D 型肝炎病毒抗原與核酸分子交互作用之研究

Studies of the Interaction of Hepatitis Delta Antigen and Nucleic Acids

中文摘要

NdAg 與 N7 分別爲包含 D 型肝炎病毒抗原（HdAg）N 端起始第 1 至第 88 個與第 24 至第 75 個胺基酸之蛋白。由過去的研究顯示，NdAg 與 N7 皆可與各式核醣核酸結合形成複合體，並具備了核醣核酸監護子的活性，即能防止 RNA 摺疊成錯誤結構或幫助 RNA 解開不正確的結構，惟 N7 之核醣核酸監護子的活性較差。本研究欲分別探討 NdAg 及 N7 兩蛋白與核醣核酸的交互作用是否具序列及結構的特異性（sequence or structural specificity）、一分子蛋白之結合區的長度爲何、以及兩蛋白與核醣核酸的交互作用是否爲協同作用（cooperativity），進而分析比較此二者與各式核醣核酸作用的方式之異同。本研究以 filter binding assay 測定蛋白與核醣核酸之結合能力。由結果得知 NdAg 可與各式核醣核酸交互作用，並且與較長之核醣核酸結合能力較佳。NdAg 與各式核酸之結合力均優於 N7，但是，N7 與核酸之結合反應協同性較高。另一方面，分析不同長度 rU 及 dT 與 NdAg 及 N7 結合的情形，我們得知 NdAg 或 N7 之核酸結合區分別爲 8 nt-10 nt 及 6 nt-8 nt。最後，我們在不同濃度 NaCl 進行結合實驗發現，N7 蛋白與核酸結合時，正負電荷間之交互作用大於 NdAg 蛋白。

英文摘要

NdAg and N7 proteins contain the amino acids ＃1-88 and ＃24-75 of hepatitis delta antigen (HdAg), respectively. NdAg and N7 form complexes with a variety of nucleic acids. In addition, they promote the unfolding and refolding of RNA molecules by acting as a RNA chaperone, but the RNA chaperone activity of N7 is lower than that of NdAg. In this study, I used filter binding assay to characterize the nucleic acid binding properties of NdAg and N7. The structural and sequence requirements of nucleic acid molecule, the binding site size of each protein, and the cooperativity of the binding reaction were analyzed. I found that the interaction between NdAg and nucleic acid did not have sequence and structural sepecificity, but NdAg bound longer RNA with higher affinity. NdAg bound nucleic acids with higher binding affinity than that of N7, but the nucleic acid binding of N7 was more cooperative. In addition, I estimated the binding site size of ndAg and N7 proteins by determining the binding affinity of each protein to different sizes rU or dT. The results showed that the binding site of NdAg and N7 were 8 nt-10 nt and 6 nt-8 nt, respectively. Finally, the results of the salt-dependent binding experiments showed that the binding of N7 to nucleic acids was more sensitive to the elevation of ionic strength than the binding of NdAg to

nucleic acids.