavage of the C2-H bond of the thiazolium ring to form an ylide like structure.

For a deeper understanding of mechanistic aspects of the ThDP catalysis further simulations including coenzyme-apoenzyme-substrate interaction are required in order to investigate the possible function of the substrate as an activator in the catalytic process.

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