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Construction of Two-Stranded α-Helix Peptides Based on Influenza Virus M1 Protein Selectively Bound to RNA

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Abstract—Various 2α-helix peptides were designed and synthesized based on the RNA-binding region of matrix protein M1 in influenza virus. The binding properties of the peptides to model ssRNA, ssDNA, dsDNA, and virus RNA were examined by the fluorescence studies of a dansyl group incorporated into the peptides. The peptide containing the hydrophilic residues of M1 RNA-binding region bound RNAs selectively. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Type A influenza virus is an enveloped virus that contains eight separated segments of single-stranded (ss) RNA. The inner surface of the viral membrane envelope is covered with matrix protein (M1).[1] M1 (252 amino acids) is the most abundant structural protein and has an ability to bind ssRNA mainly by electrostatic interaction with no sequence specificity.[2,3] M1 also plays a central role of viral assembly and transcription inhibition, though these details are as yet unknown.[4,5] From previous studies, an RNA-binding domain of M1 has been supposed to be the region of amino acid sequence (91–111).[3,6,7] Moreover, the X-ray crystal structure of M1 has revealed that the protein forms dimer, and that the residues (91–105) which overlap the putative RNA-binding domain take an α-helix structure and two α-helices from each monomer are oriented in antiparallel.[8,9] These two α-helices that include 12 positively charged residues are exposed on the surface of the M1-dimer.

There have been reported several binding motifs selectively to RNA, such as Arg-rich motif of Rev or Tat, RGG box of hnRNP U, and KH motif of hnRNP K.[10] Generally, RNA-binding proteins seldom target fully double-stranded (ds) tracts for recognition, but interact with secondary structural domains such as hairpin loops, internal loops, and bulges of RNA.[11] In most cases, an RNA-binding domain of protein forms a suitable conformation to recognize a structured RNA, and amino acids orientated exactly in the protein structure make specific contacts to RNA bases. Although the information of protein–dsDNA interaction has been abundant, that of protein–RNA interaction is not sufficient, especially on the conformational aspects of protein and RNA. Therefore, it is important to better understand properties of RNA–protein interaction and recognition in various cases. In this study, in order to examine how the putative RNA-binding domain of M1 interacts with RNA/DNA, we attempted to design and synthesize a variety of cationic peptides with a 2α-helix structure based on this binding domain (Fig. 1). The designing approach using RNA-binding motifs in native proteins is worthful for discovering a novel property in RNA–protein interaction.

Results and Discussion

Considering the antiparallel arrangement of the RNA-binding α-helices in the M1 structure, we designed a panel of peptides with an antiparallel two-stranded α-helix structure (Fig. 1). The peptide MM has the two-stranded 17-peptide sequence simplified from that of M1 (89–105)

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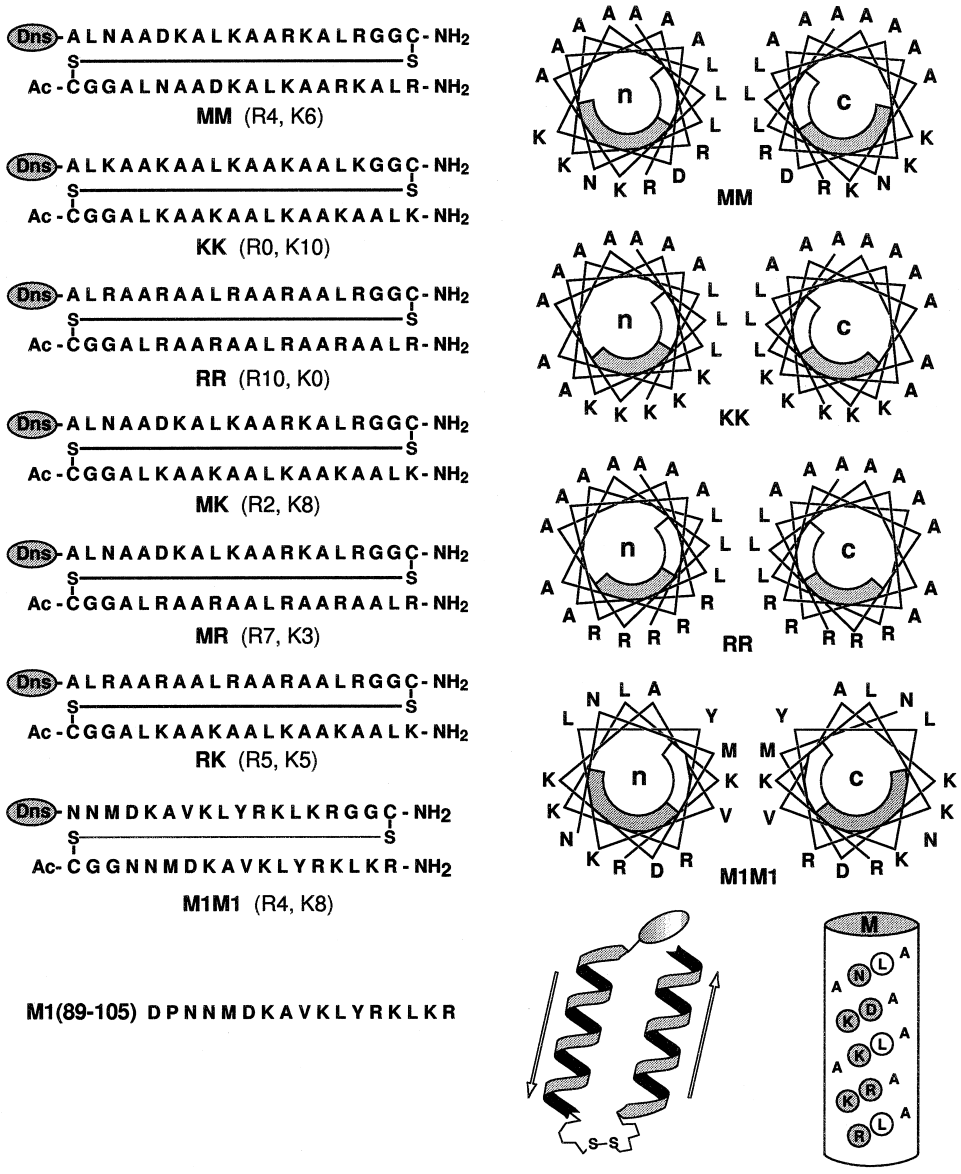

