A Molecular Switch of Chemokine Receptor Selectivity

CHEMICAL MODIFICATION OF THE INTERLEUKIN-8 Leu25 → Cys MUTANT*

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Interleukin-8 (IL-8), a member of the CXC chemokine family, is a key activator of neutrophils. We have previously shown that two novel CC chemokine-like properties, namely monocyte chemotactic activity and binding to CC CKR-1, are introduced into IL-8 by mutating Leu25 to the conserved tyrosine present in CC chemokines. To further investigate the role of this position in receptor selectivity, we have mutated Leu25 to cysteine. The protein folds correctly with two disulfide bonds and a free thiol group at Cys25. This mutant behaves overall like wild-type IL-8, with little change in neutrophil chemotaxis and IL-8 receptor binding, and has no effect on CC CKR-1. These data are consistent with cysteine being approximately isosteric with the natural amino acid leucine. However, modification of the cysteine by addition of a fluorescent N-methyl-N-(2-N-methyl-N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)aminoethyl)acetamido (NBD) group lowers potency in neutrophil chemotaxis and affinity in IL-8 receptor binding assays by 2 orders of magnitude. This Leu25 → Cys-NBD mutant introduces monocyte chemotactic activity and the ability to displace labeled macrophage inflammatory protein-1α from the recombinant CC CKR-1 receptor. Additionally, we show a specific interaction between the fluorescent mutant and the N-terminal 34-amino acid peptide from CC CKR-1. This confirms the importance of this region in IL-8 in receptor binding and in conferring specificity between CXC and CC chemokines. Circular dichroism spectra of the IL-8 mutants having CC chemokine-like activity show a consistent drop in α-helical content compared with the spectra for wild-type IL-8. This suggests that distortion of the C-terminal helix may play a role in chemokine receptor-ligand selectivity.

Chemokines are a large family of 8–10-kDa proteins that are important in the recruitment and activation of leukocytes in inflammatory diseases. CXC chemokines, for example interleukin-8 (IL-8), play a key role in acute inflammation by attracting and activating neutrophils. Two receptors, IL-8R-A (1) and IL-8R-B (2), have been identified that bind with nanomolar affinity to IL-8. The regions necessary for receptor activation and subsequent signaling through G-proteins have been localized at the flexible amino terminus of IL-8 (Glu4-Leu5-Arg6) by mutagenesis and peptide synthesis studies (3, 4).

Members of the CC chemokine family, such as macrophage inflammatory protein-1α (MIP-1α) and RANTES, activate a variety of cell types including monocytes during chronic inflammation. The CC chemokines mediate this effect through a different family of receptors including CC CKR-1, which binds MIP-1α and RANTES (5, 6); CC CKR-2, which binds MCP-1 and MCP-3 (7–9); CC CKR-3, which binds RANTES, MIP-1α, and MIP-1β (10); and CC CKR-4, which responds to RANTES, MCP-1, and MIP-1α (11). As in CXC chemokines, it is the amino-terminal region of the ligand that is responsible for receptor activation. Truncated N-terminal mutants of MCP-1 have been shown to be antagonists of MCP-1-induced monocyte chemotaxis (12). Mutants of RANTES that have an additional methionine residue at the amino terminus have been shown to be antagonists of TNP-1 and T-cell activation (13).

To date, no natural human CXC ligand has been found to bind a CC chemokine receptor. To investigate the molecular basis of this selectivity, we have compared the primary sequences of CXC and CC chemokines. In the region of IL-8 corresponding to the inner β-sheet, there is a leucine residue, corresponding to Leu25, that is conserved as a small hydrophobic amino acid in CXC chemokines, but is always a tyrosine in CC chemokines. We have made the Leu25 → Tyr mutant and shown its ability to attract peripheral blood monocytes and displace MIP-1α from its CC CKR-1 receptor, two activities that IL-8 does not possess (14). We have further investigated the molecular basis of this receptor selectivity by making the Leu25 → Cys mutation. Cysteine is approximately isosteric with leucine and would be expected to have similar activity compared with wild-type IL-8. However, the free thiol group is chemically reactive and can be modified with a variety of reagents such as the fluorescent group N,N′-dimethyl-N-(iodoacetyl)-N′-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)ethylenediamine (IANBD-amide) that we added onto Cys25.

