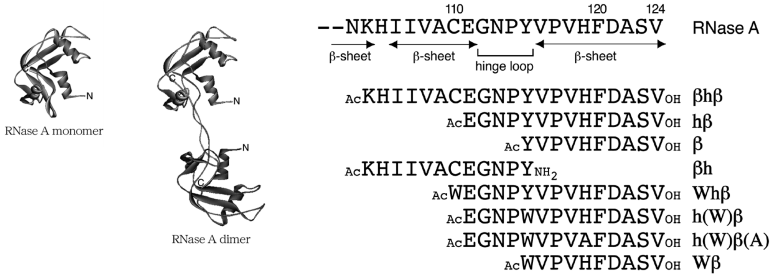
Communication

An Oligopeptide Containing the C-Terminal Sequence of RNase A Has a Potent RNase A Binding Property

Shu-ichi Nakano, and Naoki Sugimoto

J. Am. Chem. Soc., **2003**, 125 (29), 8728-8729• DOI: 10.1021/ja034659r • Publication Date (Web): 27 June 2003



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 06/27/2003

An Oligopeptide Containing the C-Terminal Sequence of RNase A Has a Potent RNase A Binding Property

Shu-ichi Nakano[†] and Naoki Sugimoto*[,†,‡]

High Technology Research Center, and Department of Chemistry, Faculty of Science and Engineering, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan

Received February 13, 2003; E-mail: sugimoto@konan-u.ac.jp

Many proteins assemble with partner proteins in cells, forming multimers in which intermolecular noncovalent interactions are established between the protein interfaces. Analogous to the protein assembly, a complement between a protein surface and a small ligand is widely used to develop protein-binding compounds. Although combinatorial chemistry is one of the successful strategies for drug discovery, enormous efforts are still necessary for the identification of lead compounds as well as for the design and synthesis of the compound libraries. The three-dimensional (3D) domain swapping is another type of intermolecular noncovalent interaction among identical proteins.[1] Although bovine pancreatic ribonuclease A, RNase A (124 amino acids), is a monomeric enzyme that hydrolyzes RNAs,[2] it also forms dimers and oligomers after lyophilization in 40−50% acetic acid.[3] Liu et al. reported the crystal structures of two types of 3D-domain-swapped RNase A dimers: the minor dimer exchanges its N-terminal α-helix,[4] and the major (or predominant) dimer exchanges its C-terminal *β*-sheet.[5] It is also reported that the formation of the RNase A dimer was inhibited by lyophilization in 50% acetic acid in the presence of the S peptide (residues 1−20 in the N-terminal α-helical domain of RNase A).[4,6] Here, we show that oligopeptides containing the sequence of the C-terminal *β*-sheet and the following hinge loop of RNase A bind to RNase A without lyophilization and are a potent inhibitor for the domain-swapped RNase A dimers.

The crystal structure of the major dimer of RNase A, swapping its C-terminal *β*-sheet, exhibits a dimeric form stabilized by interactions between the hinge loops without substantial contact at the interface.[5] We thus designed four oligopeptides,[7] ***β*h*β***, **h*β***, ***β***, and ***β*h**, that have the C-terminal sequence of RNase A as is shown in Figure 1. Size-exclusion chromatography[8] was carried out to separate the domain-swapped RNase A dimers from the monomers. As was previously reported,[3] we confirmed an ∼20% yield of dimers after lyophilization of RNase A in 50% acetic acid without oligomers larger than the dimers.[9] Elution profiles of the RNase A after lyophilization in 50% acetic acid in the presence of these peptides revealed that the amount of the RNase A dimer was significantly reduced with ***β*h*β*** or **h*β***, but not with ***β*** or ***β*h** (see Supporting Information). It is noteworthy that the lyophilization making the protein and peptide concentrations extremely high did not affect the amount of dimers in the case of ***β*** and ***β*h**, indicating the reduction of the dimers by the peptides in a sequence-dependent fashion. Importantly, we also found that the peak at 7.9 mL disappeared with the addition of ***β*h*β*** or **h*β*** without lyophilization and the elimination of the peak was dependent on the amount of the peptide (Figure 2). This suggests that the oligopeptides that have amino acids of the C-terminal *β*-sheet and the hinge loop can bind to RNase A and eliminate the domain-swapped RNase A dimers. This is consistent with the crystal structure of the major RNase A dimer that exchanges its C-terminal *β*-sheet accompanied with the hinge loop−hinge loop interactions.[5] In addition, the minor dimer that exchanges its N-terminal domain[4] also appeared to be eliminated by these peptides, perhaps due to a coupling motion between the C-terminal *β*-sheet and the N-terminal α-helix.[10]

