SYNTHESIS OF SOME PEPTIDES CORRESPONDING TO THE ACTIVE REGION OF RANTES FOR CHEMOTAXIS AND EVALUATION OF THEIR ANTI-HUMAN IMMUNODEFICIENCY VIRUS-1 ACTIVITY

Yasuhiro NISHIYAMA,*[,a] Tsutomu MURAKAMI,[b] Keisuke KURITA,[a] and Naoki YAMAMOTO[b]
Department of Industrial Chemistry, Faculty of Engineering, Seikei University,[a] Musashino-shi, Tokyo 180, Japan and Department of Microbiology, Tokyo Medical and Dental University School of Medicine,[b] Bunkyo-ku, Tokyo 113, Japan.

Five peptides corresponding to the amino-terminal sequence of RANTES (regulated upon activation, normal T-cell expressed and secreted), which is known to be the critical region for chemotaxis, were synthesized, and their anti-human immunodeficiency virus (HIV)-1 activity was examined to obtain a lead compound useful for the development of chemokine receptor-directed anti-HIV-1 drugs. A decapeptide corresponding to positions 1-10 [Ac-(^{10}Ala-RANTES 1-10)-NH_2] showed significant anti-HIV-1 activity. Ac-(^{10}Ala,^{11}Ala-RANTES 5-14)-NH_2 was also active, but less potent than the former. Other peptides corresponding to the positions 6-14, 7-14, and 8-14 did not show anti-HIV-1 activity. These results indicate that the sequence 1-10 of RANTES is important for the anti-HIV-1 effect.

KEYWORDS: RANTES; active region; anti-HIV-1 activity; peptide; AIDS

RANTES (regulated upon activation, normal T-cell expressed and secreted), one of the CC-chemokines, is a chemotactic and activating agent for a variety of leukocytes including T-lymphocytes.[1] It has been reported that RANTES, macrophage inflammatory protein (MIP)-1α, and MIP-1β inhibit infection with human immunodeficiency virus (HIV)-1 *in vitro* by interacting with CC-chemokine receptor-5 (CCR-5), a coreceptor for macrophage-tropic HIV-1.[2-4] It has also been demonstrated that the initiation of signal transduction from CCR-5 is not required for viral entry,[5] and therefore, the occupancy of suitable sites of CCR-5 with low-molecular-weight ligands may possibly be effective for the treatment of HIV-1 infected individuals. To develop such a novel class of anti-HIV-1 agents, structural requirements for the anti-HIV-1 activity of chemokines should first be clarified. Recently, a study on RANTES-related peptides revealed that the amino-terminal region of RANTES was critical for receptor-binding and chemotactic activity.[6] This finding prompted us to

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1                                   10                                  20
Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys-Cys-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro-
                                    30                                  40
Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Tyr-Thr-Ser-Gly-Lys-Cys-Ser-Asn-Pro-Ala-Val-Val-
                                    50                                  60
Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys-Ala-Asn-Pro-Glu-Lys-Lys-Trp-Val-Arg-Glu-

Tyr-Ile-Asn-Ser-Leu-Glu-Met-Ser
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Fig. 1. Amino Acid Sequence of Human RANTES

* To whom correspondence should be addressed.

study the role of this region for anti-HIV-1 activity. This communication deals with the synthesis and anti-HIV-1 activity of five peptides related to the active region for chemotaxis of RANTES.

First, Ac-(^{10}Ala-RANTES 1-10)-NH_2 **1** and Ac-(^{10}Ala,^{11}Ala-RANTES 5-14)-NH_2 **2**, in which the original Cys at position 10 and positions 10 and 11 were replaced with Ala to prevent disulfide formation, were synthesized, and their anti-HIV-1 activity was examined. These peptides have been synthesized by the multipin method for the chemotaxis study described above, but used without purification and sufficient characterization.[6] To obtain purified and fully characterized peptides, we synthesized these peptides by the conventional solid-phase method using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.[7] The desired sequences were constructed on Rink amide resin[8] by *N*-benzotriazolyl-oxytris-(dimethylamino)-phosphonium hexafluorophosphate[9] coupling in the presence of 1-hydroxybenzotriazole and *N*,*N*-diisopropylethylamine. After *N*-terminal acetylation with acetic anhydride, the protected peptide-resins were treated with trifluoroacetic acid containing 5% anisole, and the crude products thus obtained were purified to homogeneity by preparative reversed-phase HPLC. The *m/z* values on FAB-MS of the purified products were consistent with the theoretical values.[10]