We show here that the Leu25 → Cys mutant binds to IL-8R-A and IL-8R-B and activates neutrophils while having no effect on monocytes. Chemical modification of the cysteine residue with a fluorescent NBD group, however, introduces monocyte chemotactic activity and the ability to displace MIP-1α from its CC CKR-1 receptor. This fluorescent probe has been used to study heterodimer formation when unlabeled IL-8 is added as well as binding to a 34-amino acid peptide from the C terminus of the CC CKR-1 receptor. Finally, by comparison of the CD spectra, we can show that IL-8 mutants showing CC chemokine activity have a lower α-helical content compared with IL-8. This indicates that a distortion of the C-terminal helix is important in...
altering the selectivity between CXC and CC chemokines and suggests a possible mechanism for receptor selectivity.

**EXPERIMENTAL PROCEDURES**

Reagents—Unless otherwise stated, all chemicals were purchased from Sigma.

Construction and Expression of IL-8 Leu25→ Cys—Human IL-8, cloned by polymerase chain reaction from a peripheral blood monocyte cell line, was expressed in Escherichia coli B-cells using the trp promoter (19). Mutant proteins were made by resynthesizing a cassette containing the Ndel and SacI sites coding for amino acid 1–50. The Leu25→ Cys mutant was constructed using the following oligonucleotides, and the sequence was verified by the dideoxy chain termination method: 5′-CCCTCCACCCCCAATTTTGGAAAATTTGGGGTG-3′ (upper strand) and 5′-GGTCCACCTCC-AATCCACCTCATAATGTTGATAAATTTGGGGTG-3′ (lower strand). Both wild-type IL-8 and the Leu25→ Cys mutant are highly expressed using this system.

Purification of IL-8 Leu25→ Cys—The purification using 100 g of E. coli cells, wet weight, was similar to the method described previously (14). The glutathione used in the renaturation step was shown to be attached to the thiol and was consequently removed by dissolving the protein lysophylase in PBS (140 mM NaCl, 3 mM KCl, 8 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.4), adding dithiothreitol to a final concentration of 1 mM, and incubating for 1 h at 37 °C. The sample was desalted on a PD-10 gel filtration column in the same buffer, diazylated against 0.1% trifluoroacetic acid, and lyophilized. The molecular mass of the final protein was verified by electrospray ionization mass spectrometry (15).

Analytical Methods—Amino acid analysis was performed after gas-phase hydrolysis of 1 nmol of protein in 6 M HCl containing 1 mg/ml of 1,1′-dithiobis(2-nitrobenzoate) (Ellman’s reagent) (16). For the determination of accessible free thiols, 1 mg of protein was dissolved in 0.1 M Tris-HCl, pH 7.5, containing 1% sodium dodecyl sulfate. A fresh solution of 5,5′-dithiobis(2-nitrobenzoate) (0.01 M in 0.05 M sodium phosphate buffer, pH 6.5) was added to the sample to a final concentration of 1 mM, and incubating for 1 h at 37 °C. The reaction was stopped by the addition of 5,5′-dithiobis(2-nitrobenzoate) (0.01 M in 0.05 M sodium phosphate buffer, pH 6.5) to a final concentration of 10 mM, and the increase in absorbance at 412 nm was monitored until a stable value was reached. The calculation of the number of free thiol groups was carried out using an absorbance coefficient of ε = 13,000 M−1 cm−1. To determine the total number of sulfhydryl groups, 1 mg of protein was reduced with 1 mM dithiothreitol and denatured in 6 M guanidine hydrochloride at 60 °C for 1 h. Excess dithiothreitol was removed on a Brownlee C-8 HPLC column (220× 2.1 mm), and the protein was lyophilized. The lyophilysate was taken up in the above-mentioned Tris buffer, and the modified residue was Cys25 and not one of the four other cysteines to IANBD-amide, and peptide mapping was carried out to verify that the correct molecular mass of the mutant protein coupled to CC CKR-1 receptor on 3×105 COS-7 cells was competed by addition of varying concentrations of unlabeled chemokine. The activity remained on the filters after washing was counted as described previously (14).

Fluorescence Studies of the Interaction of IL-8 Leu25→ Cys—The fluorescence of the NBD group was measured on a Jasco FP-777 spectrofluorometer (Omnialb AG, Geneva) using a quartz cuvette of 1-cm path length with an excitation wavelength of 472 and detecting emitted light at 536 nm. The protein was dissolved at 25 μg/ml in PBS, pH 7.4. The effect of added IL-8 was studied by incubating the Leu25→ Cys-NBD mutant with increasing concentrations of wild-type IL-8.