Figure 1. Amino acid sequences of the designed peptides and M1 (89–105). Helix wheels of MM, KK, RR and M1M1, and antiparallel two-α-helix structure and helix net of M are illustrated; n and c denote the N- and C-terminals, and open and shaded bars indicate hydrophobic and hydrophilic faces, respectively, in the wheels. The numbers of Arg and Lys residues are indicated.

by keeping the seven hydrophilic residues including three Lys and two Arg residues per helix, which are thought to be important for the RNA binding. The peptides KK and RR contain either five Lys or five Arg residues per helix for the hydrophilic residues. The peptides MK, MR, and RK composed of heterogeneous two-helix sequences were also designed. At the interface of two helices, three hydrophobic Leu residues were used to facilitate the two-stranded-helix assembly in solution. All other residues were simplified by replacing with Ala residues. Two peptide segments were dimerized in antiparallel by the disulfide linkage of Cys residues at N- and C-termini. Therefore, Cys-Gly-Gly (CGG) and Gly-Gly-Cys (GGC) linkers were placed at N- and C-termini of the peptides. The peptides were synthesized by the Fmoc solid-phase method.[12] A dansyl (5-dimethylamino-naphthalene-1-sulphonyl (Dns)) moiety as a fluorescence probe was combined with N-terminus of the peptides to detect the RNA/DNA interaction by means of spectroscopic methods. Antiparallel dimerization for the synthesis of peptides was achieved by activation of one Cys residue using 2,2′-dithiodipyridine.[13] The peptides were purified by RP-HPLC, and identified by MALDI-TOFMS and amino acid analysis.

All the peptides showed an α-helix structure (helix content: 64–76%) in trifluoroethanol, although in aqueous solution the contents were approximately 20%,[14] indicating that the peptides have a potential of forming α-helices when they bind RNA/DNA. The binding property of the peptides to RNA/DNA was examined using the fluorescence change of Dns. By the addition of poly[rA] (average size ca. 25mer by PAGE) to a solution of MM, the intensity of the Dns fluorescence remarkably increased and the peak shifted to the shorter wavelength (from 546 to 513 nm), suggesting that the

peptide can bind the ssRNA and the Dns group shifts into more hydrophobic environments by the binding (Fig. 2). In contrast, the fluorescence was not changed by the addition of $[dA]_{20}$, indicating that the MM peptide does not interact with the ssDNA. Since it has been elucidated that the peptides form large aggregates when they interact with RNA/DNA (300–1500 nm),[15,16] the binding curve cannot be analyzed quantitatively (ex. binding constant), thus the qualitative analysis was performed to characterize the binding preference of the peptides to different nucleic acids (Fig. 3). The aggregation ability of M1-peptide/RNA is interesting with respect to the role of M1 protein regulating the formation of virus RNP.[7,15,16]

