行政院國家科學委員會補助專題研究計畫成果報告

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※利用鈷錯合物研究 RNA 分枝狀的結構及其生物上之功能(2/2)※
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計畫類別：個別型計畫
計畫編號：NSC－90－2113－M－032－026
執行期間： 90 年 8 月 1 日 至 91 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 國九十一年十月 十六日

行政院國家科學委員會專題研究計畫成果報告

利用鈷錯合物研究 RNA 分枝狀的結構及其生物上之功能(2/2)

Exploration of branched RNA structures and their biological functions using $Co(HAPP)^{2+}$ complex

計畫編號：NSC 90-2113-M-032-026
執行期限：90 年 8 月 1 日至 91 年 7 月 31 日

主持人：鄭建中 副教授 淡江化學系

一、中文摘要

RNA 結構的鑑定，為生物研究上一具挑戰性的課題。特別是那些有分枝狀的 RNA 結構，通常能夠決定其 RNA 的生物功能。因為 RNA 的結構的研究不僅是受限於高難度操作，加上缺乏適當的結構分析探針，造成 RNA 結構之鑑定，為一相當挑戰之研究課題。本實驗室發現的鈷錯合物不僅能有效偵測突狀 DNA 結構，亦能偵測 RNA 突狀結構。此錯合物有可能是目前在文獻上唯一能直接測到 RNA 突狀結構的金屬探針。在第二年計劃中，已完成的有下列三項項目：研究不同大小結構之 RNA 突狀結構，不同金屬錯合物對這些分枝狀 RNA 結構的作用；研究鎚形 ribozyme；及 internal loop 結構。本計畫的結果將會對研究 RNA 突狀結構的專一選擇性及其相關生物功能具有重要的地位。

關鍵詞：突狀、ribozyme、DNA 斷裂、鈷錯合物

Abstract

The determination of RNA structure is one of most challenge topics in biological systems. These branched RNA structures usually are capable of dominating RNA functionality. The difficult handle problem in RNA and the lack of proper tool make these complicated RNA structures appear to be untouchable. We have demonstrated that a novel octahedral $Co^{II}(HAPP)(TFA)_2$ is sensitive to the shape/size of conformation in DNA/RNA bulges, which is *the first example ever to resolve directly in RNA bulge and internal loop conformation.*

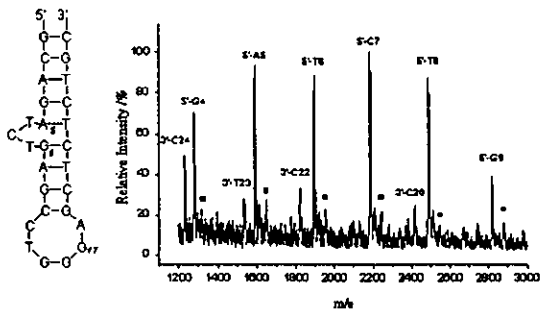
Keywords: cobalt, bulge RNA, internal loop, branch

二、緣由與目的

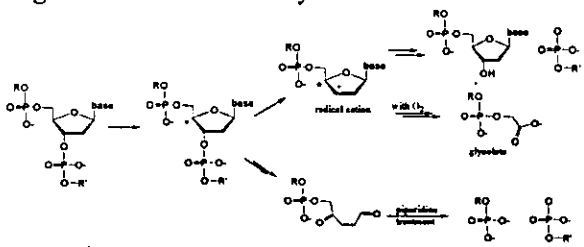
Branched structures are crucial motifs in nucleic acids chemistry in the formation of tertiary structures and the recognition of nucleic acid-binding-proteins in biological systems, particularly in RNA. Among these branched structures, internal loop and bulge conformation usually play an important role in determining the geometrical construction of tertiary structures of RNA and their biological functions. For instance, the internal loop and bulge structures in ribozymes are found to in charge of the metal binding domain as the result to splice RNA. In order to obtain the interaction, the X-ray crystal structures analysis, NMR studies, and FRET approach are capable of providing the further structural information. An alternative way to rapidly reveal the prelimarily information of these branched structures is to use chemical probes. Owing to the existence of 2'-OH group, the recognition of chemical probes frequently show the a little bit of differences between DNA and RNA structural analysis. Many wonderful chemical nucleases have been reported to nick the specific DNA and RNA sequences or nucleotides. These chemical probes have the obscurity in distinguishing the variety of single-stranded regions such as the unobstructed single-stranded sites, bulge, hairpin loop, and internal loop in RNA structure,. Lately, a novel octahedral Co^{II} complex, $Co^{II}(HAPP)(TFA)_2$ has been reported to serve as a specific probe for DNA bulges. This complex also has been demonstrated to have the low affinity towards double-stranded DNA as well as single-stranded DNA. Therefore, it is important to show this cobalt macrocyclic complex is capable of processes the highly specificity in recognition of RNA structures.