The interaction of the fluorescent NBD mutant with the amino terminus of CC CRK-1 was studied using a synthetic peptide corresponding to the N-terminal extracellular domain of this receptor. This amino-terminal peptide, M34A (Neosystem S. A., Strasbourg, France), has the following sequence: 1METPNTTEDYDTTTEFDYGDATPCQK-VRPLM (17). A 96-well multiscreen filter plates containing 3 × 105 HL-60 cells, 0.2 mg/ml M34A, labeled IL-8, and varying concentrations of chemokine. After incubation for 4°C, the cells were washed, and the bound radioactivity was counted (14). The data obtained were fitted using Grafit Version 3.01 (18) with simple weighting to the equation describing a competition for a single binding site: B = Bmax (1 + [1/IC50]).

CC CRK-1 Receptor Binding Assay—Binding of 0.3 nM 125I-labeled MIP-1α to CC CRK-1 receptors on 3×105 COS-7 cells was competed by addition of varying concentrations of unlabeled chemokine. The activity remaining on the filters after washing was counted as described previously (14). IL-8 Receptor Binding Assay—This assay was performed using HL-60 cells transfected by electroporation with the cDNA sequence for IL-8R-A and IL-8R-B. A final volume of 150 μl was added to 96-well multiscreen filter plates containing 3 × 105 HL-60 cells, 0.23 nM 125I-labeled IL-8, and varying concentrations of chemokine. After incubation at 4°C, the cells were washed, and the bound radioactivity was counted (14). The data obtained were fitted using Grafit Version 3.01 (18) with simple weighting to the equation describing a competition for a single binding site: B = Bmax (1/IC50).

Far-ultraviolet Circular Dichroism—Far-ultraviolet circular dichroism was measured on a chiral model 820A spectrometer using quartz cuvettes of 1-cm path length with an excitation wavelength of 200 and detecting emitted light at 200 nm. The reaction that monitors the conversion of tetrazolium blue into its formazan product was stopped with 100 mM Tris-HCl, pH 7.4, and the fluorescence was monitored at 560 nm. The peptide mapping was carried out using an absorbance coefficient of 0.1% trifluoroacetic acid.

The protocol for the monocyte chemotaxis assay using the 48-well micro-Bordhan chamber was described previously (14).

Far-ultraviolet Circular Dichroism—CD spectroscopy measurements were made in the far-ultraviolet range (198–250 nm), where the spectrum of proteins in solution is sensitive to their secondary structure. The measurements were done on an Aviv Model 62DS circular dichroism spectrometer using quartz cuvettes of 1-cm path length at 20.5 °C. Lyophilized samples were resuspended in 10 mM Tris-HCl, pH 7.5, at 0.03 mg/ml. A modified method of Hennessey and Johnson (20) was used to quench the CD spectrum. The samples were treated with 10 mM of the peptide mapping was carried out using the CD spectrum of the peptide. These points were then fitted with a reference data set of 15 proteins of known three-dimensional structure (21).
RESULTS

Mutagenesis and Expression—A trp expression system was used to express human recombinant IL-8 in E. coli B-cells (19). The IL-8 Leu25 → Cys mutant was assembled using an oligonucleotide cassette method. The purification and renaturation methods were identical for both proteins. The molecular mass of the IL-8 Leu25 → Cys mutant was found to be 8676.3 ± 0.9 Da instead of 8372 Da. This additional mass of 304 could be attributed to the presence of glutathione (predicted additional molecular mass = 305.3) used in the renaturation step attached to the free thiol. Upon removal of the glutathione, electrospray ionization mass spectroscopy for the mutant gave the expected mass of 8371.0 ± 1.3 Da. 100 g of cell paste yielded ~5 mg of pure protein, which was judged to be 98% pure by SDS-polyacrylamide gel electrophoresis (Fig. 1).

Analysis of IL-8 Leu25 → Cys—The determination of free sulfhydryl groups was performed using 5,5'-dithiobis(2-nitrobenzoate) with wild-type IL-8 as a control (16). The total number of thiols and the free number of thiols were found to be 3.95 and 0.02 for wild-type IL-8, respectively. This increased to 4.63 and 0.65 for the Leu25 → Cys mutant protein. Amino acid analysis of the IL-8 Leu25 → Cys protein confirmed the expected composition.

