

D 型肝炎病毒抗原 N 端區的結構與其核酸結合性質之研究

Studies of structure and nucleic acid binding properties of the N-terminal domain of hepatitis delta antigen

中文摘要

含 D 型肝炎病毒抗原 N 端第 1 至第 88 個氨基酸的蛋白稱為 NdAg。NdAg 含極多鹼性氨基酸，其具極強核酸結合力。NdAg 具 leucine zipper-like 序列，其極有可能形成 coiled-coil 結構。NdAg 亦具核糖核酸監護子的活性，其可協助各式核糖核酸摺疊成新的構型。本研究擬對 NdAg 的結構以及 NdAg 與和核酸交互作用後蛋白或核酸分子結構之變化作深入之探討，以期了解 NdAg 蛋白之結構與功能。本研究發現 NdAg 蛋白中 D 型肝炎病毒抗原第 1 至第 13 個氨基酸序列被刪除、第 1 至第 23 個氨基酸序列被刪除或 leucine zipper-like 序列內氨基酸發生插入或置換突變，均使 NdAg 蛋白的結構發生變化或令 NdAg 蛋白喪失其 α -螺旋結構。然而這些突變蛋白仍具核酸結合能力及核酸監護子活性。含 D 型肝炎病毒抗原第 24 至第 75 個氨基酸序列之蛋白即 N7 蛋白雖不具 α -螺旋結構，但當 N7 蛋白與核酸交互作用後， α -螺旋結構可被誘發。ac2450am 被核酸誘發出 α -螺旋結構的能力較 N7 蛋白差，故 D 型肝炎病毒抗原的第 50 至第 75 個氨基酸對於 NdAg 蛋白與核酸分子之交互作用應該十分重要。由 CD 光譜之變化及波長 260 nm 吸光值之增強可知，NdAg 蛋白可改變核酸分子之結構，我們並以電子顯微鏡觀察到 NdAg 蛋白可使雙股核酸分子產生 bubble 或枝鏈，此外 NdAg 蛋白亦可讓 supercoiled form 之質體轉型為 relaxed form。上述這些性質可能與 NdAg 蛋白之核酸監護子的功能有關。

英文摘要

NdAg is a fusion protein containing the first 88 amino acids of hepatitis delt

a antigen. NdAg is rich in basic amino acids, it binds nucleic acid molecules with broad specificity. NdAg contains leucine-zipper like sequence that may be involved in the formation of coiled-coil. NdAg is an RNA chaperone, it promotes the unfolding and refolding of RNA molecules. The aim of this thesis is to investigate the structure and function relationship of NdAg. The deletion of the first 13 amino acids or the first 23 amino acids of hepatitis delta antigen from NdAg together with the introduction of insertion or substitution mutation(s) to the leucine-zipper like sequence of NdAg destabilized or abolished the α -helical structure of NdAg. Nevertheless, these alterations had small effect on the nucleic acid binding and RNA chaperone activities of NdAg. Truncated mutant N7 and peptide ac2450am that contained amino acids #24-75 and amino acids #24-50 of hepatitis delta antigen, respectively, did not have stable α -helical structure. The α -helical structure of both N7 and ac2450am could be induced in the presence of nucleic acid molecules. The nucleic acid binding activity of N7 was stronger than that of ac2450am, therefore amino acids #51-75 of hepatitis delta antigen seems to be important for the protein-nucleic acid interaction. The circular dichroism spectroscopy and UV absorbency studies illustrated that NdAg altered the structure of nucleic acid molecules. In addition, the electron microscope examination indicated that NdAg not only relaxed the supercoiled plasmid DNA but also melted double-stranded nucleic acids. These properties of NdAg may be important to the RNA chaperone activity of hepatitis delta antigen.