Synthesis of Peptides Mimicking Chemokine Receptor CCR5 and Their Inhibitory Effects against HIV-1 Infection

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Peptides mimicking chemokine receptor CCR5 were synthesized and their anti-HIV-1 activities evaluated. Prepared compounds, especially a sulfated derivatives, showed significant anti-HIV-1 activities. Furthermore, a hybrid molecule linked to an N-carbomethoxycarbonyl-prolyl-phenylalanine (CPF) moiety had a greater effect.

Key words mimicking peptide; anti-HIV-1 activity; CPF; sulfation; chemokine receptor; hybrid molecule

CD4 is the primary cellular receptor for human immunodeficiency virus type 1 (HIV-1), but CD4 alone is not sufficient to allow the entry of HIV-1 into cells. In 1996, the cellular coreceptors that HIV-1 requires in conjunction with CD4 were identified as members of the chemokine receptor family of seven-transmembrane G protein-coupled receptors.1) This discovery of distinct chemokine receptors explains the differences in cell tropism between viral strains. Thus CXCR4 supports entry of T cell (T)-tropic HIV-1 strains, whereas CCR5 supports macrophage (M)-tropic HIV-1 strains. Recently, these chemokine receptors have received much attention as attractive targets for new antiviral therapies.2) The mechanism of entry of HIV-1 into cells is thought to be: first, gp120-CD4 conjugation induces conformational changes in the gp120 subunit, including exposure of the V3 loop, and then conjugation with the coreceptor occurs.3) It is known that these coreceptors are rich in tyrosines and acidic amino acids at their N-terminal region, and this contributes to the ability of HIV-1 to fuse with and enter into target cells.4) A strongly positive region of the V3 loop has been shown to be important for the association with CCR5. Consequently, it can be considered that some of these positively charged residues may directly and complementarily interact with a sequence of highly negatively charged acidic amino acids. It is known that N-carbomethoxycarbonyl-prolyl-phenylalanine (CPF)5) mimics CD4 and binds selectively to gp120. We previously reported the synthesis of the hybrid compounds linked to the CPF moiety as HIV protease inhibitors3) and showed that these compounds bind selectively to infected cells, probably based on an interaction between CPF and gp120. Therefore we focused on the synthesis of peptides mimicking CCR5 with a CPF moiety. As a part of a program aimed at the development of new HIV-1 inhibitors, we would like to report the design and synthesis of peptides mimicking CCR5 for prevention of HIV-1 infection based on a strategy of binding to gp120.

Results and Discussion

In the amino acid sequences of the N-terminal domain of CCR5, we first chose the region of Tyr10-Glu18 including tyrosines, glutamic acid, and aspartic acid as a target peptide moiety. The peptide of natural type 2a from 1 and its mimicking peptides 2b—e consisting of acidic amino acids as well as unnatural-type amino acids as the spacer unit were prepared (Chart 1).7) Resistance to the action of protease was expected from the latter compounds.

Compounds 2a—e had no expected anti-HIV-1 activities8) against M-tropic strains of HIV-1; on the other hand, these compounds showed significant anti-HIV-1 activities against T-tropic strains. Thus the percentage of HIV-1 antigen-positive cells with 2a—e was 23.3—28.3%, whereas that with CPF was 27.7%. Furthermore, the anti-HIV-1 activities of 2a—e were increased by the addition of CPF as an additive (11.0—26.7%).

To enhance the selectivity of binding to HIV-1 and hence the anti-HIV-1 activity, a hybrid compound 3 of 2a and CPF was designed and prepared (Chart 2). The synthesis of the hybrid molecule was straightforward and the construction of the covalent linkage between the CPF moiety and the peptide moiety was carried out using spacers derived from o-aminophenol. However, compound 3 prepared in this way exhibited the same level of anti-HIV-1 activity as that of 2a.

Recently, it was suggested that the hydroxyl groups of ty-
rosines in the N-terminal region of CCR5 are modified in the form of sulfates. 10) For the purpose of achieving increased anti-HIV-1 activity, some sulfated analogues were prepared. The sulfates11) 4a—c and 5 were derived from 1 and 3, respectively, and these transformations are also shown in Chart 2.12) The results of biological assay for sulfated compounds 4a—c and 5 are listed in Table 1. As can be seen from the table, in spite of the low activity of 4a and 4b, compounds 4c and 5, especially 5, showed significantly higher anti-HIV-1 activity.

Conclusions

In this study, it was confirmed that enhancement of anti-HIV-1 activities with mimicking peptides was achieved by the addition of CPF. Additionally, the sulfation of the parent peptide, and more effectively, the conjugation of the sulfated peptide with CPF, caused an increase in anti-HIV-1 activity. These results suggest that highly negatively charged sulfated groups interact strongly with a positively charged region of the V3 loop. The observations in this study will be helpful for a better understanding of the mechanism of HIV-1 infection, and hence development of new types of drugs against AIDS.

References and Notes

7) 2a: FAB-MS m/z 1167 (M+1)⁺; 2b: FAB-MS m/z 827 (M+1)⁺; 2c: FAB-MS m/z 857 (M+1)⁺; 2d: FAB-MS m/z 857 (M+2)⁺; 2e: FAB-MS m/z 887 (M+1)⁺.
9) F: FAB-MS m/z 1669 (M+Na⁺).
12) 4c: FAB-MS m/z 1567 M⁺; 5: FAB-MS m/z 1849 (M—3SO₂H+2Na—2H)⁺.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>—SO₂X</th>
<th>Drug concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>H</td>
<td>232 258 225 219</td>
</tr>
<tr>
<td>4b</td>
<td>Na</td>
<td>207 236 255 251</td>
</tr>
<tr>
<td>4c</td>
<td>NEt₃</td>
<td>57 138 245 n.t.</td>
</tr>
<tr>
<td>5</td>
<td>NEt₃</td>
<td>2 40 223 n.t.</td>
</tr>
</tbody>
</table>

Table 1. The Anti-HIV-1 Activities of 4a—c and 5

References and Notes

7) 2a: FAB-MS m/z 1167 (M+1)⁺; 2b: FAB-MS m/z 827 (M+1)⁺; 2c: FAB-MS m/z 857 (M+1)⁺; 2d: FAB-MS m/z 857 (M+2)⁺; 2e: FAB-MS m/z 887 (M+1)⁺.
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