三、結果與討論

The 4'-H sugar oxidation pathway induced by $CoHAPP^{2+}/H_2O_2$ is further confirmed by the analysis of DNA cleavage fragments in MALDI-TOF (MALDI, matrix-assisted laser desorption and ionization；TOF, time-of-flight) mass spectrometry. The MALDI-TOF-MS analysis is known to offer an efficient and accurate tool for the unambiguous characterization of DNA cleavage products. The assignments and *m/e* values of possible HIV-DNA cleavage products and their expected m/e values were listed in supplementary data. Indeed, MALDI-TOF mass spectrum exhibited a higher resolving ability in the determination of several DNA fragment ion series compared to the gel electrophoresis method. The dominant scission products were attributed to the cleavage at the deoxyribose 3'-phosphate bond, resulting in fragments of 5'-NNN-3'-PO_4 in different lengths. The relative fragment distribution agrees well with the gel electrophoresis result, and clearly demonstrates that fragmentation resulting from cleavage at bulge position of A5, T6, C7, T8 and G9 is a major pathway in the reaction.

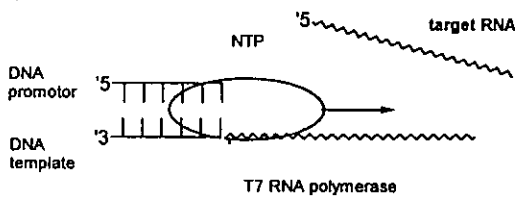


Furthermore, peaks marked with the filled squares (■) in **Fig. 1** having m/e values 58 higher than the corresponding dominant 5'-NNN-3'-PO_4 fragments. In comparison to DNA cleavage products, the observed extra fragments with molecular weight 58 are most likely due to the formation of glycolate ($-CH_2CO_2$) species. These results have been repeated three times at least. Therefore, the cleavage reaction of $CoHAPP^{2+}/H_2O_2$ with HIV-DNA is evidently disclosed through the sugar oxidation pathway at the 4'-H position based on the identification of glycolate fragments (5'-N-3'PO_4-CH_2CO_2) in **Fig.2.** In fact, the formation of glycolate has been demonstrated as a 4'H sugar oxidation product in the cleavage of oligonucleotide mediated by 4'H-radical.

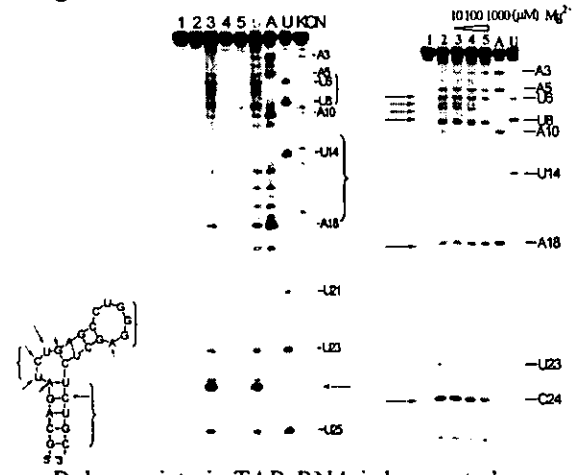


Since bulge moiety is one of the most common but important branched structures in nucleic acids, the understanding of cleavage pathway of bulge-specific chemical nuclease, $Co^{II}(HAPP)(TFA)_2$, may provide the significant structural information in the study of the complicated nucleic acids structures and their biological functions.