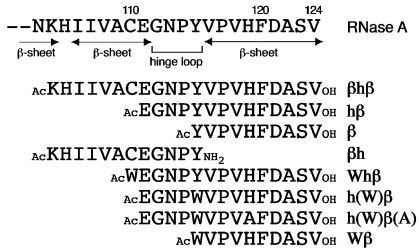


Figure 1. RNase A sequence at the carboxyl terminal domains (top), and the oligopeptide sequences and their abbreviations used in this study (below).

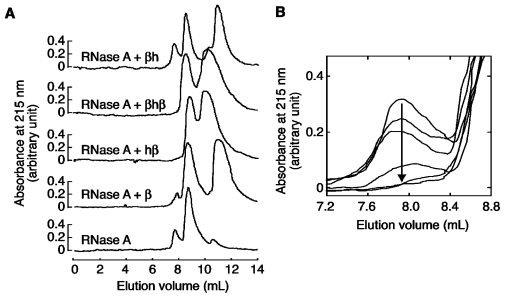


Figure 2. (A) Elution profiles of 4.6 nmol of RNase A with and without 46 nmol of peptide by 50 mM Na_2HPO_4 (pH 7.0)/300 mM NaCl. Each peptide was added to a solution containing the RNase A dimers. No lyophilization was performed after the peptide was added. The peak at 7.9 mL is assigned to the RNase A dimers, that at 8.8 mL to the RNase A monomer and RNaseA−peptide complex, and that between 10 and 12 mL to an oligopeptide. (B) The dose dependence of **h*β*** (0, 3, 6, 30, 46, and 60 nmol, from top to bottom) on the dimer peak.

On the basis of these results, we also synthesized four fluorescently labeled oligopeptides, **Wh*β***, **h(W)*β***, **h(W)*β*(A)**, and **W*β*** (Figure 1). Because RNase A contains no tryptophan (W), these oligopeptides are useful for spectrophotometrically monitoring the binding to RNase A. The emission at 380 nm from tryptophan was measured, where the emission originating from RNase A which includes six tyrosine residues was much smaller than that from the fluorescent peptides. To eliminate any nonspecific electrostatic binding, 300 mM NaCl/50 mM phosphate buffer was used. The titration of RNase A monomer with the fluorescently labeled oligopeptides without lyophilization revealed a significant decrease in the emission intensity of **h(W)*β*** and **Wh*β*** (a maximum quenching of 22% and 14%, respectively), while those of **W*β*** and **h(W)*β*(A)** were small (Figure 3A). The profiles for **h(W)*β*** and **Wh*β*** were the same, indicating that the position of the tryptophan

[†] High Technology Research Center, Konan University.
[‡] Department of Chemistry, Faculty of Science and Engineering, Konan University.