Fluorescent Labeling of IL-8 Leu25 → Cys with IANBD-amide—Analysis of the HPLC profile of the mutant protein after labeling with IANBD-amide indicated that all the initial material had reacted (Fig. 2). The IL-8 Leu25 → Cys mutant migrated as a single species on reverse-phase HPLC, with a retention time of 32.78 min. After incubation with IANBD, a new peak appeared at 34.97 min on the HPLC trace. Base-line separation of the starting material and product indicated that the neutrophil activity seen for the modified protein was not due to contamination with the IL-8 Leu25 → Cys mutant. To confirm the position of the incorporation of the NBD group, a peptide digest was performed using Glu-C protease. The fragments were analyzed by reverse-phase HPLC, detecting absorbance at 495 and 214 nm. Only one peptide had significant absorbance at 495 nm, indicating the presence of the NBD group. Sequencing of this peptide gave the sequence Glu24→Xaa-Arg-Val-Ile-Glu29. The unidentified PTH-derivative at position 25 had an absorption spectrum showing a maximum at 495 nm, confirming the incorporation of NBD at this position (Fig. 3).

Assay for Chemotactic and Receptor Binding Activity—In neutrophil chemotaxis, IL-8 shows a saturating dose-response curve with a midpoint at ~1.2 nM and a maximum efficacy of 12 (Fig. 4). The IL-8 Leu25 → Cys mutant has a midpoint at 3 nM, and the IL-8 Leu25 → Cys-NBD mutant has a midpoint at 27 nM. In these assays, RANTES showed no ability to cause neutrophil chemotaxis.

Receptor binding was assayed by displacement of 125I-labeled IL-8 from IL-8R-A or IL-8R-B on HL-60 cells. IL-8 shows equal affinity for both receptors, with IC50 values of 1.4 ± 0.1 nM for IL-8R-A and 1.9 ± 0.3 nM for IL-8R-B (Fig. 5). Under the conditions of the assay, where the concentration of 125I-labeled IL-8 is much lower than its Kd value, the IC50 values for the mutants equal the Kd values, to a first approximation (28). The IL-8 Leu25 → Cys mutant shows a decrease in affinity for both receptors, with IC50 values of 59 ± 0.5 and 19 ± 0.6 nM for IL-8R-A and IL-8R-B, respectively. The IL-8 Leu25 → Cys-NBD mutant is almost 100-fold weaker than IL-8 in binding to the receptors, with IC50 values of 170 ± 0.4 nM for IL-8R-A and 150
In a chemotaxis assay using peripheral blood monocytes, RANTES gives a bell-shaped curve with a maximum at 1 nM and maximal efficacy of 6 (Fig. 6). The IL-8 Leu$^{25}$Cys-NBD mutant protein is also able to induce monocyte chemotaxis, with a maximum at 12 nM and an efficacy similar to that of RANTES. This mutant also displaces MIP-1$alpha$ from the MIP-1$alpha$/RANTES (CCCKR-1) receptor (Fig. 7). MIP-1$alpha$ can displace the radioactive ligand from the receptor, with an IC$_{50}$ of 0.97 ± 0.03 nM, and the IL-8 Leu$^{25}$Cys-NBD mutant displaces $^{125}$I-labeled MIP-1$alpha$, with an IC$_{50}$ of 118 ± 0.8 nM. Both IL-8 and the IL-8 Leu$^{25}$Cys mutant show no activity in these two assays.

Fluorescence Studies—Addition of varying amounts of wild-type IL-8 to a solution of 7.5 nM IL-8 Leu$^{25}$Cys-NBD caused a concentration-dependent increase in fluorescence (Fig. 8). These data fitted well with the binding isotherm, $\phi = \phi_{\text{initial}} + (\phi_{\text{initial}} - \phi_{\text{final}}) \times L/[L] IC_{50} + [L]$, where $\phi_{\text{initial}} = 1217$ units was the initial fluorescence of the IL-8 Leu$^{25}$Cys-NBD in solution and $\phi_{\text{final}} = 2052$ units was the limiting fluorescence at infinite concentration of added IL-8. The IC$_{50}$ or midpoint concentration was 110 nM. When IL-8 Leu$^{25}$Cys-NBD was mixed with a 34-amino acid peptide from the amino terminus of CC CKR-1, there was a dose-dependent fluorescence decrease from 802 to 461 units (Fig. 9). The data were fitted to the binding isotherm, and a value of 156 nM was calculated for the IC$_{50}$. Similar data were obtained when RANTES specifically labeled at the N terminus was used, giving an IC$_{50}$ of 40 nM. However, no change in fluorescence was observed with N-terminally labeled IL-8, confirming that the change in fluorescence was due to a specific interaction with the N-terminal receptor peptide.

