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A Computational Study for Roles of a Coenzyme and Amino Acid Residues in NAT2-Isoniazide Complex

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1. Introduction

N-acetyltransferase 2 (NAT2) is one of the drug metabolizing enzymes which metabolizes several endogenous or exogenous compounds such as antitubercular drug isoniazide. NAT2 is the enzyme that catalyzes *N*-acetyl conjugation of allylamine derivatives, and it is an important enzyme in drug design trials because of its polymorphism [1]. The polymorphism causes individual difference of metabolic rate of allylamine. The individual whose rate is faster is called as rapid acetylator (RA), the individual whose rate is slower is slow acetylator (SA). Because the rate of SAs in Caucasians is higher than in the Japanese, NAT2 plays important roles in consideration of drug metabolisms of Caucasians. In the ligand binding site of NAT2, there is an acetyl CoA molecule as a cofactor and it plays an important role in acetylations of substrates. The cysteine residue (Cys68) in the active site also plays significant roles in drug metabolism [2]. Recently, 3D structure of human NAT2 determined by X-ray crystallography was reported [3]. In the reported structure, not acetyl CoA but CoA was included in NAT2 as the cofactor. Because the atomic distance between the sulphur atom of CoA and the sulphur of the cysteine residue in the binding site is short (2.74 Å), substitution of acetyl CoA for CoA may cause the structural change of NAT2. In this study, CoA of NAT2 crystal structure was substituted by acetyl CoA by using molecular modelling, and molecular dynamics (MD) simulation of the modelled structure was carried out in order to refine the 3D structure. By these calculations, the effects of acetyl CoA for 3D structure of NAT2 were evaluated. Furthermore, the ligand binding site of NAT2 was determined by using the refined structure, and the role of the residues constructing the site, e.g. Cys68, was also investigated. The docking study of isoniazide to NAT2 was carried out, and the 3D structure of NAT2-isoniazide complex was predicted.

2. Method

3D structure of NAT2 used in this study was derived from Protein DataBank (PDB ID: 2PFR). In 2PFR, four residues were added in the N-terminal and there are some missing atoms. These structural problems were modified by using Maestro software package [4]. In addition to these modifications, hydrogen atoms were added. The

cofactor CoA and Cys68 were considered as free thiol, and hydrogens were added for both sulphur atoms in CoA and Cys68. After the structural minimization of the PDB structure, substitution of acetyl CoA for CoA was carried out, and optimization of acetyl CoA-NAT2 structure was performed. For these calculations, AMBER9 [5] was used, and the generalized Born implicit solvent model was adopted. The cutoff of non-bonding terms was not used, and default settings of the other parameters were used. Using the optimized structures as initial, temperature-increase simulations were performed from 0 K to 300 K. The generalized Born implicit solvent model was used, and 30-ps MD simulations with a 0.5-fs time step were carried out. After the temperature-increase MD, 1 ns MD simulations with time step 1.0 fs in 300 K were performed. For the MD simulations, SHAKE method was adopted, and AMBER ff99 force field was used. To the refined structure of NAT2, isoniazide was docked by using docking program GOLD with default settings. For the comparison, docking into NAT2 with CoA (not acetyl CoA) was also carried out.

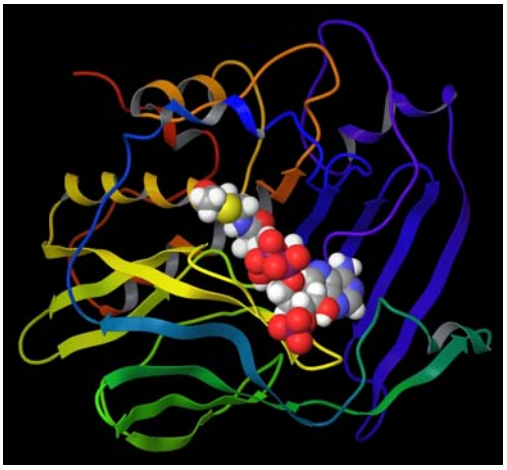

Fig. 1 The 3D structure of NAT2 refined by MD simulation

3. Results and Discussion

The refined structure of NAT2 by MD simulations is illustrated in Fig. 1. As shown in the figure, 3D structure of NAT2 was not broken by substitution of acetyl CoA for CoA. It suggests that stable simulations could be carried out for NAT2 and reasonable refined structure was obtained. Calculated root mean square deviations also indicate that the simulation stably converged in 1 ns. Comparing NAT2-acetyl-CoA complex and NAT2-CoA complex, cavity around the cofactor was larger in NAT2-acetyl-CoA than in NAT2-CoA, and the cavity seems to be the ligand binding site. Furthermore, isoniazide can be docked into NAT2-acetyl-CoA by docking study, although any solutions cannot be obtained for NAT2-CoA. The results indicate that the structures of cofactors play important roles in ligand binding of NAT2.

References

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