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Anti-HIV-1 Peptides Derived from Partial Amino Acid Sequences of CC-Chemokine RANTES

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Abstract—Fifteen acetyl-peptide-amides with partial amino acid sequences of RANTES (regulated upon activation, normal T-cell expressed and secreted), all Cys residues of which were substituted by Ala, were synthesized, and screened for anti-HIV-1 activity. Peptides corresponding to 1–10, 37–46, and 57–68 showed marked activity against CC-chemokine receptor 5-using HIV-$1_{JR\text{-}CSF}$ (% inhibition at 100 nM 69, 82, 76%, respectively). The results indicate that multiple regions, including the N-terminal part responsible for chemotactic activity, are involved in anti-HIV-1 activity of RANTES, yielding possible lead compounds for anti-HIV-1 agents.

Introduction

Acquired immunodeficiency syndrome (AIDS) caused by infection with human immunodeficiency virus type 1 (HIV-1) remains a serious disease, although two classes of chemotherapeutic agents, reverse transcriptase inhibitors and proteinase inhibitors, are effective to suppress the viral replication and consequently prolong the life span of affected people. New anti-HIV-1 drugs are being explored extensively because of limitations of the above medicines, such as severe adverse effects and appearance of drug-resistant mutants.[1]

Recent studies on coreceptors for cell entry by HIV-1 have provided us a remarkably advanced understanding of the pathogenesis of AIDS, shedding light on chemokine receptors as potential targets for anti-HIV-1 therapeutics.[2–4] CC-Chemokine receptor-5 (CCR5) and CXC-chemokine receptor-4 (CXCR4) were identified as coreceptors essential for the macrophage-tropic and T-cell tropic strains of HIV-1, respectively.[5–7] Physiological ligands for these chemokine receptors including RANTES (regulated upon activation, normal T-cell expressed and secreted) inhibit the replication of HIV-1 in peripheral blood mononuclear cells (PBMCs) effectively at nanomolar levels.[8,9] N-Terminal modified and truncated RANTES are potent anti-HIV-1 proteins devoid of the agonistic activity.[10–13] Recently, low-molecular-weight CCR5-antagonists, TAK779,[14] piperidino-piperazines,[15] 1-amino-2-aryl-4-(piperidin-1-yl)butanes[16,17] and E913,[18] and CXCR4 antagonists, AMD3100,[19] T-22,[20] ALX40–4C,[21] and 3,6,9,12-tetraazatetradecane-1,14-diylbis(zinc dithiocarbamate)-*S*,*S*′-dioxide,[22] have been found to inhibit the replication of CCR5-using and CXCR4-using HIV-1, respectively.

Short peptides with minimum structural requirements for the desired pharmacological activity hold greater potential as lead compounds for rational drug design than macromolecular proteins. Systematic peptide screening appears a powerful and logical approach to obtain such peptide leads, as exemplified by successful identification of small bioactive peptides from various proteins including laminin,[23] ras oncogen product p21ras,[24] and CD66.[25] Here we describe a peptide

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screening study to explore small anti-HIV-1 peptides from RANTES, which inhibits replication of CCR5-using HIV-1 at the lowest concentrations among anti-HIV-1 CC-chemokines.[26] This is the first paper, except our preliminary communications,[27,28] disclosing low-molecular-weight anti-HIV-1 peptides effective for CCR5-using HIV-1.

Results and Discussion

Peptide synthesis

RANTES consists of 68 amino acid residues including four Cys residues, the first two being located adjacently to form a unique sequence of the CC-chemokine family, Cys-Cys (Fig. 1).[29] We employed a screening strategy similar to that in a previous study, in which RANTES fragments were screened for the chemotactic activity,[30] to permit comparison of anti-HIV-1 activity and chemotactic activity of synthetic peptides. All Cys residues were replaced by Ala to avoid potential disulfide formation.

The desired peptides were synthesized by conventional Fmoc-based solid-phase method[31] using Rink amide resin followed by deprotection with trimethyl bromosilane–thioanisole in TFA.[32] The crude products were purified by HPLC to homogeneity (95% or higher) and characterized by FAB-MS.

Anti-HIV-1 activity

The anti-HIV-1 activity of synthetic peptides was assayed using phytohemagglutinin-stimulated PBMCs and CCR5-using HIV-$1_{JR\text{-}CSF}$ as described previously,[33] and is represented as% decrement of HIV-1 p24 in culture supernatants as compared to the amount in the culture incubated in the absence of peptides. Recombinant RANTES was included as a reference.

