Form 3

Abstract of Thesis

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Title	Studies on the folding mechanism of halophilic RNase H1 and role of type 1 RNase H in DNA repair 好塩菌RNase H1のフォールディング機構とDNA修復における1型RNase Hの役割に関する研究

Abstract of Thesis

Abstract

Halo-RNase H1 is an acidic type 1 RNase H from extreme halophilic archaeon *Halobacterium* sp. NRC-1. Studies on its folding by CD spectroscopy and determination of its crystal structure indicate that suppression of negative charge repulsion on the protein surface by salt or by binding of divalent metal ions to the bi/quad-aspartate sites is necessary for its folding. Cleavage of Okazaki fragment-like substrate and double-stranded DNA (dsDNA) containing a single ribonucleotide by Halo-RNase H1 and *E. coli* RNase H1 indicates that both enzymes exhibit 3'-junction ribonuclease (3'-JRNase) activity and cleave these substrates at the (5')RNA-DNA(3') junction. This activity is necessary to completely remove single ribonucleotides misincorporated into DNA during replication in cooperation with 5'-JRNase activity of type 2 RNase H2.

Chapter 1: General Introduction

Ribonuclease H (RNase H) cleaves the P-O3' bond of the RNA strand of RNA/DNA hybrids in the presence of manganese or magnesium ions. It can also cleave an Okazaki fragment-like substrate and dsDNA containing a single ribonucleotide at around the (5')RNA-DNA(3') junction. RNases H are divided into two families. While type 1 RNases H (RNases H1) are responsible for processing the stretches of more than three ribonucleotides, mainly formed during DNA replication of the lagging strand and are the constituents of Okazaki fragments, type 2 RNases H (RNases H2) are involved in DNA repair mechanisms for their ability to cleave single ribonucleotides misincorporated into nascent strands of DNA. RNase H1 from *Halobacterium* sp. NRC-1 (Halo-RNase H1) consists of an N-terminal domain with unknown function and a C-terminal RNase H domain. It exhibits 3'-JRNase activity. Like other proteins from halophilic organisms, Halo-RNase H1 is an acidic protein with the p*I* value of 4.2, suggesting that it is not folded in a low salt condition due to negative charge repulsion on the protein surface. However, it remains to be determined whether Halo-RNase H1 requires high concentration of salt for folding. It also remains to be determined whether Halo-RNase H1 exhibits 3'-JRNase activity for dsDNA containing a single ribonucleotide.

Chapter 2: Role of divalent metal ions in the catalysis and folding of Halo-RNase H1

Halo-RNase H1 is active in a low salt condition in the presence of moderate concentrations of divalent metal ions. The far- and near-UV CD spectra suggested that Halo-RNase H1 assumes a partially folded state (I) in the absence of salt and divalent metal ions and a fully folded state (N) in the presence of >2 M NaCl, ≥5 mM $MnCl_2$ or ≥300 mM $MgCl_2$. Determination of the enzymatic activity showed that the optimum concentration of divalent metal ions for activity was several folds lower in a high salt condition than in a low salt condition. These results suggest that divalent metal ions play a dual role in the catalysis and folding of Halo-RNase H1. In a low salt condition, Halo-RNase H1 requires divalent metal ions not only for activity but also for folding, while in a high salt condition, Halo-RNase H1 requires them only for activity.

Chapter 3: Crystal structure of Halo-RNase H1

The crystal structure of Halo-RNase H1 was determined at 1.4 Å resolution in the presence of 10 mM $MnCl_2$. The refined model consists of two molecules both lacking the N-terminal domain. The main-chain fold of Halo-RNase

H1 and the steric configuration of its four active site residues showed high similarity with other RNases H1. The surface of the protein is negatively charged due to the abundance of acidic residues. Three bi-aspartate sites were found to be present on the surface, two of which are located close to each other forming a quad-aspartate site. Molecule A in the crystal lattice containing two molecules (A and B) contacts the active site of molecule B through one bi-aspartate site, the active site of molecule A" from another lattice through its quad-aspartate site, and the quad-aspartate site of molecule A' through its active site. All these contacts are mediated with manganese ions.

Chapter 4: Divalent metal ion-induced folding mechanism of Halo-RNase H1

To understand the mechanism behind the folding of Halo-RNase H1 induced by divalent metal ions, the active site mutant (2A-RNase H1), the bi/quad-aspartate site mutant (6A-RNase H1), and the mutant at both sites (8A-RNase H1) were constructed. The far-UV CD spectra of these mutants suggest that 2A-RNase H1 mainly exists in the I state, 6A-RNase H1 exists in both the I and N states, and 8A-RNase H1 exists in the N state in a low salt condition. Another RNase H from the same organism (Halo-RNase H2) also exists in the I and N states in low and high salt conditions respectively, but does not exist in the N state in the absence of salt and presence of divalent metal ions. Halo-RNase H1 contains a quad-aspartate site, whereas Halo-RNase H2 does not contain it, suggesting that binding of divalent metal ion(s) to the quad-aspartate site induces folding of Halo-RNase H1.

