國　立　清　華　大　學

碩 士 論 文

果蠅多巴胺乙醯基轉移酶的基質通道大小對酵素活性之影響

The size effect of substrate-entrance tunnel of Dopamine N-acetyltransferase on its enzyme activity

所　別：分子醫學研究所

學　號：101080569

姓　名：楊淳宬 (Chun-Cheng Yang)

指導教授：呂平江 博士 (Dr. Ping-Chiang Lyu)

彭明德 博士 (Dr. Ming-Der Perng)

中華民國 一百零四年 六月

中文摘要

果蠅的多巴胺乙醯基轉移酶 (Dopamine N-Acetyltransferase, Dat)，是屬於苯烷基胺乙醯基轉移酶家族中的蛋白酶(EC 2.3.1.87, Arylalkylamine N-Acetyltransferase, AANAT)，它們可以催化乙醯輔酶A (Acetyl-CoA) 的乙醯基團轉移至苯烷基胺上，而產出N-乙醯苯烷基胺。近期以來，我們實驗室已經解出多巴胺乙醯基轉移酶晶體、多巴胺乙醯基轉移酶/乙醯輔酶A複合晶體兩者之蛋白質結構。隨後更進一步解出解析度高達1.2Å 的多巴胺乙醯基轉移酶的三元複合晶體 (乙醯基轉移酶/乙醯基輔酶 A/基質) 的蛋白質結構。藉由此三元複合體的結構加上先前實驗室所做的等溫滴定微量熱法實驗，我們發現乙醯基轉移酶需要先結合乙烯基輔酶A，才能夠與基質作用。因此我們發現基質與乙醯基輔酶A進入乙醯基轉移酶的入口是不相同的。在先前的研究，我們實驗室發現M121以及D142 坐落於基質通道的最狹窄處 (通道瓶頸)。當 M121 以及 D142 分別突變成色胺酸，經由酵素動力學實驗發現M121W以及D142W會阻礙基質進入到疏水性的基質結合空腔，進而證實通道的存在。在本實驗中，我們將M121以及D142分別突變成丙氨酸，使得通道瓶頸的直徑變大。另外也將M121以及D142一併突變成色胺酸，使得通道瓶頸變得比個別突變還小。經由酵素動力學的實驗，我們發現讓通道瓶頸變寬的D142A，與wt-Dat有著幾乎相同的活性，且對不同大小的測試基質的選擇性不如wt-Dat顯著。而讓通道瓶頸變更窄的M121WD142W的酵素活性則是有顯著降低的現象，從這結果我們可以發現通道瓶頸的大小確實會影響酵素的活性。然而，M121A 雖然使得通道瓶頸變大，卻造成酵素活性顯著降低。使用LIGPLOT和PyMol進行結構分析後，我們發現M121對基質可以產生S/π作用，所以當M121被改變成丙氨酸時，改變得不單純只是通道瓶頸的大小，而是直接影響基質結合。

Abstract

Drosophila dopamine N-acetyltransferase (Dat, EC 2.3.1.87) is an arylalkylamine N-acetyltransferase (AANAT), which can catalyze acetyl transfer from acetyl-coenzyme A to arylalkylamine, yielding N-acetylarylalkylamine. According to our X-ray structure, a tunnel was inside the protein with entrances at top and bottom. It has also been shown that the substrate located at the middle of Dat, and CoA located at the bottom which seemed to completely block the entrance. Dat exhibits an ordered sequential mechanism, with acetyl-CoA binding first, followed by substrate. Therefore, substrate should access to its binding site through the other entrance, and it looked like a tunnel existed. M121 and D142 located in the narrowest site of the tunnel (tunnel bottleneck). The replacement of these two residues with tryptophan resulted in a decrease in enzyme activity which may imply the hindrance to the substrate entrance by M121W and D142W. In the present study, the size effect of the substrate tunnel was on enzyme activity. We replaced M121 and D142 to alanine individually, which cause the tunnel bottleneck broader. We also made a double mutation, M121WD142W, to make the tunnel bottleneck even narrower than single mutation. The enzyme kinetic studies demonstrated that D142A had almost the same enzyme activity as that of wild type, and M121WD142W showed a significant decrease in the enzyme activity compared to wild type, M121W and D142W. These results confirmed that the size of tunnel bottleneck may affect the substrate specificity again. Although the tunnel bottleneck is broader, M121A exhibits a decrease in enzyme activity. After analysis by LIGPLOT and PyMol, we found that M121 should participate in substrate binding via S/π interactions. Therefore, changing M121 to alanine not only change the size of substrate tunnel bottleneck, but also was expected to significantly decrease the substrate binding.