As shown in Table 1, three peptides, that is, Ac-(C10A-RANTES 1–10)-NH_2, Ac-(RANTES 37–46)-NH_2, and Ac-(RANTES 57–68)-NH_2, exhibited marked anti-HIV-1 activity at submicromolar concentrations. Ac-(C10A,C11A-RANTES 5–14)-NH_2 and Ac-(RANTES 53–62)-NH_2 were also active, but less potent than the above peptides. Other peptides showed undetectable or marginal activity.

Two anti-HIV-1 peptides, Ac-(C10A-RANTES 1–10)-NH_2 and Ac-(C10A,C11A-RANTES 5–14)-NH_2, were found from the N-terminal region. A previous study showed that those peptides were potent chemotactic peptides.[30] The data from present study suggests that the N-terminal region of RANTES is important not only for chemotactic activity but also for anti-HIV-1 activity. Though these peptides are agonistic, further structure–activity studies upon these N-terminal peptides might yield more promising lead compounds for anti-HIV-1 agents with diminished chemotactic activity.

The so-called N-loop (Phe12-Pro20) was shown to play important roles in binding with CCR5.[34] Ac-(C10A,C11A-RANTES 9–18)-NH_2, which covers almost entire N-loop region including critical residues Phe12 and Ile15, showed no detectable anti-HIV-1 activity. Since this synthetic peptide contains two Ala-substitutions, it may not reflect the tertiary structure of positions 9–18 of RANTES. Thus it might be inappropriate to exclude the possibility that this region involves in the anti-HIV-1 effect of RANTES.

It is noteworthy that Ac-(RANTES 37–46)-NH_2 showed anti-HIV-1 activity comparable with RANTES. Furthermore, this peptide has no significant chemotactic activity.[30] Ac-(RANTES 37–46)-NH_2 appears a promising lead for anti-HIV-1 agents devoid of undesirable chemotactic activity.

Table 1. Anti-HIV-1 activity of synthetic peptides

Compound	% Inhibition[a]	
	10 nM	100 nM
Ac-(C10A-RANTES 1–10)-NH_2	51	69
Ac-(C10A,C11A-RANTES 5–14)-NH_2	18	37
Ac-(C10A,C11A-RANTES 9–18)-NH_2	NI	NI
Ac-(RANTES 13–22)-NH_2	NI	NI
Ac-(RANTES 17–26)-NH_2	NI	NI
Ac-(RANTES 21–30)-NH_2	10	5
Ac-(C34A-RANTES 25–34)-NH_2	NI	NI
Ac-(C34A-RANTES 29–38)-NH_2	NI	NI
Ac-(C34A-RANTES 33–42)-NH_2	10	14
Ac-(RANTES 37–46)-NH_2	72	82
Ac-(C50A-RANTES 41–50)-NH_2	NI	NI
Ac-(C50A-RANTES 45–54)-NH_2	19	5
Ac-(C50A-RANTES 49–58)-NH_2	NI	NI
Ac-(RANTES 53–62)-NH_2	53	52
Ac-(RANTES 57–68)-NH_2	35	76
Recombinant RANTES	53	95

[a]Phytohemagglutinin-stimulated PBMCs were infected by HIV-$1_{JR\text{-}CSF}$ and cultured in the presence or absence of synthetic peptides for 7 days. The anti-HIV-1 activity of peptides represents % decrement of soluble HIV-1 p24 as compared to the amount in the absence of peptides (means of 3–6 replicates). NI, no inhibition (<5%).

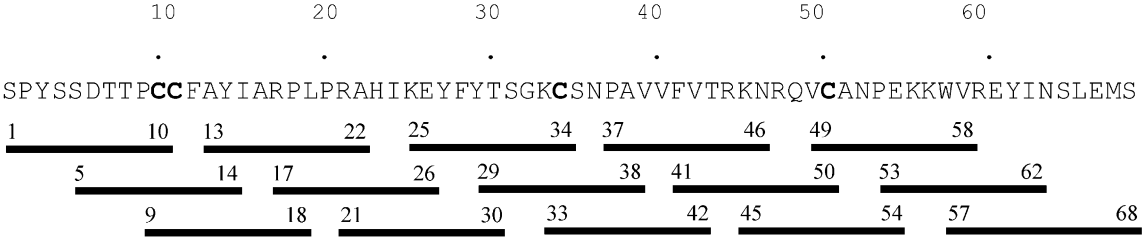

Figure 1. Amino acid sequence of human RANTES. Thick bars show the positions corresponding to synthetic peptides used in this study. All Cys residues (C) were replaced by Ala.