Chapter 5: Role of type 1 RNase H in DNA repair

An Okazaki fragment-like substrate and dsDNA containing a single ribonucleotide were cleaved by Halo-RNase H1 and another type 1 RNase H, *E. coli* RNase H1. Interestingly, both enzymes cleaved this duplex at the (5')RNA-DNA(3') junction in the presence of $MnCl_2$. Stepwise cleavage of this duplex with *E. coli* RNases H1 and H2 in this order or in the inversed order allowed to excise the ribonucleotide from this duplex. These results suggest that type 1 and type 2 RNases H are able to completely remove a single rNMP misincorporated into dsDNA in an unordered yet cooperative manner.

Chapter 6: General Discussion

For several years, ribonucleotide excision repair (RER) pathway has captured the attention of many researchers for its critical role in maintaining the integrity of the genome and the fidelity of the transferred hereditary material. Structural and functional characterization of Halo-RNase H1 from *Halobacterium* sp. NRC-1 has led me to further investigate and understand the possible role of type 1 RNase H in this repair pathway. Structurally, this thesis elaborates the unique folding behavior of Halo-RNase H1 catalyzed by divalent metal ions and explains this folding mechanism using site-directed mutagenesis approach following the structure determination. Functionally, this thesis proposes a new role for type 1 RNase H in the removal of single rNMPs misincorporated into the genome in a cooperative manner with type 2 RNase H.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文は、高度高塩菌 *Halobacterium* sp. NRC-1 由来リボヌクレアーゼ H1（Halo-RNase H1）の２価金属イオンに依存するユニークなフォールディング機構とⅠ型 RNase H（RNase H1）の DNA 修復における役割について研究したものであり、以下に示すように、序論、本論４章、および総括から構成されている。第１章（序論）では、RNase H の分類、生理機能、構造、触媒機構、基質認識機構などに関するこれまでの研究をまとめるとともに、本研究の目的と意義を述べている。第２章では、Halo-RNase H1 は低濃度の食塩（NaCl）存在下では部分的にフォールディングしたⅠ状態の構造を形成すること、高濃度（２M 以上）の NaCl あるいは 5 mM 以上の $MnCl_2$ や 300 mM 以上の $MgCl_2$ 存在下では完全にフォールディングしたN状態の構造を形成することを明らかにしている。第３章では、10 mM $MnCl_2$ 存在下でフォールディングした Halo-RNase H1 の結晶構造を決定することにより、Halo-RNase H1 の構造は他の RNase H1 の構造と良く似ているが、分子表面には bi-Asp 部位や quad-Asp 部位を構成する Asp をはじめ酸性残基が多く存在するため、強い負電荷を帯びていることを明らかにしている。また、Halo-RNase H1 はモノマーで存在するにも関わらず、結晶中ではいくつもの分子が２個の Mn^{2+} を介して相互作用しており、各界面では Mn^{2+} は一つの分子の活性部位と別の分子の bi-Asp 部位や quad-Asp 部位と配位していることを明らかにしている。第４章では、活性部位や bi/quad-Asp 部位の負電荷反発を解消した変異体を各種構築することにより、bi/quad-Asp 部位への Mn^{2+} の結合が Halo-RNase H1 のフォールディングを誘導することを明らかにしている。また、quad-Asp 部位をもたない Halo-RNase H2 のフォールディングは高濃度の食塩によって誘導されるが、２価金属イオンでは誘導されないことから、Halo-RNase H1 のフォールディングは、quad-Asp 部位への Mn^{2+} の結合により誘導され、活性部位への Mn^{2+} の結合により安定化されることを明らかにしている。第５章では、いくつかの RNase H1 が示す 3'-junction ribonuclease（3'-JRNase）活性を、大腸菌 RNase H1 と様々な合成基質を用いて解析することにより、3'-JRNas 活性は、RNA を１残基含む二本鎖 DNA を RNA の 3'側で切断すること、RNase H2 の 5'-JRNase 活性と組み合わせることによりこの RNA を完全に除去することを明らかにしている。第６章（総括）では、本研究で得られた結果に基づき、２価金属イオンに依存する Halo-RNase H1 のユニークなフォールディング機構と、DNA の複製中に間違ってとりこまれた RNA の除去修復における type 1 RNase H の役割について考察するとともに、今後の展望について述べている。

以上のように、本論文は Halo-RNase H1 のフォールディング機構と DNA 修復における type 1 RNase H の役割に関して新たな知見を見いだした点で意義深い。よって本論文は博士論文として価値あるものと認める。