CD Measurements—We have carried out CD measurements in the far-ultraviolet range (198–250 nm) of 0.03 mg/ml samples of IL-8, RANTES, MIP-1$beta$, and the three IL-8 variants at 20.5°C in 10 mM Tris-HCl, pH 7.5. As can be seen in Fig. 10, there are clear differences between the spectra obtained for CXC and CC chemokines. In addition, the spectra for the IL-8 mutants showing monocyte chemotactic activity and CC CKR-1 binding show a third class of spectra compared with the wild-
type chemokine data. The results were compared with a standard data set (21) in order to determine the relative amounts of the secondary structural elements such as \( \alpha \)-helix and \( \beta \)-sheet. To validate our results, we compared the results obtained experimentally by CD with the theoretical values from known NMR data of IL-8 (22), RANTES (23), and MIP-1\( \beta \) (24) (Table I). We see that in all three cases, the experimental and theoretical values are only different by 1%. The IL-8 Leu25 \( \rightarrow \) Cys mutant shows similar structure to IL-8, but with a slight loss of \( \alpha \)-helix. The IL-8 Leu25 \( \rightarrow \) Tyr and IL-8 Leu25 \( \rightarrow \) Cys-NBD mutants, however, show a marked decrease in the \( \alpha \)-helical content with percent averages of 6 and 8, respectively, which is even lower than that for the CC chemokines. The other two secondary structural classes remain unchanged when compared with IL-8.

### DISCUSSION

Chemokines play a key role in inflammatory diseases by selectively recruiting and activating a wide variety of cells, including leukocytes. The initial stage of this cellular activation involves the binding of chemokines to a family of seven transmembrane domain G-protein-coupled receptors. To date, two receptors have been cloned for CXC chemokines: IL-8R-A (1) and IL-8R-B (2). cDNAs for four human CC chemokine receptors have also been cloned (5–11). The available data using these recombinant receptor clones clearly show that CXC chemokines do not bind to CC chemokine receptors or vice versa. The only receptor that has been found that binds both classes of ligand is a ubiquitous chemokine receptor on erythrocytes known as the Duffy antigen (25). So far, however, this has not been shown to induce a signaling response to any chemokine.

In the attempt to understand the molecular basis of this selectivity, the three-dimensional structures of the ligands were initially studied. These studies show that the monomeric structures of both CXC and CC chemokines are similar, even though their sequence identity is lower than 25% in many cases. However, the dimeric interfaces for CXC and CC chemokines are different, suggesting that factors such as the hydrophobicity of the dimeric interface play a role in receptor selectivity. Multiple sequence alignments have enabled us to identify a conserved small hydrophobic amino acid in CXC and CC chemokines: IL-8R-A (1) and IL-8R-B (2). cDNAs for four human CC chemokine receptors have also been cloned (5–11). The available data using these recombinant receptor clones clearly show that CXC chemokines do not bind to CC chemokine receptors or vice versa. The only receptor that has been found that binds both classes of ligand is a ubiquitous chemokine receptor on erythrocytes known as the Duffy antigen (25). So far, however, this has not been shown to induce a signaling response to any chemokine.

The Leu25 \( \rightarrow \) Cys mutation introduces two novel CC chemokine-like activities into IL-8, namely monocyte chemotaxis and the ability to bind CC CKR-1.

To further investigate the role of this important residue, we have made the Leu25 \( \rightarrow \) Cys mutant. Since leucine and cysteine are approximately isosteres, we would predict that this mutation would produce only a minimal change in the activity of IL-8. However, this mutation introduces a chemically reactive group, which could be modified with a variety of reagents such as a hydrophobic fluorescent group. This in turn would enable us to monitor the binding of mutant IL-8 to form receptor complexes.

The Leu25 \( \rightarrow \) Cys mutant refolds correctly with two disulfide bonds.

