Molecular Modeling Studies on NADP-Dependence of Candida Tropicalis Strain Xylose Reductase

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Abstract: The Candida tropicalis strain CT1799 xylose reductase (XR) with protein ID ABG49458.1 is a kind of NADPH-dependent xylose reductase. It could be used to construct recombinant Saccharomyces cerevisiae strain for utilizing xylose and producing alcohol. To investigate the interaction mechanism of XR with NADP and NAD, the 3D (dimensional) structure for XR was developed. With the 3D structure, the molecular docking operations were conducted to find the most favorable bindings of XR with NADP and NAD. Based on these results, the binding pockets of XR for NADP and NAD have been explicitly defined, respectively. It was observed that Asn278 and Arg282 of XR could form hydrogen bonds with both NADP and NAD that were bonded to the same site of XR with some competitive relationship. However, according to the binding energies and conformational fitting, NADP is a more favorable coenzyme to XR. All these findings may explain why XR is NADP-dependent. The findings can be used to guide mutagenesis studies, providing useful clues to modify the enzyme for improving the utilization of xylose in producing alcohol. In addition, because the human aldose reductases have the functions to reduce the open chain form of glucose to sorbitol, a process physiologically significant for diabetic patients at the time that their blood glucose levels are elevated, the information gained through this study may also stimulate the development of new strategies for the therapeutic treatment of diabetes.

Keywords: New strategy of diabetes therapy, Alcohol-producing, Xylose reductase, Assimilation, Binding pocket, Hydrogen bonds.

INTRODUCTION

NADPH-dependent xylose reductases are classified as the monomeric aldo-keto reductase superfamily (AKRs) of proteins and enzymes. Each of the family members seems to have some kind of interactions with NAD or NADP [1]. AKRs have distinctly defined functions for reversibly catalyzing NAD(P)H-dependent reduction of D-xylose to xylitol. This kind of reaction is one of the initial steps in assimilation of xylose into the glycolytic pathway [2, 3].

Although the roles of the majority of the AKRs have not been well defined, many of them are deemed as the general detoxification catalysts, meaning that they are able to reduce the reactive carbonyl-containing compounds [4, 5]. According to the report [6], they can also control the levels of intracellular polyols in some osmotic regulations. In general, however, the reductases possess the special ability to catalyze the NAD(P)H-dependent reduction, capable of transforming the carbonyl substrates to their corresponding alcohols. Some of them will choose the directions in which the carbonyl substrates are reduced although the reactions are mainly reversible. Furthermore, little substrate specificity has been observed. However, there is an interesting finding that the hydrophobic compounds are preferred over hydrophilic compounds. Clinical interest on the AKRs has been focused on human aldose reductases because they are physiologically significant for diabetic patients at the time when their blood glucose levels are elevated [7].

Some kinetic experiments and structural investigations have been carried out on the AKRs, which were mainly directed for understanding the mechanism and inhibitions of aldose reductases. In the current study, Candida tropicalis strain Ct1799 xylose reductase with protein ID ABG49458.1 has been selected as a target for investigation. To investigate the binding mechanism of XR with NADP, an indispensable knowledge is of its 3D structure. Since no experimental structural data whatsoever is available for XR so far, we have to resort to the computational approaches. Many lines of evidences have indicated that mathematical/computational approaches, such as structural bioinformatics [8-14], molecular docking [15-20], molecular packing [21-23], pharmacophore modelling [24, 25], Mote Carlo simulated annealing approach [26], diffusion-controlled reaction simulation [27], graph/diagram approach [28-43], bio-macromolecular internal collective motion simulation [44], QSAR [13, 45-50], protein subcellular location prediction [51-57], identification of membrane proteins and their types [58, 59], identification

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of enzymes and their functional classes [60], identification of GPCR and their types [61-63], identification of proteases and their types [64], protein cleavage site prediction [65-67], and signal peptide prediction [68, 69], can timely provide very useful information and insights for drug development as well as basic research and hence are quite rewarding. The present study was initiated in an attempt to use structural bioinformatics tools to investigate the binding mechanism of XR with NADP in hope to provide useful information to stimulate the drug development in the relevant areas."

MATERIALS AND METHODS

In order to use the structural bioinformatics tools to model the 3D structure of XR (protein ID ABG49468.1), the crystal structure of xylose reductase AKR2B5 (PDB code 1K8C) was chosen as templates, which was first released in 2002 [70], with a resolution of 2.10 Å.