Two anti-HIV-1 peptides were found from the C-terminal region, which plays a crucial role in binding with cell surface glycosaminoglycans (GAGs) to form and maintain chemokine gradients required to recruit leukocytes to sites of inflammation.[35] Several recent studies suggest that the GAG-binding domain of chemokines plays an important role in the anti-HIV-1 effect as well.[36–40] From data available in the above literatures, the anti-HIV-1 effect of C-terminus-derived peptides found here appears unlikely to be attributed to interaction with chemokine receptors. Even if it is the case, the anti-HIV-1 effect of the C-terminal peptides is not an inconsistent observation, since HIV-1 itself is held to interact with cell-surface GAGs[41–44] and GAG-biding compounds, in principle, may compete with virus. The C-terminal peptides might be useful to potentiate anti-HIV-1 agents including RANTES-derived anti-HIV-1 peptides by reflecting the chemokine delivery mechanism mentioned above, as exemplified by the enhanced biological activity of SDF-1 N-terminal peptide (corresponding to positions 5–14) conjugated with the peptide derived from the C-terminal GAG-binding domain (corresponding to 55–67) via a tetraglycine linker.[45]

Conclusion

Consequently, the results obtained here indicate that multiple regions including the N-terminal part responsible for the chemotactic activity are involved in the anti-HIV-1 effect of RANTES. Low-molecular-weight anti-HIV-1 peptides effective for CCR5-using HIV-1, unlike those effective for CXCR4-using virus,[46,47] have not been found to date, and the anti-HIV-1 peptides found here, particularly Ac-(RANTES 37–46)-NH_2, potentially serve as leads to realize the rational design of anti-HIV-1 agents effective for CCR5-using species.

Experimental

HPLC was conducted with a Waters 600 system equipped with a UV/VIS detector (220 nm). A and B in the mobile-phase system refer to 0.05% aq TFA and 0.05% TFA in MeCN, respectively. Columns and flow rates for analysis and purification were YMC (Kyoto, Japan) R-ODS (4.6×250 mm), 1.0 mL/min and D-ODS (20×250 mm), 10 mL/min, respectively.

Protected amino acids, TFA, *m*-cresol, thioanisole, trimethyl bromosilane, and ethanedithiol were purchased from Watanabe Chemical Industries (Hiroshima, Japan). DMF was of peptide synthesis grade. Piperidine and Et*N*iPr_2 were distilled from KOH and ninhydrin before use and stored at 4 °C. Recombinant RANTES was purchased from R&D System (Minneapolis, MN). Other reagents were of reagent grade and used without purification.

Peptide synthesis

General procedures. Side-chain protecting groups used were as follows: *t*Bu for Ser, Thr, Tyr, Asp and Glu; Boc for Lys and Trp; Trt for Asn, Gln and His; 4-methoxy-2,3,6-trimethylbenzesulfonyl for Arg. Starting from Rink amide resin (0.35 mmol/g; 143 mg, 0.05 mmol), solid-phase synthesis was carried out according to the following protocol: (1) DMF (4 mL), 1 min×5; (2) 20% piperidine in DMF (4 mL), 3 min×2, 20 min×1; (3) DMF (4 mL), 1 min×10; (4) Fmoc-amino acid (0.20 mmol), BOP (89 mg, 0.20 mmol), 0.5 M HOBt in DMF (0.40 mL), 1.0 M Et*N*iPr_2 in DMF (0.60 mL), DMF (3 mL), 45 min; (5) DMF (4 mL), 1 min×5; repeat from step (1). After removal of the terminal Fmoc group, acetic anhydride (19 μL, 0.20 mmol) and 1.0 M Et*N*iPr_2 in DMF (0.20 mL, 0.20 mmol) were added to the resin suspended in DMF (4 mL), and the mixture was agitated for 60 min. The resulting peptide resin was washed with DMF (4 mL; 1 min×10), CH_2Cl_2 (4 mL; 1 min×5), MeOH (4 mL; 1 min×5), and *n*-hexane (4 mL; 1 min×5), and then dried over $CaCl_2$ in vacuo. The protected peptide-resins obtained were treated with trimethyl bromosilane (0.26 mL, 2.0 mmol), thioanisole (0.24 mL, 2.0 mmol), *m*-cresol (0.10 mL), ethanedithiol (0.20 mL) in TFA (1.20 mL) at an ice-bath temperature for 90 min. After evaporation in vacuo, diethyl ether was added to the residue to afford a precipitate, which was collected by centrifugation and washed thoroughly with diethyl ether. The crude products were purified by HPLC. After lyophilization, the purified materials were dissolved in 0.01 N-HCl and lyophilized again.

