The Role of Acetyl coenzyme A in Progressing the Catalytic Cycle of Insect Arylalkylamine N-acetyltransferase

Chu-Ya Wu (吳竹雅), I-Chen Hu (胡宜忱), Yi-Zong Lee (李翊榮), Yi-Chen Yang (楊伊琛), Wei-Cheng Ding (丁韋丞), Hui-Chun Cheng (鄭惠春), Shih-Che Sue (蘇士哲) & Ping-Chiang Lyu (呂平江)

Institute of Bioinformatics and Structural Biology, Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan, 30013, R.O.C.
s102080827@m102.nthu.edu.tw

Abstract

Drosophila melanogaster dopamine N-acetyltransferase (Dat) belongs to arylalkylamine N-acetyltransferase (AANAT, EC 2.3.1.87), that transfer acetyl group to monoamines. Insect AANATs participate in the neurotransmitters inactivation, cuticle sclerotization, and pigmentation. AANATs follow an ordered sequential mechanism in catalysis process, in which Acetyl coenzyme A (Ac-CoA, cofactor) binds first then binds substrate. We have solved x-ray structure of ternary complex of truncated Dat (Dat21-230, tDat) with two bound products (CoA and Ac-PEA). The crystal was obtained from co-crystallization of tDat, Ac-CoA, and PEA. Our complex structure indicates that the products of N-acetylation did not release from tDat and still bind to its active site. It prompted us to investigate how the next catalytic reaction begins. Here, we have combined several biophysical methods to study the catalytic cycle of tDat, including X-ray diffraction, isothermal titration calorimetry (ITC) and nuclear magnetic resonance spectroscopy (NMR). Our ITC experiments showed that Ac-CoA could kick out the two bound products of tDat/CoA/Ac-PEA ternary complex and ready to initiate the next catalytic reaction. We have finished NMR backbone assignments of tDat with sequence coverage above 97% completeness. NMR [1H, 15N]-heteronuclear single quantum coherence (HSQC) spectra were employed to monitor the titration behavior of adding Ac-CoA into the ternary complex tDat/CoA/Ac-PEA. When the molar ratio was higher than 1:2 (CoA:Ac-CoA), the HSQC spectrum exhibited no more change and was highly similar to that of the tDat/Ac-CoA binary complex. NMR chemical shift perturbation (CSP) analysis of this Ac-CoA competition experiment revealed that adding Ac-CoA can influence both the binding pockets of CoA and Ac-PEA on ternary form. Most significant resonance changes or broadening appeared at acetyl group binding site and active loop. Our results revealed the essential roles of Ac-CoA in progressing the catalytic cycle of Insect AANATs.

Keywords - Dopamine N-acetyltransferase(Dat), Arylalkylamine N-acetyltransferase(AANAT), Order bi-bi sequential mechanism, TRANSFERASE.