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

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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 decapptide corresponding to positions 1-10 [Ac-(10)Ala-RANTES 1-10)-NH2] showed significant anti-HIV-1 activity. Ac-(10)Ala, 11-Ala-RANTES 5-14)-NH2 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) 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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\begin{array}{c}
\text{Ser-Pro-Tyr-Ser-Ser-Asp-Thr-Thr-Pro-Cys-Cys-Phe-Ala-Tyr-Ile-Ala-Arg-Pro-Leu-Pro} \\
\text{Arg-Ala-His-Ile-Lys-Glu-Tyr-Phe-Thr-Ser-Gly-Lys-Cys-Ser-Asn-Pro-Ala-Val-Val} \\
\text{Phe-Val-Thr-Arg-Lys-Asn-Arg-Gln-Val-Cys-Ala-Asn-Pro-Glu-Lys-Lys-Trp-Val-Arg-Glu} \\
\text{Tyr-Ile-Asn-Ser-Leu-Glu-Met-Ser}
\end{array}
\]

Fig. 1. Amino Acid Sequence of Human RANTES

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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-(\textsuperscript{10}Ala-RANTES 1-10)-NH\textsubscript{2} \textbf{1} and Ac-(\textsuperscript{10}Ala,\textsuperscript{11}Ala-RANTES 5-14)-NH\textsubscript{2} \textbf{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.\textsuperscript{6} To obtain purified and fully characterized peptides, we synthesized these peptides by the conventional solid-phase method using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry.\textsuperscript{7} The desired sequences were constructed on Rink amide resin\textsuperscript{8} by N-benzotriazolyl-oxytris-(dimethylamino)-phosphonium hexafluorophosphate\textsuperscript{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.\textsuperscript{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 (JRCSF) 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.\textsuperscript{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 \textbf{1} was thus confirmed to exhibit significant anti-HIV-1 activity. Peptide \textbf{2} was also active, but less potent than \textbf{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, \textit{i.e.,} the sequence before the first disulfide bridge (1-9) or the one after that (10-14), Ac-(\textsuperscript{10}Ala,\textsuperscript{11}Ala-RANTES 6-14)-NH\textsubscript{2} \textbf{3}, Ac-(\textsuperscript{10}Ala,\textsuperscript{11}Ala-RANTES 7-14)-NH\textsubscript{2} \textbf{4}, and Ac-(\textsuperscript{10}Ala,\textsuperscript{11}Ala-RANTES 8-14)-NH\textsubscript{2} \textbf{5} were synthesized, purified, and characterized\textsuperscript{10} in a similar manner. Peptides \textbf{3}, \textbf{4}, and \textbf{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 |
|-----------------------------------------------|----|----|
|                                                | 10 nM | 100 nM |
| Ac-SPYSSDTTPA-NH\textsubscript{2} \textbf{1}  | 54   | 64   |
| Ac-SDTTPAFAFAY-NH\textsubscript{2} \textbf{2} | 15   | 45   |
| Ac-DTTPAFAFAY-NH\textsubscript{2} \textbf{3} | 0    | 12   |
| Ac-TTPAFAFAY-NH\textsubscript{2} \textbf{4} | 0    | 0    |
| Ac-TPAFAFAY-NH\textsubscript{2} \textbf{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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REFERENCES AND NOTES

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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