Form 3

Abstract of Thesis

Name （Permanasari, Etin Diah）	
Title	Studies on the N-terminal substrate binding domains of bacterial RNases H1 and H2 （細菌由来RNase H1とRNase H2のN末端基質結合ドメインに関する研究）

To examine whether bacterial RNases H2 have a substrate binding domain, which is different from hybrid-binding domain (HBD) of RNase H1 and TATA-box binding protein (TBP)-like domain of RNase H3, the amino acid sequences of various RNases H2 were compared with one another. HBD and TBP-like domain have been shown to be important for substrate binding, activity and/or stability of RNase H1 and RNH3 respectively. None is known for substrate binding domain of RNases H2. In this study, only the bacterial RNase H2 sequences were compared, because eukaryotic RNases H2 are heterotrimeric and their catalytic subunits require other two subunits for activity. The result indicates that some bacterial RNases H2 have an N- or C-extension, which shows little amino acid sequence similarity to HBD or TBP-like domain, suggesting that these extensions are folded into a unique structure. N-extensions of bacterial RNases H2 show significant amino acid sequence similarities with one another (at least 20%), suggesting that they share a similar structure. In contrast, C-extensions of bacterial RNases H2 vary in size and amino acid sequences, suggesting that they have various structures. None of the crystal structures of N-extensions has been determined. In contrast, the crystal structure of C-extension of *Thermotoga maritima* RNase H2 (TmaRNH2) has been determined as that of TmaRNH2-substrate complex. According to this structure, C-extension is present as a part of the C-terminal domain, is folded into a hairpin helix structure (TmaHH), and does not directly contact the substrate. In this study, *Bacillus stearothermophilus* RNase H2 (BstRNH2), *T. maritima* RNase H2 (TmaRNH2), and *Aquifex aeolicus* RNase H2 (AaeRNH2) were chosen as representatives of bacterial RNases H2 containing N-extension, C-extension, and neither extension respectively, and characterized. AaeRNH2 was as active as and more stable than other two enzymes, suggesting that it does not require an additional domain for activity and stability. To analyze the role of N- and C-extensions, BstRNH2 lacking N-extension (BstRNH2ΔN) and TmaRNH2 lacking C-extension (TmaRNH2ΔC) were constructed and characterized. These proteins exhibited decreased activities and substrate binding affinities as compared to those of their intact partners. The far-UV CD spectra of these proteins were similar to those of their intact partners, suggesting that the removal of these extensions does not significantly alter their protein structure. Because C-extension of TmaRNH2 does not contact the substrate and is present as a part of the C-terminal domain, this extension is probably required to make the conformation of the C-terminal domain functional. N-extension of BstRNH2 probably exists as a domain with unique structure (N-terminal domain, BstNTD) and functions as a substrate binding domain. In addition, when BstNTD was attached to the N-terminus of TmaRNH2ΔC, BstNTD partly restored its activity. This result supports the hypothesis that BstNTD functions as a potent substrate binding domain. To examine whether the attachment of a substrate binding domain of bacterial RNases H to the N-terminus of RNH^{HIV} restores its activity, N-extension of BstRNH2 and HBD of *T. maritima* RNase H1 (TmaRNH1) were attached to the N-terminus of RNH^{HIV}. We did not construct the fusion protein, in which TBP-like domain is attached to the N-terminus of RNH^{HIV}, because TBP-like domain binds to substrate in a similar manner to that of HBD. TmaHBD-RNH^{HIV} and BstNTD-RNH^{HIV} greatly increased the activity and substrate binding affinity of the isolated RNase H domain of HIV-1 reverse transcriptase (RNH^{HIV}) when they were attached to the N-terminus of RNH^{HIV}. RNH^{HIV} is nearly inactive and binds to substrate very weakly. The attachments of TmaHBD and BstNTD increased the Mn^{2+}-dependent activity of RNH^{HIV} by 500- and 13-fold respectively. They also increased the substrate binding affinity of RNH^{HIV}. These results suggest that N-extensions of bacterial RNases H2 function as a substrate binding domain, and substrate binding domains of RNases H can be used as a tag for binding of heterologous proteins to RNA/DNA hybrid.

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、細菌由来 RNase H1 と RNase H2 の N 末端や C 末端に存在するドメインの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 2 章、および総括から構成されている。第一章（序論）では、RNase H の分類、生理機能、構造、触媒機構などに関するこれまでの研究をまとめると共に、細菌由来 RNase H1 の N 末端に存在する Hybrid binding domain（HBD）や細菌由来 RNase H3 の N 末端に存在する TATA-box binding protein-like domain（TBP-like domain）の構造と機能に関してこれまで行われてきた研究の背景に触れ、本研究の目的と意義を述べている。第二章では、様々な細菌由来 RNase H2 のアミノ酸配列を比較することにより、RNase H2 は、*Bacillus stearothermophilus* 由来 RNase H2（Bst-RNH2）など N 末端に伸長領域（N-extension）を有するもの、*Thermotoga maritima* 由来 RNase H2（Tma-RNH2）など C 末端に伸長領域（C-extension）を有するもの、Aquifex aeolicus 由来 RNase H2（Aae-RNH2）のように N-extension も C-extension も含まないもの、の 3 種類に分類されることを明らかにしている。また、Bst-RNH2 の N-extension 欠損変異体や Tma-RNH2 の C-extension 欠損変異体を構築し、酵素活性、基質結合能、安定性を解析することにより、Bst-RNH2 の N-extension と Tma-RNH2 の C-extension は Mn^{2+}存在下における酵素活性や基質の切断部位特異性には重要ではないが、Mg^{2+}存在下における酵素活性、基質結合、安定性には重要であることを明らかにしている。さらに、Aae-RNH2 が高い活性および耐熱性を示すことから、すべての RNase H2 が N-extension や C-extension を活性や安定性に必要とするわけではないことを提案している。第三章では、Bst-RNH21 の N-extension （BstNTD）や *T. maritima* 由来 RNase H1（TmaRNH1）の HBD（TmaHBD）を HIV-1 逆転写酵素の RNase H ドメインの N 末端に付与した融合タンパク質を構築し、酵素活性、基質結合能、安定性を解析することにより、HIV-1 逆転写酵素の RNase H ドメインは DNA ポリメラーゼドメインから切り離されると基質結合能を失い、活性を失うこと、BstNTD や TmaHBD をその末端に付与することにより、基質結合能が回復し、Mn^{2+}存在下における酵素活性が大きく向上することを明らかにしている。また、これらの結果に基づき、BstNTD や TmaHBD が hybrid binding tag として利用できることを提案している。第四章（総括）では、本研究で得られた結果に基づき RNase H2 の N-extension や C-extension の役割、さらには様々な基質結合ドメインの付加による RNase H の分子多様性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 RNase H2 の N 末端や C 末端に存在する伸長領域の役割に関して構造生物学的な観点から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。