D 型肝炎病毒抗原 N 端區域與核酸分子交互作用之研究

Studies of Interaction between the N-terminal Domain of Hepatitis Delta Antigen and RNA

中文摘要

NdAg 爲含 D 型肝炎病毒抗原第 1 至第 88 個胺基酸之蛋白，其可與各式核糖核酸結合，並具有核醣核酸監護子的活性，可協助各式核醣核酸摺疊成新的構型。NdAg 序列第 2 至第 27 個胺基酸可與 HDV RNA 結合，第 13 至 48 個胺基酸含 leucine-zipper like 序列，另第 67 至 88 個胺基酸爲核位訊息。本研究欲深入了解 NdAg 上各個區域對蛋白結構、RNA 結合能力及 RNA 監護子活性的重要性。我們構築各式 NdAg 蛋白的突變體利用雙旋光分光光譜 (CD) 分析蛋白結構，再利用 filter binding assay 偵測蛋白的 RNA 結合力，同時由蛋白促進 hammerhead ribozyme 它割反應的能力評估其 RNA 監護子活性。NdAg 具有 a-螺旋結構，刪除 NdAg 第 2 至第 13 或第 60 至第 88 個胺基酸，對此結構無影響。但若於 leucine-zipper like序列上刪除第14至第23或第35至第43個胺基酸，或於第27-28 個胺基酸間插入 2 個 alanine，均會使 a-螺旋結構消失。故可知 leucine-zipper like 序列對 NdAg 之結構十分重要。另經由 RNA 結合力的測試結果可知，刪除第 2 至第 13 個胺基酸會使 RNA 結合力大幅下降，另 leucine-zipper like 序列之刪除或插入突變亦會降低 RNA 結合力，刪除第 35 至第 43 個胺基酸對結合力影響最巨。蛋白 RNA 結合力的強弱與其 RNA 監護子活性的高低是呈正相關的。RNA 結合力低之突變體亦可促進 hammerhead ribozyme 它割反應，唯需較高濃度的蛋白。最後，經由沉澱實驗得知，NdAg 與 RNA 結合後可形成巨大複合物。

英文摘要

NdAg contains the first 88 amino acids of hepatitis delta antigen (HdAg). NdAg interacts with a variety of RNAs, in addition, it facilitates RNA structural rearrangement by acting as an RNA chaperone. NdAg contains HdAg's cryptic RNA binding domains (aa 2-27), leucine-zipper like sequence (aa 13-48), and nuclear localization signals (aa 67-88). In this thesis, I constructed NdAg mutants for investigating the role of different structural/functional domains on the structure, the RNA binding activity, and the RNA chaperone activity of NdAg. The a-helical structure of NdAg was not affected when aa 2-13 or aa 60-88 were deleted. However, the deletions of aa 14-23 and aa 35-43, as well as the insertion of two alanine residues to aa 27-28 disrupted the a-helical structure. These findings confirm the importance of the leucine-zipper like sequence for the structure of NdAg. The deletion of the N terminal cryptic RNA binding domain, as well as insertion and deletion mutations in the leucine-zipper like sequence decreased the RNA binding activity of NdAg. NdAg

and all of its mutants constructed in the study promoted the assembly of the hammerhead catalytic domain between a trans-acting hammerhead ribozyme and its cognate substrate, but the RNA binding activity and the stimulatory activity of NdAg and its mutants are correlated. The formation of the RNA-protein complexes seems to be a prerequisite for the stimulatory activity on hammerhead ribozyme catalysis of NdAg and its mutants, and the sedimentation experiment suggests that there are more than one form of NRA-NdAg complexes.