In the case of poly[rA], the fluorescence intensity of all peptides (0.75 μM) increased by 15 to 25 times as compared to that without RNA (Fig. 3), which is independent of the amino acid compositions and sequences of peptides. All the peptides bound the RNA with the fluorescence intensities approaching the plateau at 5.0–10 μM nucleotide (nt) concentration. These results suggest that the peptides have a similar affinity for the RNA. This might be related to the formation of large aggregates of peptides bound to the nucleic acid. It should be noted that the MM peptide did not show the significant fluorescence increment for other dsDNA or ssDNA, suggesting that the peptide selectively binds the RNA. The other peptides, KK, RK, MR and MK, displayed the increased binding properties to $[dA]_{20}$·$[dT]_{20}$, but faint binding preferences to ssDNAs, $[dA]_{20}$ and $[dT]_{20}$, under the concentration range, except for the RR peptide. Only the RR peptide preferably binds all nucleic acids, although the fluorescent increment for $[dA]_{20}$ was small. From these results, seven hydrophilic residues (3K, 2R, N, and D) in MM have a potential for discriminating the RNA from others. The peptides having the M-segment (MM, MK, MR) attained a higher selectivity for the RNA than the peptides without M (RK, KK, RR).

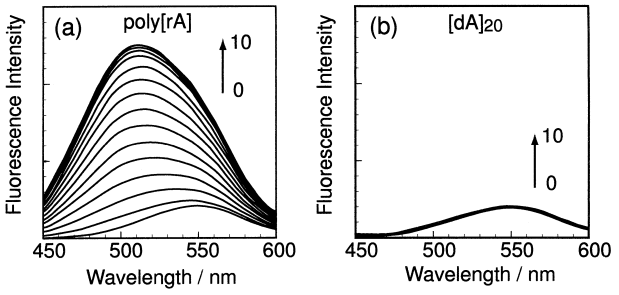

Figure 2. Fluorescence titration of MM peptide with poly[rA] (a) and $[dA]_{20}$ (b) in 20 mM Tris–HCl (pH 7.4) with 50 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA, at 25 °C. [Peptide] = 0.75 μM; [nt] = 0–10 μM; λ_{ex} = 340 nm.

Next, the binding of peptides (MM, KK, RR) to native RNA was examined using 172 nts RNA from the influenza virus (172vRNA), which contains a 31 nts stem region with a panhandle structure and a 141 nts single stranded loop.[3] The 172vRNA was prepared using T7 polymerase from the corresponding DNA. Consequently, the peptides MM, KK and RR showed the fluorescence increments with almost the same ability (Fig. 3). The MM peptide binds vRNA as well as poly[rA]. In addition to these RNAs, KK bound $[dA]_{20}$·$[dT]_{20}$, while RR showed all preferences. Interestingly, the peptide having completely the native sequence of the RNA-binding region (91–105), M1M1 (Fig. 1), exhibited a strong binding preference to the dsDNA as well as RNA (Fig. 3). These results imply that the design using three hydrophobic Leu residues in MM successfully makes the peptide have the RNA selectivity. In native M1 protein, M1 forms a dimer