10.1021/ja034659r CCC: $25.00

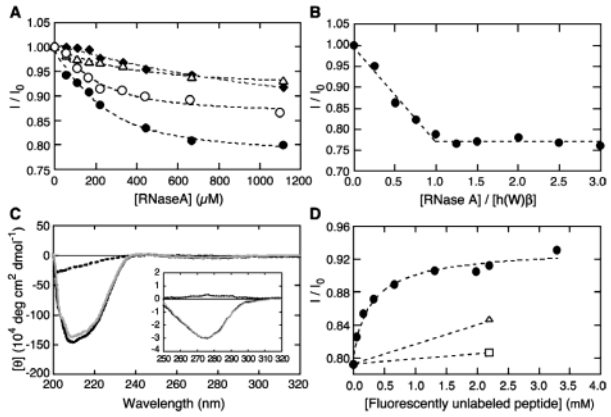


Figure 3. (A) RNase A titration profiles of the emission at 380 nm (an excitation at 280 nm) of **h(W)*β*** (●), **Wh*β*** (○), **h(W)*β*(A)** (◆), and **W*β*** (△). The peptide concentration was 220 μM. The binding reaction was performed in 50 mM Na_2HPO_4 (pH 7.0)/300 mM NaCl for >15 min at 22 °C. The fitting curve gives K_d (dissociation constant) values of 110 ± 40 μM for **h(W)*β*** and 84 ± 38 μM for **Wh*β***. (B) The plot of **h(W)*β*** binding to RNase A monitored at 380 nm after lyophilization in water. The peptide concentration was constant at 220 μM. (C) CD spectra of RNase A (gray line), **h(W)*β*** (thick dots), and an equimolar mixture of **h(W)*β*** and RNase A after lyophilization in water (thick line). All spectra were measured using 20 μM samples in 50 mM Na_2HPO_4 (pH 7.0)/300 mM NaCl at 25 °C. The inset is the spectra attributed to the chromophores of aromatic side chains obtained by the use of 250 μM samples. (D) Restoration of the **h(W)*β*** quenching by the addition of fluorescently unlabeled peptides (**h*β***, ***β***, and ***β*h**) was monitored at 380 nm. The fluorescently labeled **h(W)*β*** (220 μM) and RNase A (220 μM) were lyophilized in water, and then mixed with **h*β*** (●), ***β*** (△), or ***β*h** (□) followed by incubation at 22 °C for 1 h. The fitting curve gives a K_d value of 180 ± 80 μM for **h*β***.

only slightly affected the peptide binding (K_d = 110 ± 40 μM for **h(W)*β*** and 84 ± 38 μM for **Wh*β***). The smaller quenching of **W*β*** is consistent with the reduced inhibition of the RNase A dimer formation by ***β*** in Figure 2. In addition, **h(W)*β*(A)**, replacement of the single amino acid in the β-sheet region of **h(W)*β***,[11] resulted in a significant decrease in the quenching, indicating that RNase A recognizes the amino acids in the β-sheet region of **h(W)*β***. To obtain the binding stoichiometry, a solution of RNase A mixed with **h(W)*β*** was lyophilized in water before measurement to promote association.[12] The plot in Figure 3B reveals an equimolar binding of **h(W)*β*** to RNase A with a maximum quenching of ~25%, identical to that observed at the high RNase A concentrations in Figure 3A. The CD (circular dichroism) spectra in Figure 3C indicate that the global conformation of RNase A is retained by the **h(W)*β*** binding.[13] To further confirm the peptide binding to RNase A, restoration of the **h(W)*β*** quenching was monitored in the presence of the fluorescently unlabeled peptide (**h*β***, ***β***, or ***β*h**). As shown in Figure 3D, only **h*β*** significantly inhibited the binding of **h(W)*β*** to RNase A,[14] confirming that **h*β*** and **h(W)*β*** are bound in the same region of RNase A.

In conclusion, the oligopeptides containing the C-terminal sequence of RNase A have a potent RNase A binding property and inhibit the formation of the domain-swapped RNase A dimers. The binding and inhibition were observed only for the peptides having the hinge loop amino acids and a correct sequence in the β-sheet region. These observations are consistent with the interaction found in the major domain-swapped RNase A dimer, so that the peptide binding may be promoted through the swapping reaction with the C-terminal β-sheet of RNase A. In contrast to the RNase A dimer formation that requires lyophilization in 50% acetic acid, binding of the peptide to RNase A proceeded in a buffer without lyophilization. This may be because lyophilization enhances the chance of encountering two or more partially unfolded RNase A molecules, while the formation of the peptide−RNase A complex requires only a single partially unfolded RNase A. The design of the protein-binding peptide shown here is much simpler than other methods such as the combinatorial method. The 3D-domain swapping in proteins has not been quantified extensively because extreme conditions such as lyophilization in 50% acetic acid are required for the formation of the domain-swapped molecules. The present strategy using an oligopeptide may be of general application to the investigations of the 3D-domain swapping in proteins as well as for the development of an oligopeptide that specifically binds to a target protein.

Acknowledgment. This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan.

