行政院國家科學委員會補助專題研究計畫期中報告

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※ 利用試管篩選法找出與化學或生物分子具專一性鍵結之核酸(1/2) ※
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計畫類別：個別型計畫

計畫編號：NSC－91- 2113 - M- 032 - 007

執行期間： 91 年 8 月 1 日 至 92 年 7 月 31 日

計畫主持人：鄭建中

執行單位：淡江大學化學系

中 華 民 國 九 十 二 年 五 月 六 日

行政院國家科學委員會專題研究計畫期中報告

利用試管篩選法找出能與化學分子或生物分子具專一性鍵結之核酸(1/2)

In vitro selection of DNA/RNA specifically binding with chemicals and/or bio-molecules

計畫編號：NSC 91-2113-M-032-007

執行期限：91 年 8 月 1 日至 92 年 7 月 31 日

主持人：鄭建中 副教授 淡江大學化學系

一、中文摘要

核酸，在疾病診斷及治療中逐漸扮演越來越重要的角色。利用核酸的序列組合及三度空間結構，可提供出一個具有高度選擇性及專一辨識性的生物分子藥物庫。本兩年的計畫即是針對此一未來的需求，希冀發展出快速有效地方法，找出在與化學或生物分子作用時，所產生相對應的高專一性的核酸分子 aptamers。初步的目標物為唾液酸及其他的單糖分子及酪胺酸酵素為主要測試對象。利用數以億萬計的任意序列核酸分子，在與目標物作用後，篩選出其所對應之專一的 DNA 序列。藉由固定目標物在非移動相的樹酯上，與任意序列之 DNA 作用後，再進行分離及純化。所得出微量的 DNA 產物，將利用聚合酵素連鎖反應放大到所需的量後；進而分析出該核酸分子的序列及其結構。所對應之 RNA，則可以 DNA 為模版，利用 RNA 聚合酵素，製造出所對應之 RNA。

關鍵詞：試管篩選法，唾液酸，酪胺酸酵素，核酸，聚合酵素連鎖反應

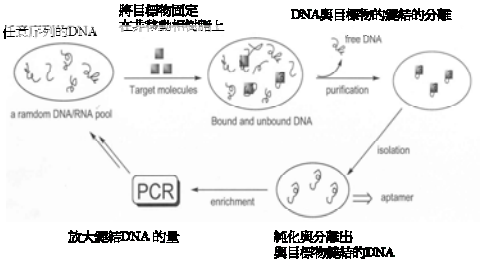
Abstract

Nucleic Acids are getting important in the development of therapy and/or diagnostic purposes. Since nucleic acids are capable of providing the specific affinity toward target molecules through their sequence and tertiary structures, it will be an excellent candidate for “novel” reagents for the clinical purposes. The basic concept of in vitro selection is that a large population (~10^{14}) of random DNA/RNA sequence is targeted against a ligand to generate highly specific recognition of DNA/RNA sequence. The target molecules such as sialic acid and tyrosinase were immobilized in the solid support. DNA pools containing certain random DNA nucleotides were reacted with the chemical-modified solid support. After proper purification and PCR enrichment, the corresponding DNA sequence will be isolated with high specificity and optimal DNA affinity to the target molecules. The corresponding RNA can be prepared by the transcription by RNA polymerase.

Keywords: in vitro selection, aptamer, selex, sialic acid,tyrosinase, helical complex

二、緣由與目的

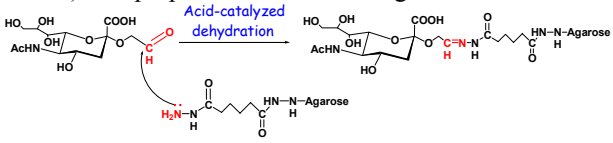
Nucleic acids are getting important in the development of novel drugs for the therapeutic and diagnostic purposes. In 1990, Tuerk and Gold released the information regarding to the selection of a specific DNA to the targeted molecule from a random pools. It provides a new consideration in the development of DNA therapy. Besides many challenging points, many chemical companies are reluctant to release their study of DNA sequence due to the future marketing profile. The basic concept of in vitro selection is that a large population (~10^{14}) of random DNA/RNA sequence is targeted against a ligand to generate highly specific recognition of DNA/RNA sequence. Since nucleic acids are capable of providing the specific affinity toward target molecules through their sequence and tertiary structures, it will be an excellent candidate for “novel” reagents for the clinical purposes. Aptamer represents the nucleic acids is the final product generated from in vitro selection. SELEX (or in vitro selection) is stand for the systematic evolution of ligand by exponential enrichment.



In the study of protein-DNA interaction, although the protein is expressed as the final determination product in biological systems, the identification of proteins is tedious and exhausting process. Thus, the use of DNA chips to have specific recognition and high affinity is the goals for the most researchers. Here, we attempt to use DNA and/or RNA as the binding materials instead of chemicals or protein such as antibody to fish out specifically the target protein.

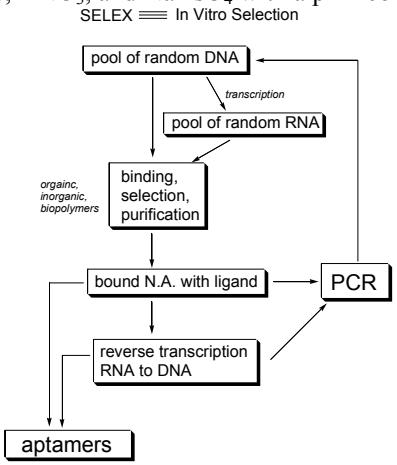
三、結果與討論

The target molecules were examined using sialic acids and tyrosinase. In order to immobilize to the solid support, the modified sialic acid (provided by Prof. Lin at the Institute of Chemistry, Academia Sinica) was prepared as the following scheme.