### Table I

Secondary structure content of CXC, CC, and mutant IL-8 chemokines

|          | \( \alpha \)-Helix | \( \beta \) Sheet | Undefined structure |
|----------|-------------------|------------------|---------------------|
| CXC      |                   |                  |                     |
| IL-8     | 22.5              | 25.3             | 52.2                |
| IL-8     | 28                | 29               | 41                  |
| IL-8 Leu25 \( \rightarrow \) Cys | 21 | 22 | 50 |

### Monocyte-activating mutants

|                     | \( \alpha \)-Helix | \( \beta \) Sheet | Undefined structure |
|---------------------|-------------------|------------------|---------------------|
| IL-8 Leu25 \( \rightarrow \) Cys |                   |                  |                     |
| IL-8 Leu25 \( \rightarrow \) Cys-NBD | 6 | 27 | 67 |
| CC chemokines       |                   |                  |                     |
| RANTES              | 13                | 14.5             | 72.5                |
| RANTES              | 11                | 18               | 72                  |
| MIP-1\( \beta \)     | 17                | 17               | 66                  |
| MIP-1\( \beta \)     | 16                | 17               | 68                  |
| MIP-1\( \alpha \)    | 17                | 14               | 69                  |
| MCP-1               | 13                | 27               | 60                  |
bonds and one free thiol that can be attributed to Cys25. There should be no disulfide bond formation across the dimeric interface since the distance between the two thiols (calculated from the structure of wild-type IL-8) is 5.8 Å. This distance is much longer than the 3 Å normally seen in disulfide bonds. The Leu25 → Cys mutant is overall similar to wild-type IL-8. It binds IL-8R-A 50-fold and IL-8R-B 10-fold weaker than wild-type IL-8 and activates neutrophils with only 2-fold less potency. No effect was seen in monocyte chemotaxis assays, and the mutant was unable to displace MIP-1α from its CC CRK-R1 receptor.

The modification of the free cysteine with the fluorescent NBD group was shown to be stoichiometric. HPLC purification confirmed that there was no unlabeled starting material in the final product. Addition of the bulky aromatic NBD group caused a dramatic 100-fold decrease in binding to IL-8R-A and IL-8R-B and a concomitant 30-fold decrease in potency in neutrophil chemotaxis assays. These results are consistent with those reported for the IL-8 Leu25 → Tyr mutation (14). In addition, the NBD-modified mutant can compete with MIP-1α for binding to CC CRK-R1, with a 118-fold lower potency compared with MIP-1α. The mutant protein can also signal through the receptor, as can be seen by its ability to attract monocytes.

The fluorescent NBD probe is sensitive to its local environment (26), and this property can be used to study the interaction of IL-8 Leu25 → Cys-NBD with other proteins. When the labeled mutant protein is incubated with wild-type IL-8, there is an increase in the fluorescent signal, which corresponds to a local increase in hydrophobicity around residue 25. We predict that the proteins are forming heterodimers and that the NBD group is buried in the hydrophobic pocket between the two IL-8 C-terminal helices. We are currently trying to crystallize the mutant protein to verify this hypothesis.

The four CC chemokine receptors show over 50% amino acid identity along their entire length, but have N-terminal extracellular regions that are very different. We have synthesized identity along their entire length, but have N-terminal extra-C-terminal helices. We are currently trying to crystallize the IL-8R-B and a concomitant 30-fold decrease in potency in neutrophil chemotaxis assays. When the secondary structure compositions of the wild-type chemokines such as IL-8, RANTES, and MIP-1α were calculated from the CD data, there was a very good agreement of the α-helical and β-sheet content as compared with the NMR determinations. The CD spectra of the CXC and CC chemokines are clearly different: the CC chemokines show a much lower α-helical content, consistent with a much shorter C-terminal helix. However, the IL-8 mutants that bind to CC CRK-R1 and that can induce monocyte chemotaxis (Leu25 → Tyr and Leu25 → Cys-NBD) show a characteristic third class of CD spectra with a lower α-helical content compared with wild-type IL-8. Since in the IL-8 structure, Leu25 is close to the C-terminal helix, it is tempting to suggest that this introduction of a large aromatic and hydrophobic group close to the helix is causing some distortion of the helix sheet interface. This helical distortion in turn may lead to a distortion of the amide bonds and a characteristic new CD spectrum. We are currently solving the structures of these mutants by x-ray crystallography in order to investigate the three-dimensional basis of the change in receptor selectivity.

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