I.磷酸葡萄糖異構酵素與抑制劑 5PAH 形成複合體之結構及功能研究

II.轉錄活化因子 Jun 之結合蛋白 JAB1 的質體構築、表現、純化及結晶學研究

Structural and Functional Study of PGI Complexed with 5PAH Cloning, Expression, Purification and Crystallographic Study of JAB1

中文摘要

I.磷酸葡萄糖異構酵素與抑制劑 5PAH 形成複合體之結構及功能研究

摘要

磷酸葡萄糖異構酵素(phosphoglucose isomeraes;PGI; E.C 5.3.1.9)為醣解代謝及生醣作用中的重要酵素，其催化六磷酸葡萄糖與六磷酸果醣之間的異構作用。關於 PGI 的晶體結構，包括人類、兔子及枯草桿菌的 PGI 已有高解析度的結構被提出，並提供在分子層次上對於 PGI 作用機制的了解，但對於 PGI 催化區與其受質結合的方位，研究學者卻有相異的證據提出，使得目前對於 PGI 與受質進行催化時所參與作用之胺基酸催化機制仍不清楚。另外，PGI 也被發現具有一些生長因子的特性，如神經白細胞素及癌細胞所分泌的自體分泌因子，因此研究 PGI 與其受質之間的結構將有助於了解 PGI 在代謝反應及細胞生長中所扮演的角色。5PAH (5-phosphospho-D-arabino hydroxamate)為過渡態(transition state)時可能形成的中間產物順式-烯二醇的類似物環，為目前對 PGI 具有抑制效果最大之化合物，其 Ki 值為 1╳10-7 M。本研究的 PGI 源自於枯草菌(bacillus stearothermophilus)，名為 PgiB，其與 5PAH 形成之複合體晶體，經由 X 光繞射實驗，可得解析度為 2.3 埃 的結構資料，PgiB-5PAH 複合體屬於正交晶系 (Orthorhombic) ，空間群(Space group)為 I222。以分子置換法的方式確定 PgiB-5PAH 複合體結構之相位，並建立其晶體結構。分析 PgiB-5PAH 複合體結構及 5PAH 結合位置，提出 PGI 之作用機制，主要是由 Arg202、Glu285 及另一次單位的 His306 參與催化六磷酸葡萄糖與六磷酸果糖之反應。

II.轉錄活化因子 Jun 之結合蛋白 JAB1 的質體構築、表現、純化及結晶學研究

摘要

JAB1 (Jun activating binding protein) 被發現為轉錄活化因子(c-Jun 或 Jun D) 的共同活化因子 (coactivator)，可增強 AP-1 (activating protein 1) 原致癌蛋白質活化基因的表現，與腫瘤的形成與轉化有密切關係。由前人的研究可知 JAB1 與細胞中多種代謝途徑有關，包括植物光調節反應、調控細胞生長週期及調控 DNA 轉錄作用。但目前只有生化上的研究來探討 JAB1 的功能，由於缺乏 JAB1 結構上的資料，因此對於其作用機制仍不清楚。老鼠肌肉之 JAB1 全長為 334 個胺基酸，經刪除 N 端及 C 端不同數目的氨基酸的突變型及野生型 JAB1，嘗試以大腸桿菌大量表現，發現 Jab1 基因在僅含有組織氨基酸標籤(His・Tag) pET-15b 的表現載體上，無法被大腸桿菌之表現系統所表現。將野生型 JAB1(wt)及刪除 N 端 101 個胺基酸之突變型

JAB1(ΔN101)，經基因重新構築於含有NusA標籤 (NusA·Tag)的pET-43.1b表現載體，以固定化鈷金屬親和層析的純化方式，已得到大量高品質的NusA-JAB1融合蛋白質。利用凝血酵素與腸激酵素切割融合蛋白的作用中，並不能得到單獨的JAB1蛋白質。因此以NusA-JAB1融合蛋白進行結晶實驗，目前已觀察到微晶體的形成，正嘗試調整不同的結晶影響因子，期望能盡早獲得高品質的晶體，並解出JAB1高解析度之蛋白質立體結構，以了解JAB1在分子層次內的作用機制，提供治療癌症藥物設計上更多的資訊。

英文摘要

Structural and Functional Study of PGI Complexed with 5PAH

Abstract

Phosphoglucose isomeraes (PGI) catalyzes the reversible isomerization of glucose 6-phosphate to fructose 6-phosphate. The PGI structures of Human、rabbit and Bacillus stearothermophilus have been published at high solution. Those provide evidences to understand the molecular mechanism of PGI. However the data show that the phosphate group of substrates binding to PGI has different orientation. There is unclear the role of residues at catalyze site. In addition, PGI has been show to have functions equivalent to neurolenkin, autocrine motility factor, and maturation factor. Here, we study the X-ray crystal structure of Bacillus stearothermophilus PGI complexed with 5PAH (5-phosphospho-D- arabinohydroxamate), A potent chemo-therapeutic agent that mimics the high energy intermediate product of PGI. 5PAH is also the best inhibitor of the isomerization reaction reported to data with a ki of 1×10-7 M. The PGI-5PAH complex structure has been determinate at 2.3 angstron resolution. It is belong to orthorhombic lattice. The space group is I222. The phase was determinate by molecular replacement method. The position of 5PAH in the enzyme active site predict the residues of Arg202、Glu285、Lys420 and His306 from the another subunit were involved in the PGI reaction proceeds.

Cloning, Expression, Purification and Crystallographic Study of JAB1

Abstract

JAB1 (Jun activating binding protein 1) enhances the transcriptional activity of c-Jun and JunD homodimeric complexes by stabilizing them on their cognate AP-1 (activating protein 1) DNA binding sites. JAB1 is also known as COP9 signalosome subunit 5 (CSN5), which is a component of the COP9 signalosome regulatory complex (CSN). The complex is essential for both plant and animal developments. Recently, JAB1 has been identified to associate with numbers of diverse target proteins that play roles in many cell processes, including the regulation of the JNK-mediated MAP kinase pathway, nuclear hormone signaling, and cell cycle progression. In this study, the JAB1wt and JAB1 mutant (ΔN101) of mouse musculus have been cloned into the NusA-fusion expression vector and overexpressed in

Escherichia coli. The high quantity of the pure wild type JAB1 and its mutant have been obtained using a His-Tag affinity column. But it can't obtain the JAB1 along after the restriction protease digestion. The crystallization trial of both fusion proteins has been initiated by the hanging- and the sitting-drop vapor-diffusion methods. There have microcrystals of JAB1-NusA fusion protein need to modify. The crystal structure of JAB1 and its mutant will provide valuable insight into the molecular mechanism of JAB1 recognition of the target proteins.