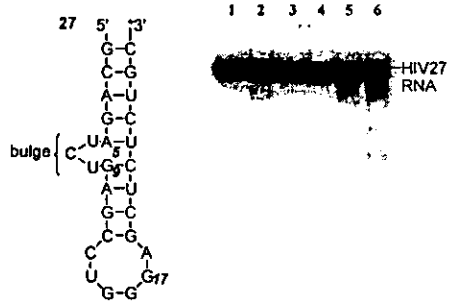
Bulge-Specific RNA Cleavage. The presence of branched moieties such as bulge, internal loop, and helical junctions in RNA arise more complicate conformations in the study of RNA secondary and tertiary structures. The cleavage in RNA structures commonly is correlated to the accessibility of their conformation as a result to provide the structural information. The exploration of RNA structures and the biological functions thereof is of great interest to many chemists as well as biologists. Therefore, it will be of great interest to explore the bulge specific recognition in RNA using $CoHAPP^{2+}$. A variety of modified TAR-RNA was used as the targeted RNA in the study of the interaction with $CoHAPP^{2+}$.



The target RNAs were prepared by *in vitro* transcription using DNA promoter, various DNA templates, and T7 RNA polymerase. The reaction of target RNA and Co^{II} complexes were performed under the similar condition in reaction with DNA as mention previously. In the absence of aniline treatment, the major cleavage sites were found to at U6, C7, U8, which is at the bulge site, and C24. The slightly cleavage sites were at A5 and A18.



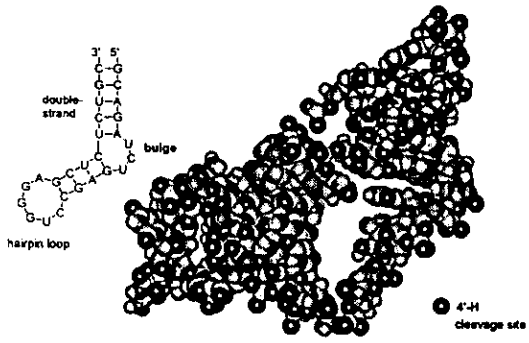
Bulge moiety in TAR-RNA is known to be flexible to have the conformational change upon the addition of binding ligands such as *tat*-peptide, argininamide, Mg^{2+}, and Ca^{2+} ions revealed by NMR and X-ray crystallography studies. In the absence of ligands, the bulge structure is capable of inducing the bending of the helical conformation around 93° in TAR-RNA, measured by gel electrophoresis. In the presence of external ligands, the interaction between the ligand and nucleobase at the bulge conformation triggers the bulge conformational change in order to overcome the steric hindered environment. As a result, the bending angle in TAR-RNA helixes is decreased and the TAR-RNA helical structure is changed to more like co-axial conformation.



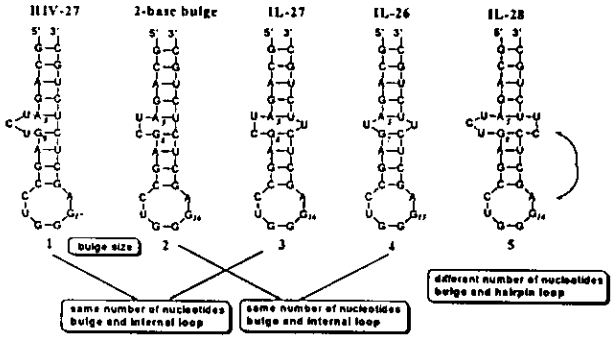
Since the bulge conformation in TAR-RNA is sensitive to the additional binding ligands, RNA conformational change upon the addition of $CoHAPP^{2+}$ complex was examined by using the non-denaturing gel electrophoresis at 4°C. The induction of nucleic acids conformational change commonly required either by a strong binding mode or by a high concentration of ligands. It suggests that $CoHAPP^{2+}$ is sensitive to the conformational change at the bulged RNA structure.

