D 型肝炎病毒抗原蛋白胺端區域之核酸監護子活性的研究

The study of the nucleic acid chaperone activity of the N-terminal domain of hepatitis delta antigen

中文摘要

D 型肝炎病毒抗原蛋白的胺端 1 到 88 個胺基酸(簡稱爲 NdAg)具有核酸堅護子的活性，我的研究在探討 NdAg 在執行核酸堅護子活性時的機制。我利用 melting temperature (Tm) analysis 分析以化學法合成 DNA oligomers 所形成互補序列間的穩定度，再選取特定的 DNA oligomers 進行核酸監護子的活性分析。我發現 NdAg 能選擇性地促使較高和較低 Tm 值範圍的 DNA oligomers 形成較穩定的雙股，另外 NdAg 對不同濃度的 DNA oligomers 都具有類似的活性。NdAg 亦能促使股交換反應，讓可和底股形成雙股的頂股取代原較不穩定雙股的頂股。然而 NdAg 執行股交換反應時並沒有方相性。

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.