The sequence of XR contains 324 amino acids, while that of 1K8C contains 322 amino acids. Sequence alignment performed by our in-house software SIMM (Shanghai Molecular Modeling) for the two sequences is shown in Fig. (**1**). The sequence identity of the two proteins is about 76%.

Based on the sequence alignment (Fig. **1**) and the atomic coordinates of the crystal structure 1K8C, the 3D structure of XR was developed by the "segment matching" or "coordinate reconstruction" approach [71-74]. This kind of approach had been used to model the 3D structures of caspase-8 [75], Cdk5-Nck5a* complex [76], caspase-9 [77], and BACE1 [8, 78] before their crystal structures were available or released to the public, timely providing useful information for both basic research and drug development in the relevant areas [8, 78-81]. The segment match approach has also been successfully used to predict the 3D structures for CARDs (caspase recruitment domains) of Apaf-1, Ced-4 and Ced-3 based on the solution structure of the RAIDD CARD [82], as well as the â -secretase zymogen [83]; the structural information derived from the latter has convincingly elucidated the mechanism why the prodomain of â -secretase did not suppress the activity like in a strict zymogen, as reported by Benjannet *et al*. [84] and Shi *et al*. [85]. Furthermore, the segment match approach was also used to develop the 3D structures of extracellular domains for the subtypes 1, 2, 3, and 5 of GABA-A receptors [9], BACE2 [86], GFAT [10], human ion channels [87], α7 nicotinic acetylcholine receptor [11], cathepsin-E [88], human G alpha 13 and human TXA2 receptor [12], DNA-CBF3b complex [89], H5N1-NA [90] as well as CYP2C19 [17, 91], greatly stimulating the development of the related areas (see, e.g., [17, 92-94]).

After 500 steps energy minimization of the initial computational structure, the final 3D structure thus obtained for XR is shown in the ribbon drawing colored according to its secondary structures (Fig. **2**) and the ligand NADP shown in the ball-and-stick drawing. In addition, some assessments were performed on our computational structure using SWISS Model Server.

In the current study, the molecular docking with the Monte Carlo simulated annealing [26, 95], also known as a Metropolis algorithm [96], was adopted to investigate the interactions of XR with NADP. Before beginning the docking operation, 1000 steps energy minimization were performed to the initial computational structure in the explicit solvent. The binding pocket for both ligands was found according to the template 1K8C because the xylose reductases have high homology and their binding pockets are quite conservative. During the docking process, the ligand was

Fig. (1). The sequence alignment of XR (Chain-1) with 1K8C (Chain-2), where the amino acids are colored according to the following four types: (**1**) acidic, red; (**2**) basic, dark blue; (**3**) hydrophobic, green; (**4**) hydrophilic, light blue. The sequence identity of the two enzymes is about 76%. (For the color drawing of this figure, the reader is referred to the web version of this paper).