Supporting Information Available: Elution profiles of the RNase A after lyophilization in 50% acetic acid in the presence of ***β*h*β***, **h*β***, ***β***, or ***β*h** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Benett, M. J.; Choe, S.; Eisenberg, D. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3127−3131. (b) Schlunegger, M. P.; Bennett, M. J.; Eisenberg, D. *Adv. Protein Chem.* **1997**, *50*, 61−122. (c) Chen, Y. W.; Stott, K.; Perutz, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1257−1261. (d) Schiering, N.; Casale, E.; Caccia, P.; Giordano, P.; Battistini, C. *Biochemistry* **2000**, *39*, 13376−13382. (e) Hakansson, M.; Svensson, A.; Fast, J.; Linse, S. *Protein Sci.* **2001**, *10*, 927−933. (f) Rousseau, F.; Schymkowitz, J. W.; Wilkinson, H. R.; Itzhaki, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5596−5601. (g) Liu, Y.; Eisenberg, D. *Protein Sci.* **2002**, *11*, 1285−1299.

(2) Raines, R. T., *Chem. Rev.* **1998**, *98*, 1045−1066.

(3) (a) Fruchter, R. F.; Crestfield, A. M. *J. Biol. Chem.* **1965**, *240*, 3868−3874. (b) Gotte, G.; Bertoldi, M.; Libonati, M. *Eur. J. Biochem.* **1999**, *265*, 680−687. (c) Park, C.; Raines, R. T. *Protein Sci.* **2000**, *9*, 2026−2033. (d) Nenci, A.; Gotte, G.; Bertoldi, M.; Libonati, M. *Protein Sci.* **2001**, *10*, 2017−2027.

(4) Liu, Y.; Hart, P. J.; Schlunnegger, M. P.; Eisenberg, D. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3437−3442.

(5) Liu, Y.; Gotte, G.; Libonati, M.; Eisenberg, D. *Nat. Struct. Biol.* **2001**, *8*, 211−214.

(6) (a) Goldberg, J. M.; Baldwin, R. L. *Biochemistry* **1998**, *37*, 2556−2563. (b) Raines, R. T.; McCormick, M.; Van Oosbree, T. R.; Mierendorf, R. C. *Methods Enzymol.* **2000**, *326*, 362−376. (c) Dwyer, J. J.; Dwyer, M. A.; Kossiakoff, A. A. *Biochemistry* **2001**, *40*, 13491−13500.

(7) All oligopeptides were synthesized on a solid support based on the Fmoc strategy as previously described (Nakano, S.; Sugimoto, N. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 2205−2210.). All oligopeptides were confirmed by identification with MALDI-TOF MS (PE Biosystems Voyager).

(8) RNase A was purchased from Sigma, and its molecular weight was confirmed by MALDI-TOF MS. Size exclusion chromatography was performed using a TOSOH TSK-GEL G2000SWxL (7.8 mm × 30 cm) column with a flow rate of 1 mL/min at room temperature. The running buffer was 50 mM Na_2HPO_4 (pH 7.0)/300 mM NaCl. RNase A without lyophilization showed only one peak at 8.8 mL corresponding to the RNase A monomer. The domain-swapped RNase A dimer was prepared as previously reported.[3]

(9) The chromatographic analysis for the fraction of the domain-swapped dimers generated no monomer peak, indicating a slow dissociation rate of the dimers. Size exclusion chromatography also showed that lyophilization of RNase A in water gave no oligomeric products.

(10) Cole, R.; Loria, J. P. *Biochemistry* **2002**, *41*, 6072−6081.

(11) The Ala substitution was performed with the histidine corresponding to the acid catalyst in the protein.

(12) The titration curve in Figure 3B differs from that in Figure 3A, indicating that the concentrated RNase A and the peptide promoted their association during the lyophilization in water. It also suggests a slow dissociation rate of the peptide from RNase A because both experiments were carried out under the same conditions except for the lyophilization process.

(13) The CD spectra also indicated that **h(W)*β*** formed a random-coil structure, and the sum of the individual spectrum for **h(W)*β*** and RNase A was not the same as that for the equimolar mixture of **h(W)*β*** and RNase A.

(14) The restoration by ***β*** to some extent is consistent with the slight inhibition of the protein dimer by ***β*** in Figure 2.

JA034659R