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Novel NMR approach to detect the changes in the hydrogen bonds in protein upon binding to ligand using deuterium isotope shifts

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Drosophila melanogaster DAT (dopamine N-acetyltransferase) is one of the arylalkylamine N-acetyltransferase (AANAT). DAT catalyses the transfer of the acetyl group in acetyl-CoA to various arylalkylamines, including dopamine, serotonin, phenylethylamine and tryptamine. However, it is not clear how DAT transfer the acetyl group. The crystal structure of DAT-Acetyl-CoA complex has already been solved (Fig.1). X-ray crystal structure showed the hydrogen bond between the DAT catalytic residues and co-factor/substrate is important for the catalytic reaction. The co-factor binding may induce structural change of substrate binding site and allows the substrate to bind to the binding site. The hydrogen bond strengths are changed, according to the allosteric structural change of binding site.

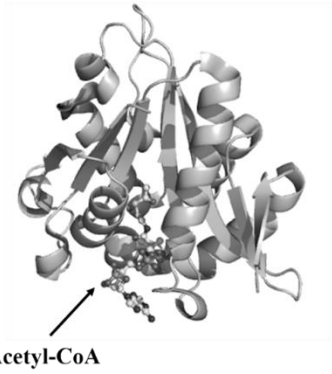

Fig.1 Crystal structure of the dopamine N-acetyltransferase–acetyl-CoA complex

In attempting to explore the difference of the hydrogen bond strength, we compared the ^{1}H-^{15}N HSQC spectrum between 6% D_2O and 50 % D_2O in apo-, binary-, and ternary-form (Fig.2). The systematic comparison of the ^{1}H-^{15}N based isotope shifts in various sets of co-factor and substrate give us the structural details in the allosteric change caused by co-factor and substrate.

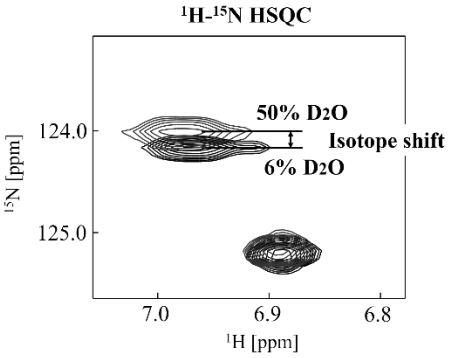

Fig.2 ^{1}H-^{15}N based isotope shift

Reference

Cheng KC, Liao JN, Lyu PC. (2012) *Crystal structure of the dopamine N-acetyltransferase-acetyl-CoA complex provides insights into the catalytic mechanism, Biochemical Journal, 446,* 395-404