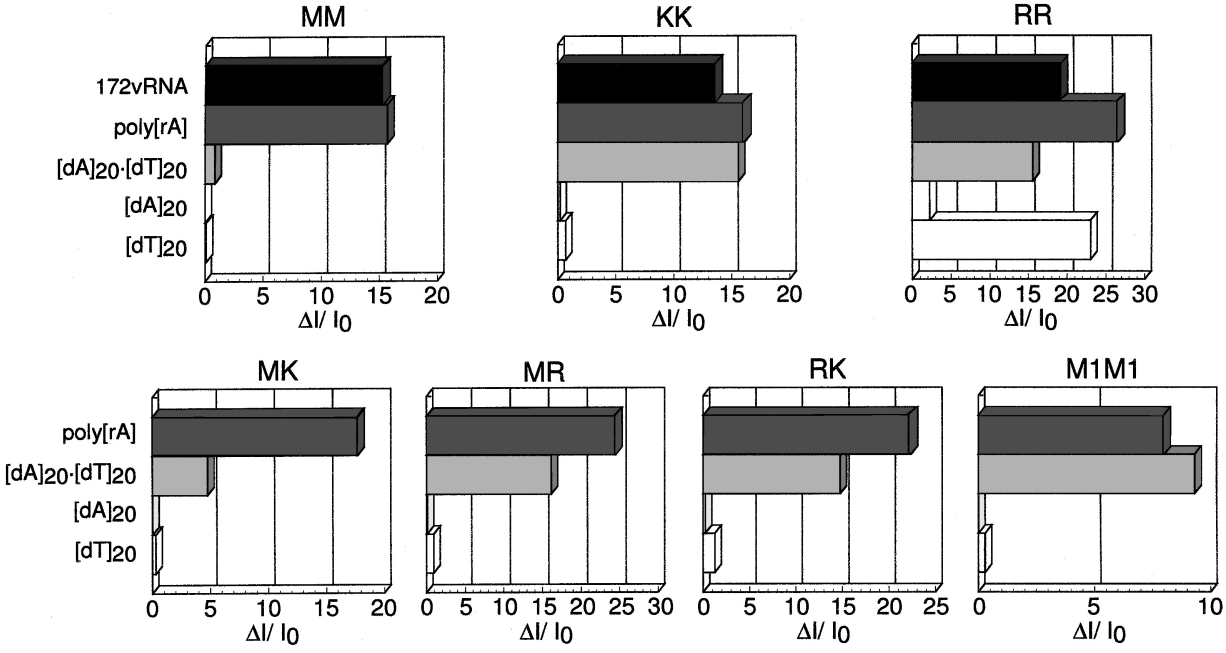

Figure 3. Fluorescence change of peptides at 500 nm by the addition of various nucleic acids (10 μM nt). Conditions are the same as those in Figure 2.

conformation and the resulting two-stranded helices can accommodate preferably to RNA. However, picking the peptide 2α-structure from M1 protein dimer is not sufficient to arrange the residues important for the RNA-binding. The design using Leu residues as an interface to facilitate the formation of the 2α-helix structure is effective to provide the peptide having selectivity to RNA as the native protein may operate. It is noteworthy that the one segmental peptide such as the half of M1M1 has a lower activity of binding to the vRNA.[16] The proper designing in two-stranded α-helices will lead to construction of a new motif selectively bound to RNA.

References and Notes

1. Lamb, R. A.; Choppin, P. W. *Annu. Rev. Biochem.* **1983**, *52*, 467.
2. Wakefield, L.; Brownlee, G. G. *Nucleic Acids Res.* **1989**, *17*, 8569.
3. Watanabe, K.; Handa, H.; Mizumoto, K.; Nagata, K. *J. Virol.* **1996**, *70*, 241.
4. Hankins, R. W.; Nagata, K.; Bucher, D. J.; Popple, S.; Ishihama, A. *Virus Genes* **1989**, *3*, 111.
5. Elster, C.; Larsen, K.; Gagnon, J.; Ruigrok, R. W. H.; Baudin, F. *J. Gen. Virol.* **1997**, *78*, 1589.
6. LaCasse, E. C.; Lefebvre, Y. A. *Nucleic Acids Res.* **1995**, *23*, 1647.
7. Ye, Z.; Liu, T.; Offringa, D. P.; McInnis, J.; Levandowski, R. A. *J. Virol.* **1999**, *73*, 7467.
8. Sha, B.; Luo, M. *Nature Struct. Biol.* **1997**, *4*, 239.
9. Sha, B.; Luo, M. *Acta Cryst.* **1997**, *D53*, 458.
10. Burd, C. G.; Dreyfuss, G. *Science* **1994**, *265*, 615.
11. Mattaj, I. W. *Cell* **1993**, *73*, 837.
12. Chan, W. C.; White, P. D. *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*; Chan, W. C., White, P. D., Eds. Oxford University Press: New York, 2000; p 41.
13. Futaki, S.; Kitagawa, K. *Tetrahedron* **1997**, *53*, 7479.
14. CD measurements were carried out in 20 mM Tris–HCl (pH 7.4) with 50 mM NaCl or TFE at 25 °C. α-Helicity (%): MM (17% in buffer, 73% in TFE); KK (14, 64); RR (25, 76); MK (19, 73); MR (22, 66); RK (19, 71). Concentration dependence of CD was not observed from 1.0 to 12 μM.
15. The mean particle size of peptide-RNA/DNA complex was measured by dynamic light scattering studies: poly[rA] 300–400 nm (all peptides); $[dA]_{20}$·$[dT]_{20}$ 1200–1500 nm (KK, RR, MK, MR, RK); $[dA]_{20}$ 600 nm (RR); $[dT]_{20}$ 400 nm (RR). The particle sizes of aggregates appeared to be regulated by a type of nucleic acid. Aggregated particles were not observed for the peptides of which fluorescence intensities were not changed.
16. Kuribayashi, H.; Takahashi, T.; Watanabe, K.; Nagata, K.; Ueno, A.; Mihara, H. *Nucleic Acids Symp. Ser., No. 39*; Oxford University Press: Oxford, 1998; p 63.