The first thing is to immobilize the target molecule to the solid support. The attachment of sialic acid to solid supports was accomplished by the coupling

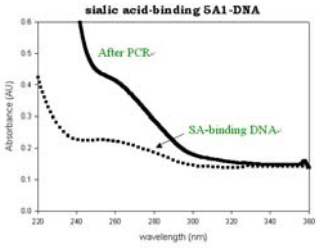
reaction of sialic acid derivative and hydrazine-modified resin at room temperature for 24 hr. After thoroughly washing with methanol and water. The bound sialic acid to solid support was confirmed by the Warren assay in a solution of periodate, HNO_3, and $NaAsO_4$ with a pink color.



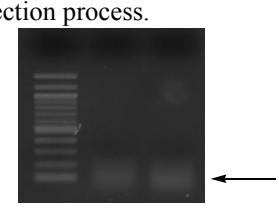
試管篩選法的流程示意圖

The modified resin is further subjected to the reaction with a DNA pool containing numbers of DNA sequence in 3 mM solution. The template DNA sequence was designed as the list. 5'-AGC-GCT-$(N)_{30}$-TAG-CGC-GTA-TAG-TGA-GTG-CTA-TTA-3'. The DNA primer for PCR is prepared as the following list : 5'-TAA-TAG-CAC-TCA-CTA-TA-3'. Since DNA has the unique UV absorption at 260 nm, the bound DNA and modified solid support is monitored by the measurement of UV absorption at 260 nm. After stirring overnight, the unbound DNA was eluted through a column with water several times until no UV absorption at 260 nm. Then, the high concentration solution of NaCl was used to elute the bound DNA from the solid support.

The trace amount of DNA was allowed to react with the primer to undergo PCR enlargement. The selection result showed the selection result and PCR enhancement. Thus, we have demonstrated our capability in this DNA selection.



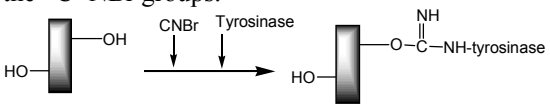
Furthermore, one may argue the possibility the side chain of the resins or resins itself may bind with DNA to induce such effect. In fact, the control reaction without the sialic acid was done at the same time. However, no similar result was observed. It suggests that the side chain of resins and itself did not affect the DNA selection process.



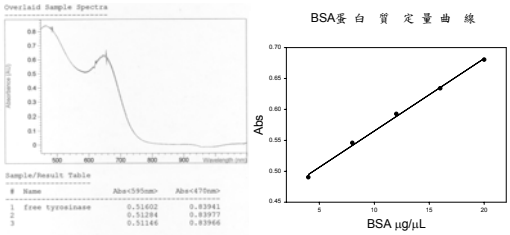
與唾液酸作用篩選後，利用 PCR 放大 DNA 的量，在瓊膠電泳的所呈現 DNA 位置。

II. Interaction of tyrosinase and DNA

Under the similar condition, the selection of tyrosinase was achieved. The immobilized tyrosinase was accomplished by using CNBr-activated Sepharose in aqueous solution. The amino groups of tyrosinase undergo the coupling reaction with the –C=NBr groups.

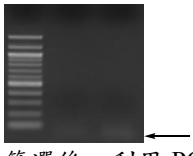


The identification of the interaction of tyrosinase and DNA was used by the Bradford Assay using coomassie blue dye. After the proper treatment to remove DNA from the solid support, the bound DNA shown the characteristic UV absorption at 260 nm.



而在 Tyrosinase 定量實驗中，根據 BSA 標準曲線方程式，將 Tyrosinasey 在 595nm 的 UV 吸收值代入，以內插法求得 Tyrosinase 的濃度為 5.5931 μ g/mL。

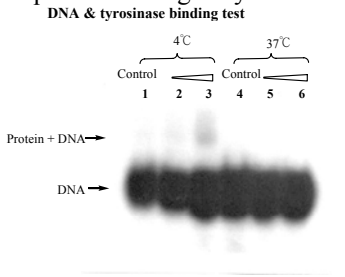
Furthermore, the binding affinity of tyrosine with DNA was confirmed by gel-mobility assay. The result shows that the reaction temperature (4 and 37°C) is important to the stability of the protein-DNA adduct. As well, the buffer and salt concentration may influence the binding strength in protein-DNA interaction.



與 tyrosinase 作用經篩選後，利用 PCR 放大 DNA 的量，在瓊膠電泳的所呈現 DNA 位置。

Since DNA is a random DNA sequence with 30 unknown nucleotides, the concentration of the binding DNA sequence will be extremely low to the overall DNA concentration of 4^{30}. That is a possible reason the binding affinity in gel mobility assay is quite low. However, it is a positive result for the specific DNA

sequence binding of tyrosinase.



目前已完成如上圖所示的流程，共計三次。因為根據統計至少要重複三次以上，才能篩選到較具專一性的 DNA 序列。但每重複一次的量相當少外，還有許多實驗技術尚待克服。目前結果相當樂觀，也發展到一些新的分離方法。因此，除了最後 DNA 序列及其結構鑑定外，研究 DNA-protein 及 DNA-substrates interaction 將會是下一重點之一。

四、計畫成果自評

相符程度：約完成 88%之原計畫。

預期目標：達成 90%之預期目標及其他。

學術及應用價值：本計畫的開發，從生物技術的改良、到研究物質與 DNA 鍵結、蛋白質與 DNA 作用、到碳水化合物的辨識性，不僅具有相當潛力的學術價值，亦具有相當重要的商業價值，可發展生物晶片，生物藥物，DNA 藥物等重要商機。

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