The anti-HIV-1 activity of these peptides was examined in comparison to that of recombinant RANTES (rRANTES). Phytohemagglutinin-activated peripheral blood mononuclear cells were infected with macrophage-tropic HIV-1 virus (JR_{CSF}) and cultured in the presence or the absence of synthetic peptides. After 7 days, the amount of soluble HIV-1 p24 in each culture supernatant was determined by ELISA. Details of the anti-HIV-1 assay were described in a previous paper.[11] The anti-HIV-1 activity of peptides was represented as % decrement of p24 from the amount in the absence of peptides, and is shown in Table 1. Peptide **1** was thus confirmed to exhibit significant anti-HIV-1 activity. Peptide **2** was also active, but less potent than **1**. These results clearly indicate the importance of the amino-terminal region of RANTES for anti-HIV-1 activity.

To discuss which amino acid sequence is important for anti-HIV-1 activity, *i.e.*, the sequence before the first disulfide bridge (1-9) or the one after that (10-14), Ac-(^{10}Ala,^{11}Ala-RANTES 6-14)-NH_2 **3**, Ac-(^{10}Ala,^{11}Ala-RANTES 7-14)-NH_2 **4**, and Ac-(^{10}Ala,^{11}Ala-RANTES 8-14)-NH_2 **5** were synthesized, purified, and characterized[10] in a similar manner. Peptides **3**, **4**, and **5** were then assayed and found to exhibit no appreciable anti-HIV-1 activity at the concentrations examined, as listed in Table 1. These data provide evidence that the amino-terminal sequence before the disulfide plays an important role in anti-HIV-1 activity.

Table 1. Anti-HIV-1 Activity of RANTES Amino-terminal Peptides

		% inhibition	
		10 nM	100 nM
Ac-SPYSSDTTPA-NH_2	**1**	54	64
Ac-SDTTPAAFAY-NH_2	**2**	15	45
Ac-DTTPAAFAY-NH_2	**3**	0	12
Ac-TTPAAFAY-NH_2	**4**	0	0
Ac-TPAAFAY-NH_2	**5**	0	0
rRANTES		46	88

We have thus clarified that the amino-terminus 1-10 of RANTES, which is known to be the critical region for chemotaxis, is also important for anti-HIV activity. This finding is seemingly inconsistent with the anti-HIV-1 activity of RANTES 9-68, which lacks the first eight amino acids.[12] However, the anti-HIV-1 activity of RANTES[2] and aminooxypentane (AOP)-RANTES,[13] which have the amino-terminal sequence, is higher than that of RANTES 9-68. Moreover, RANTES 19-28, 21-30, 23-32, 41-50, and 43-52 are known to exhibit moderate chemotactic activity.[6] This strongly suggests the existence of multiple receptor-binding domains in RANTES. Some of them might contribute to inhibition of viral entry, and the amino-terminus 1-10 is probably one of them. Ac-(^{10}Ala-RANTES 1-10)-NH_2 **1** is the smallest anti-HIV-1 chemokine analogue at present and would be an important lead compound for the development of chemokine receptor-directed anti-HIV-1 agents. For this purpose, further structure-activity relationship (SAR) study to reduce chemotactic activity and to enhance anti-HIV-1 activity is required. Systematic SAR study of peptides **1** is now under way in our laboratories.

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10) **1**, *m/z* 1066 (MH^+); **2**, *m/z* 1084 (MH^+); **3**, *m/z* 997 (MH^+); **4**, *m/z* 882 (MH^+); **5**, *m/z* 781 (MH^+).
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