Unlike the unstable conformation of 3-base bulge structures in DNA, the NMR study has been revealed TAR-RNA structure and its derivatives. When the

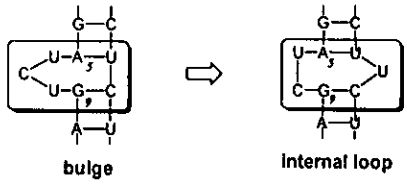
structure is viewed from the minor groove, the formation of the pocket is obviously observed in the 3-base bulge of TAR-RNA structure. Based on the mechanism study in DNA, CoHAPP^{2+} displayed the preference of sugar oxidation at the 4'-H position at the ribose-sugar moiety. Therefore, the cleavage sites of CoHAPP^{2+} in TAR-RNA are allowed to superimpose with the NMR structures of TAR-RNA. As a result, the possible explanation for the bulge-specific recognition by CoHAPP^{2+} in the RNA bulge pocket was considered to be an octahedral conformation of metal complex, which may provide the nucleobase-stacking effect by 1,10-phenanthroline ligand but the presence of the axial ligands prohibited the deeply intercalation.



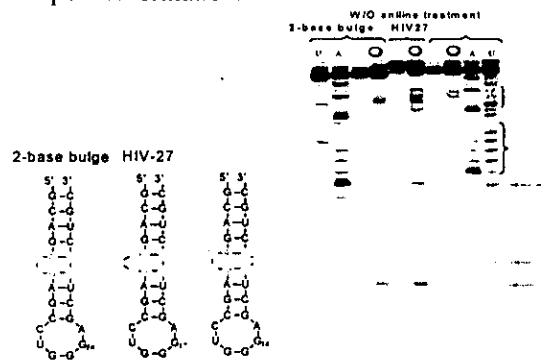
According to the above result of bulge-specific binding, the opposite site of bulge region has less effect in the specific recognition. However, it showed the cleavage site at C24 in TAR RNA suggesting the orientation of this residue may be close to the hydroxyl radical reaction center. In order to confirm the hypothesis, a modified TAR-RNA with an asymmetric internal loop was designed and prepared.



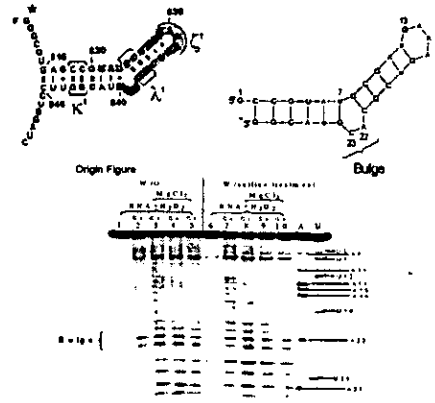
As a result, CoHAPP^{2+} still recognize specifically at the internal loop region and insensitive to the hairpin loop.



The cleavage at the C24 residue was suppressed in reaction with internal loop of RNA using CoHAPP^{2+} in the presence of H_2O_2. The major cleavage site is still at the bulge site (U6 and C7). However, when the internal loop is the mismatch C-U base-paired, no cleavage was observed. The mismatch base-paired is known to have the nucleobase-in interaction without the pocket formation.



It suggests that the asymmetry internal loop (N>1) may form a pocket-like conformation instead of the random linear form. The internal loop structure is one of most common structures in RNAs. Therefore, using the recognition in the RNA pocket, CoHAPP^{2+} complex has been demonstrated to extend its capability not only in RNA bulge-specific recognition but also in RNA internal loop structures.



Summary

Since bulge moiety and internal loop are the most common but important branched structures in nucleic acids, particular in RNA, this bulge-specific chemical nuclease, $Co^{II}(HAPP)(TFA)_2$, may provide the significant but preliminary information in branched RNA structures.

四、計畫成果自評

相符程度：約完成 85%之原計畫。

預期目標：達成 85%之預期目標及其他。

學術及應用價值：

The use of unambiguous structure of TAR-RNA and its analogs, $Co^{II}(HAPP)^{2+}$ was found to be a bulge-specific probe in RNA and insensitive to the hairpin loop region in the analysis of RNA cleavage fragments,. This result may lead to be the first look in the directly revealing the orientation of RNA branched structure structures such as bulges and internal loops.

Up to date, there is no proper tool to examine the variety of single-stranded regions in the secondary and tertiary RNA structures. However, these branched moieties are the most common but important structures in nucleic acids, particular in RNA, this specific chemical nuclease may provide the significant but preliminary information in probing unusual, huge, and unsolved RNA structures.