Ac-SPYSSDTTPA-NH_2 [Ac-(C10A-RANTES 1–10)-NH_2]. Yield, 16 mg (30%). t_R 22.51 min (98%; A:B 95:5 for 5 min, then 95:5 to 40:60 in 55 min); *m*/*z* (FAB) 1066 (MH^+).

Ac-SDTTPAAFAY-NH_2 [Ac-(C10A,C11A-RANTES 5–14)-NH_2]. Yield, 15 mg (28%). t_R 25.45 min (99%; A:B 95:5 to 50:50 in 45 min); *m*/*z* (FAB) 1084 (MH^+).

Ac-PAAFAYIARP-NH_2 [Ac-(C10A,C11A-RANTES 9–18)-NH_2]. Yield, 36 mg (64%). t_R 18.69 min (>99.5%; A:B 80:20 to 40:60 in 40 min); *m*/*z* (FAB) 1117 (MH^+).

Ac-AYIARPLPRA-NH_2 [Ac-(RANTES 13–22)-NH_2]. Yield, 39 mg (67%). t_R 18.68 min (99%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1168 (MH^+).

Ac-RPLPRAHIKE-NH_2 [Ac-(RANTES 17–26)-NH_2]. Yield, 45 mg (71%). t_R 12.93 min (>99.5%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1257 (MH^+).

Ac-RAHIKEYFYT-NH_2 [Ac-(RANTES 21–30)-NH_2]. Yield, 18 mg (26%). t_R 19.31 min (99%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1368 (MH^+).

Ac-KEYFYTSGKA-NH_2 [Ac-(C34A-RANTES 25–34)-NH_2]. Yield, 22 mg (35%). t_R 17.35 min (95%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1234 (MH^+).

Ac-YTSGKASNPA-NH_2 [Ac-(C34A-RANTES 29–38)-NH_2]. Yield, 24 mg (46%). t_R 9.99 min (97%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1036 (MH^+).

Ac-KASNPAVVFV-NH_2 [Ac-(C34A-RANTES 33–42)-NH_2]. Yield, 12 mg (22%). t_R 22.13 min (>99.5%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1072 (MH^+).

Ac-PAVVFVTRKN-NH_2 [Ac-(RANTES 37–46)-NH_2]. Yield, 41 mg (73%). t_R 24.17 min (>99.5%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1171 (MH^+).

Ac-FVTRKNRQVA-NH_2 [Ac-(C50A-RANTES 41–50)-NH_2]. Yield, 45 mg (71%). t_R 15.10 min (99%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1259 (MH^+).

Ac-KNRQVAANPE-NH_2 [Ac-(C50A-RANTES 45–54)-NH_2]. Yield, 28 mg (48%). t_R 7.00 min (95%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1167 (MH^+).

Ac - VAANPEKKWV - NH_2 [Ac - (C50A - RANTES 49–58)-NH_2]. Yield, 16 mg (27%). t_R 18.32 min (95%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1182 (MH^+).

Ac-PEKKWVREYI-NH_2 [Ac-(RANTES 53–62)-NH_2]. Yield, 20 mg (29%). t_R 20.50 min (96%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1388 (MH^+).

Ac-WVREYINSLEMS-NH_2 [Ac-(RANTES 57–68)-NH_2]. Yield, 15 mg (19%). t_R 33.51 min (95%; A:B 90:10 to 40:60 in 50 min); *m*/*z* (FAB) 1567 (MH^+).

Anti-HIV-1 activity

Anti-HIV-1 activity of synthetic peptides was measured as reported previously.[32] Briefly, PBMCs (50×10^5) stimulated with phytohemagglutinin (1 mg/mL) and then infected by HIV-$1_{JR\text{-}CSF}$ (MOI = 0.001; 37 °C, 2 h) were incubated in the presence and absence of peptides at 37 °C for 7 days, and p24 concentrations in the supernatants were determined by ELISA (Cellular Products).

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