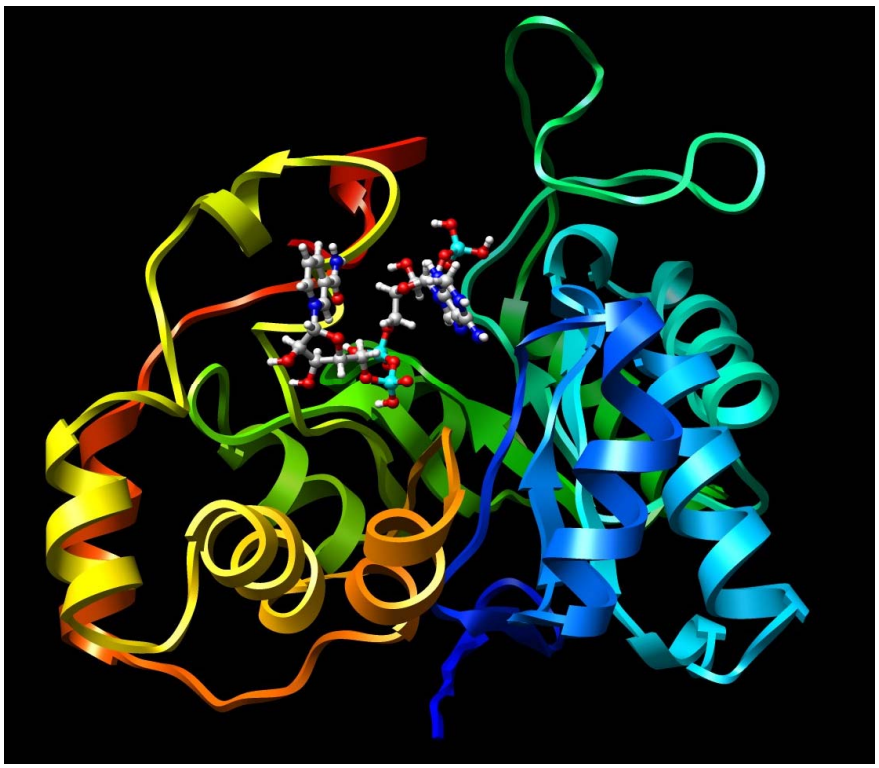

Fig. (2). Illustration to show the computed 3D structure of XR with NADP, where the XR is in the ribbon drawing colored according to its secondary structures and the NADP is in the ball-and-stick drawing. (For the color drawing of this figure, the reader is referred to the web version of this paper).

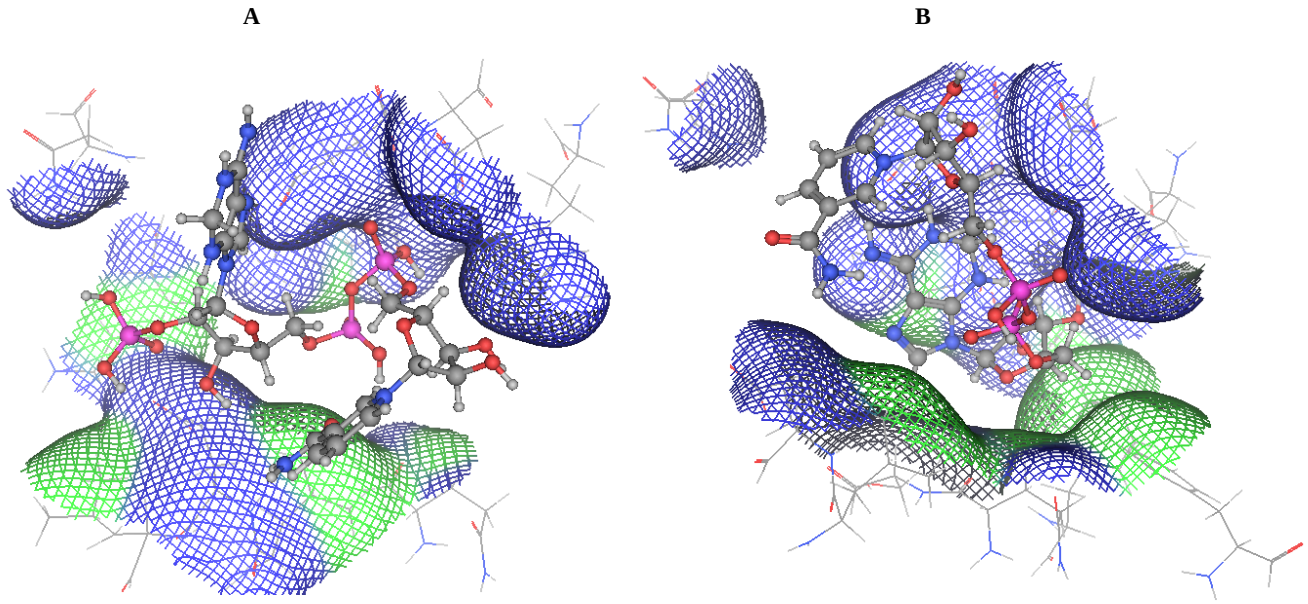

Fig. (3). Illustration showing the lipophilic and hydrophilic surfaces of the binding pocket (or cavity) of XR for (**A**) NADP and (**B**) NAD, where the lipophilic and hydrophilic surfaces are colored in green and blue, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

flexible. The program generated a diversified set of conformations by making random changes of the coordinates of NADP [96-98]. When a new conformation of NADP was generated, the search for the favorable bindings was conducted within a specified 3D box, using either simulated annealing [95] or tabu search [99, 100]. Both methods seek to optimize the purely spatial contacts as well as electrostatic interactions. The interaction energy was calculated using the van der Waals and electrostatic potential fields. In all our computations, the CHARMM33 force field parameters were utilized [101]. For facilitating comparison, using the same approach as described above, the docking of NAD to XR

