学 位 論 文 の 要 旨

氏名　藤原　純子

学　位　論　文　名　A Study on Novel Biochemical Properties of Deoxyribonuclease I

発　表　雑　誌　名　別紙のとおり
(巻，初頁～終頁，年)

著　　者　　名　別紙のとおり

論 文 内 容 の 要 旨

INTRODUCTION

Deoxyribonuclease I (DNase I, EC 3.1.21.1) is present principally in organs associated with the digestive system. It used to be simply regarded as a digestive enzyme secreted by the exocrine glands, but other functions *in vivo* have recently been revealed. For example, endogenous DNase I has been suggested as a candidate endonuclease responsible for internucleosomal DNA degradation during apoptosis. To reveal the role of DNase I, investigation of the biochemical and molecular properties of DNase I is important.

Actin, which binds to DNA-rich fibers, potently inhibits the enzymatic activity of recombinant human DNase I. It has been suggested that two amino acid residues (Val67 and Ala114) are mainly responsible for actin binding in human and bovine DNase I. Actin-binding capacities have been shown to differ among species. However, to date, the mechanism for the inhibitory effect of actin in vertebrate DNase I remains unclear.

In addition, DNase I is known to be a glycoprotein and two potential N-linked glycosylation sites are known for mammalian enzymes. Asn18 (Asn-Ala/Asp -Thr) and Asn106 (Asn -Asp -Thr/Ser) are well conserved in almost all mammalian enzymes. Although, N-glycosylation

plays important roles not only in protein folding but also in function, roles of N-linked sugar moieties in DNase I are still unclear.

Therefore, the aim of the present study was to (1) investigate the molecular mechanism of the actin-binding site in the vertebrate DNase I family using wild-type and substitution mutants from mammalian, reptilian and amphibian DNases I, (2) elucidate the degree of N-glycosylation and its role in enzymatic activity and thermal stability.

MATERIALS AND METHODS

Expression of wild and substituted mutant DNases I in COS-7 cells and purification of the enzymes A DNA fragment containing the entire coding sequence of each DNase I cDNA was prepared from total RNA derived from the pancreas by reverse transcriptase PCR amplification. Each DNase I cDNA was cloned into a mammalian expression vector and transiently expressed in COS-7 cells. Purification using Con A-WGA mixture-agarose column was performed.

Construction of substitution mutants To investigate the role of amino acid at 67, mutants of rat snake (Ile67Val) and viper snake (Val67Ile) enzymes were constructed. For the role of amino acid at 114, mutants of viper snake (Phe114Ala), rat snake (Phe114Ala), African clawed frog (Phe114Ala), and porcine (Ser114Ala;Ser114Phe) enzymes were constructed. Substitution mutants were constructed by splicing by overlap extension method.

Deglycosylation analysis The purified DNases I were subjected to enzymatic deglycosylation by either Peptide N-glycosidase F (PNGase F) or endoglycosidase H (Endo H). To evaluate thermal stability, samples were incubated at several temperatures following deglycosylation.

Analytical methods and electrophoresis DNase I activity was assayed by the test tube method or the single radial enzyme diffusion method. Activity staining for DNase I was done using a DNA-casting polyacrylamide gel electrophoresis method, followed by enzymatic deglycosylation.

RESULTS AND DISCUSSION

Inhibitory effects of G-actin on the enzyme activities of wild-type and mutant DNases I

Human (Val67/Ala114) and viper snake (Val67/Phe114) enzymes are inhibited by actin, whereas porcine (Val67/Ser114), rat snake (Ile67/Phe114), and African clawed frog (Ile67/Phe114) enzymes are not. After substitution, the rat snake (Ile67Val) was inhibited by actin, while the viper snake (Val67Ile) was not. Strikingly, for viper snake (Phe114Ala), rat snake (Phe114Ala) and African clawed frog (Phe114Ala) expressed no protein. The mutant of porcine (Ser114Ala) enzyme was inhibited by actin, but not the porcine (Ser114Phe) enzyme.

Purification of recombinant DNases I and effect of deglycosylation on enzyme activities

A single-step purification of recombinant DNases I was achieved using a Con A-WGA agarose column. This procedure allows DNases I to be easily and reproducibly isolated and purified to electrophoretic homogeneity.

The degree of N-linked glycosylation in purified mammalian recombinant DNase I was investigated by PNGase F or Endo H digestion. The recombinant enzyme was cleaved by PNGase F, not by Endo H, indicating that the recombinant enzymes are modified by N-linked complex-type carbohydrate moieties. In the human recombinant DNase I, activity was decreased by PNGase F-treatment, while that of the porcine DNase I remained unaffected. The thermal stability of the human enzyme was extremely susceptible to heat following PNGase F-treatment, as was the porcine enzyme to a lesser extent.

CONCLUSION

These results suggest that (1) Val67 may be essential for actin-binding in vertebrate; Phe114 may be related to the folding of DNase I in reptiles and amphibians; Ala114 may be indispensable for actin-binding in mammals and that (2) N-linked complex-type carbohydrate moieties may contribute to the enzymatic activity and/or thermal stability of mammalian recombinant DNases I.

別紙

氏名　藤原　純子

論文名

1. Actin-Inhibition and Folding of Vertebrate Deoxyribonuclease I are Affected by Mutations at Residues 67 and 114.

2. Single-Step Purification by Lectin Affinity and Deglycosylation Analysis of Recombinant Human and Porcine Deoxyribonucleases I Expressed in COS-7 Cells.

3. Two Deoxyribonuclease I Gene Polymorphisms and Correlation Between Genotype and its Activity in Japanese Population.

発表雑誌名
(巻，初頁～終頁，年)

1.Comparative Biochemistry and Physiology Part B:Biochemistry and Molecular Biology Vol. 143, 70-75, 2006.

2. Biotechnology Letters Vol. 28, 215-221, 2006.

3. Legal Medicine Vol. 9, 233-236, 2007.

著者名

1. Junko Fujihara, Yoko Hieda, Yuying Xue, Norihito Nakagami, Shinji Imamura, Koji Takayama, Kaori Kataoka, Haruo Takeshita

2. Junko Fujihara, Yoko Hieda, Yuying Xue, Izumi Okui, Kaori Kataoka, Haruo Takeshita

3. Junko Fujihara, Hisakazu Takatsuka, Kaori Kataoka, Yuying Xue, Haruo Takeshita