期刊發表：研究結果已投稿一篇，另一篇正進行中。

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第三十五屆國際配位化學會議會後報告

淡江大學化學系 副教授 鄭建中

第三十五屆國際配位化學會議(35th International Conference on Coordination Chemistry)於 2002 年 7 月 19 日至 26 日在德國的海德堡(Heidelberg，Germany)召開，由德國的海德堡大學及德國的化學學會主辦，會期共六天，共有 59 個國家，參加者學者共約一千二百多人參加，我國有 11 位化學人士前往參加。本次會議分有六個主題，例如：生物無機化學（bioinorganic chemistry），金屬藥物(metal in medicine)，金屬催化劑(metal in catalysis)，沃納型化學(Werner type complexes），超分子配位化學（Supramolecular coordination chemistry），和材料及奈米化學（materials and nanochemistry）。大會除安排 9 場重要知名人士的大會演講外，另有 216 場分散在各個主題的邀請演講，為各個領域的研究結果之發表會。其他則一律為壁報論文，共約有一千多篇以上壁報論文發表，規模頗大。節錄一些在本次會議中較有心得及較值的注意的演講如下所述。

首先大會頒發 Wilkinson Award 給德國的 Achim Muller 教授，他利用了 368 個鉬金屬建構出檸檬形狀的立體結構分子，作為奈米材料的研發用。雖然，有機分子已有利用 C60 為巨大分子開發出許多新的化學，但無機方面的材料則是更有無窮的潛力。原因是金屬具有氧化還原能力；不同配位數目及結構的特色，造成利用金屬所建構的分子材料及奈米結構更具有多樣性。

以色列的 I. Willner 教授則是利用金屬的配位化學做出模擬光化學作用的酵素。利用蛋白質工程技術，固定相的矽膠，電子轉移錯合物，及金粒子材料的特色，不僅模擬植物光合作用製造化學分子外，亦測試將水分子轉化成氧氣的可行性，及發展出可感光的人工眼珠等等，使金屬配位化學更具有解決自然科學問題的能力。

另外，德國的 P. Fromme 教授對植物產生光合作用的分子結構，做了相當

精闢的分析。不僅是得到照光系統的晶體，甚至有至外太空零重力下，所製造出來的晶體，來做研究及解析，對提供未來新的能源開發，光電池研發，及利用光能而產生氧氣的主題，都具有相當的前瞻性。

日本的 M. Fujita 教授更是利用金屬的配位化學來作分子建築。利用鉑金的 cis 結構，當作是銜接器，組合一些基本的配位基，而呈現出結構相當特殊的超巨大分子。接著利用分子 panneling 的特性，合成出一個三角平版，再利用自我組裝的方法，製造出配位性的奈米管（coodination nanotubes）及碗型（bowls）及球型結構（sphere）結構。同時也發現在這些孔洞下，即所謂的 molecular flask（分子燒瓶），所做的化學反應，如照光反應，或 Diel-Alders 反應的產物都很特別，不僅增加立體選擇性，產物的產率，甚至一些鈍性的化學加成反應都會發生。

此次會議已逐漸反應出目前世界潮流趨勢，即將利用金屬錯合物的配位特色，除了使用在生命科學外，最近也開始營建出分子材料，新型的奈米結構，金屬液晶材料等，應用到日常生活中。再者，世界各國對新生代的化學家給於相當的提攜及教育，尤其是在大學部及研究生方面，更是不遺餘力。在邀請的演講中，許多新生代的的化學家更是力爭上游的一直冒出。對他們而言，身為一個化學系學生為榮，因為他們瞭解化學家創造了許多目前重要的事物從解開生命分子作用到為奈米材料的開發，是在創造時代，開創新世界。在台灣，大部分的人則是認為化學系不知未來能做什麼的冷門科系，因此所得的結果就不相同。事實上，不曉得是否是一種巧合，化學研發能力強弱，正好是一個國家是否強大的重要指標之一。在新世紀中，由於化學，逐漸與材料及生物的融合，造成給下一代新生的化學家相當大的壓力及知識膨脹的包袱。因此，如何從中學及大學培養一批新時代的化學家，使其早些接觸尖端科學，及培養其對科學的興趣，將會對台灣的在發展新的材料及生物技術會有很大的助益，