Table 1. Residues in Forming the Binding Pockets of XR for NADP and NAD, Respectively

Ligands	Binding Pocket Residues[a]						
NADP	Lys27	Asn29	Gln225	Leu228	Glu229	Leu230	Lys276
	Ser277	**Asn278**	Asn279	**Arg282**			
NAD	Asn29	Phe222	Gln225	Ser226	Leu228	Glu229	Phe242
	Pro275	Lys276	Ser277	**Asn278**	Asn279	**Arg282**	

[a]Residues with bold-face type mean that they have hydrogen bonding interaction with the ligand.

was also performed; the information thus obtained would be useful for understanding the mechanism about NADPH-dependence of XR. The most favorable interactions of XR with NADP and NAD thus obtained are given in Figs. (**3A** and **B**), respectively.

Similar docking methods were also used for studying the binding mechanism of CoV M^{pro} with ligands [15, 25], alpha 7 nAChR dimer with GTS-21 [92], and calmodulin with chrysin [102], finding anti-SARS drugs [103, 104], seeking for agaritine derivatives [19], designing inhibitors against SAH enzyme [105], searching for new agonists against Alzheimer's disease [20], as well as investigating the drug resistance of H5N1 virus [94].

RESULTS AND DISCUSSIONS

The binding pockets of XR for NADP and NAD are shown in the Table **1**. Here, the constituents of the binding pocket of a protein receptor to a ligand are defined by those residues that have at least one heavy atom (i.e., an atom other than hydrogen) with a distance ≤ 5 Å from a heavy atom of the ligand. Such a criterion was originally used to define the binding pocket of ATP in the Cdk5-Nck5a* complex [76] that has later proved quite useful in identifying functional domains and stimulating the relevant truncation experiments [81]. The similar approach has also been used to define the binding pockets of other receptor-ligand interactions [11, 15, 25, 78, 90, 91, 94, 104, 106].

For NADP, the binding pocket is formed by 11 residues, among which Asn278 and Arg282 have hydrogen bond interactions with NADP. There are 6 hydrogen bonds existing between XR and NADP. For NAD, the corresponding binding pocket is formed by 13 residues, more than NADP. Asn278 and Arg282 also have formed 2 hydrogen bonds with NAD. Compared the two binding pockets, we can find that they are highly similar. Nine residues, Asn29, Gln225, Leu228, Glu229, Lys276, Ser277, Asn278, Asn279, and Arg282, have been shared by the two binding pockets. Also, it is interesting to find that Asn278 and Arg282 have hydrogen bond interactions with both NADP and NAD. So, we think that NADP and NAD bond to the same site of XR with competitive relationships. Judging from the binding energies, as shown in Table **2**, the binding energy of NADP is much higher than the corresponding energy of NAD. Compared with NAD, NADP has a phosphate radical, as shown in Fig. (**4**), which is propitious to disperse charges so as to decrease the electronic energies. Also, the phosphate radical can make the contact of XR and NADP more sufficient. In addition, as mentioned above, NADP forms more hydrogen bonds with XR than NAD. So, NADP is considered to be more preferable, which may be the explanations for the NADP-dependence of XR.

The lipophilic and hydrophilic surfaces of the binding pockets (or cavities) of XR for NADP and NAD are shown in Fig. (**3**). We can find that the two binding pockets are almost the same as mentioned above, which are mainly hydrophilic. In addition, the conformations of NADP and NAD may explain why NADP is preferred. The molecular conformation of NAD is folded with a high potential energy, which is disadvantageous for NAD to interact with XR; while the molecular conformation of NADP stretches well, and is advantageous for the interactions between XR and NADP.

CONCLUSION

The binding pockets of XR for NADP and NAD are found, which are formed by 11 and 13 residues, respectively.

Table 2. List of the Interaction Energies (kcal/mol) for XR with NADP and NAD

Ligands	E (electronic)	E (van der Waals)	E (binding)
NADP	-18.53	-14.27	-32.79
NAD	-7.65	-17.78	-25.43

The van der Waals interactions were computed with $E_{vdw} = \sum_{i<j} e_{ij} \left[\frac{(1+a)R_{ij}}{r_{ij} + aR_{ij}} \right]^{nij} \left[\frac{n_{ij}}{m_{ij}} \frac{(1+b)R_{ij}^{m_{ij}}}{r_{ij}^{m_{ij}} + bR_{ij}^{m_{ij}}} - \frac{m_{ij} + n_{ij}}{m_{ij}} \right] s(r_{ij}) T_{ij} I_{ij}^{vdw}$

where e_{ij}, R_{ij}, M_{ij} and n_{ij} are the force-field parameters. To avoid the occurrence of zero denominator, the buffering constants *a* and *b* are introduced. *S* is the smoothing function; I^{vdw} the interaction scale factor defined to be 0 and 1; T_{ij} the interaction scale factor used to scale particular non-bonded interactions.

