利用生物資訊方法來探討白點症病毒之胸腺嘧啶激酶－胸腺嘧啶磷酸激酶

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摘要

蝦白點症是危害養殖業最嚴重的疾病之一，它是由白點症病毒所造成的，會使得養殖生物大量的死亡。自從 1993 年由中國爆發以來，迄今雖已有十多年的時間，但對於此病毒仍不具有足夠的了解，也因此直至今日尚無有效的藥物控制疫情，只能利用防範的方式避免疫情的擴散。因此我們對於白點症病毒需要有更多的了解。

在病毒的複製過程中，胸腺嘧啶激酶與胸腺嘧啶磷酸激酶在核苷酸的生合成中扮演著關鍵性的角色，因此我們希望藉由生物資訊的方法模擬白點症病毒胸腺嘧啶激酶－胸腺嘧啶磷酸激酶的三級結構，而後利用此模擬結構進行化合物的篩選，同時以分子生物技術達成分子選殖與表現白點症病毒胸腺嘧啶激酶－胸腺嘧啶磷酸激酶，並分析其特性。

目前已成功地以人類胸腺嘧啶磷酸激酶(PDB 1NMZ)做為模版，模擬出白點症病毒的胸腺嘧啶磷酸激酶，並利用此結構以及入塢交互作用(DOCK)的方式於化學分子資料庫(MDDR)篩選出可能最適化的五十個化合物。

另一方面，藉由 PCR 技術，我們成功地獲得白點症病毒之胸腺嘧啶激酶－胸腺嘧啶磷酸激酶的基因，並利用重組基因方式與麥芽醣結合蛋白(MBP)產生融合蛋白(fusion protein)，利用大腸桿菌表現系統與親合性管柱層析純化後，得到單一蛋白質分子量約 85 kDa 的融合蛋白，並藉由膠體內切割方式(in gel digestion)結合液相管柱層析與質譜鑑定，確定表現出來的為胸腺嘧啶激酶－胸腺嘧啶磷酸激酶。但所表現之融合蛋白並不具有明顯的酵素活性。

藉由生物資訊以及分子生物技術的結合，我們可以對蛋白質進行結構上的預測、抑制劑的推測，和生化活性的測試，以期發現有效的抑制性藥物。

Bioinformatic approaches to study thymidine kinase and thymidylate kinase of white spot syndrome virus (WSSV TK-TMK)

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Abstract

The crustacean-infected white spot syndrome (WSS) has widely distributed in most Asian countries as well as in the Gulf of Mexico and Southeast USA, where penaeid shrimps were cultured. It is an economically significant shrimp disease, which causes high shrimp mortalities and severe damage to shrimp cultures. The disease is caused by a virus called white spot syndrome virus (WSSV).

A goal to kill white spot syndrome virus is to exploit a biochemical difference between the infected virus and the host tissue in order to interfere selectively with the infected process. Thymidine kinase and thymidylate kinase play crucial roles in pyrimidine salvage pathway and fulfill the requirement for developing virus specific chemotherapeutic agents. In this study, bioinformatic strategies include homology simulation and ligand docking, using the human thymidinylate kinase (PDB code: 1NMZ) as template, have been successfully applied to construct WSSV-TMK homology model and utilized for screening fitness compounds for antiviral drugs application. Fifty compounds were sifted out from the MDDR and CMC databases, respectively, based on the GOLD docking program.

In parallel, the WSSV-*tktmk* gene has been successfully amplified from the virus genome, over-expressed as a fusion to the maltose-binding protein (MBP), and purified by amylase affinity column as a ~85 kDa MBP-TK-TMK fusion protein. The peptide mapping coupled with tandem mass spectrometric determination identified nine peptides, which correspond to the WSSV-TK-TMK protein, thus confirmed its identity. However, the purified TK-TMK showed little enzymatic activity toward its native substrate, deoxythymidine or thymidine monophosphate.

Thus, in combination of bioinformatics and molecular biology approaches will enable us to predict protein structure, perform ligand docking for virtual inhibitor screening, analyze the enzymatic activity of the target protein, and finally reach the goal for discovery of efficacious inhibitor of WSSV TK-TMK.