The electrostatic interactions were computed with $E_{ele} = \frac{e^2}{d 4\pi\varepsilon_0} \sum_{i<j} \frac{q_i q_j}{(r_{ij} + b_{ele})^k} s(r_{ij}) T_{ij} I_{ij}^{ele}$

where T_{ij} is the interaction scale factor, defined exactly the same as in the van der Waals interaction; q_i is the partial charge on atom *i*; b_{ele} the buffering constant intended to avoid zero denominators; *k* is either 1 or 2 depending on whether the constant dielectric or distance-dependent dielectric form is used, respectively; *d* the dielectric constant; and I^{ele} the interaction scale factor defined to be either 0 or 1.

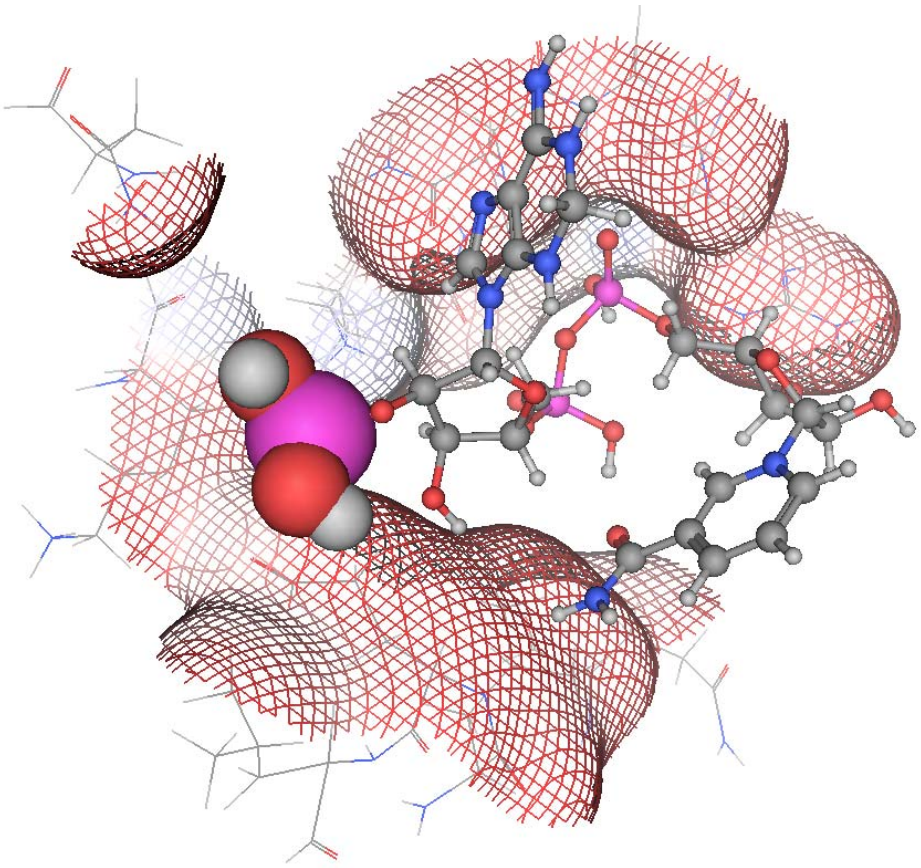

Fig. (4). Illustration showing the binding pocket surfaces of XR for NADP, where the exposed, lipophilic, and hydrophilic surfaces are colored in red, blue, and green, respectively. Also, the phosphate radical of XR is rendered in space-filling representation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper).

There are 9 residues, Asn29, Gln225, Leu228, Glu229, Lys276, Ser277, Asn278, Asn279, and Arg282, appearing in the two binding pockets. Furthermore, Asn278 and Arg282 have hydrogen bond interactions with both NADP and NAD. So, it is considered that NADP and NAD bond to the same site of XR with competitive relationships. Judging from the binding energies and molecular conformations, NADP is more preferable. This is also why XR is NADP-dependent.

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