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Solution structure of CCL19 and identification of overlapping CCR7 and PSGL-1 binding sites

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Abstract

CCL19 and CCL21 are chemokines involved in the trafficking of immune cells, particularly within the lymphatic system, through activation of CCR7. Concurrent expression of PSGL-1 and CCR7 in naive T-cells enhances recruitment of these cells to secondary lymphoid organs by CCL19 and CCL21. Here the solution structure of CCL19 is reported. It contains a canonical chemokine domain. Chemical shift mapping shows the N-termini of PSGL-1 and CCR7 have overlapping binding sites for CCL19 and binding is competitive. Implications for the mechanism of PSGL-1's enhancement of resting T-cell recruitment are discussed.

Keywords

CCL19; CCL21; PSGL-1; Resting T-Cell; Chemokines; Protein NMR

Chemokines are a group of small-secreted signaling proteins that direct the trafficking of immune cells in the body through activation of chemokine receptors[1]. Chemokine receptors belong to the rhodopsin family of G-protein coupled receptors[1]. Activation of the chemokine receptor CCR7 by the chemokines CCL19 and CCL21 is required for the recruitment of

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ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

Author Contributions

FP, CV –NMR data collection. SG, MG, TM,FP,CV –Chemical shift assignments. SG,MG, FP, CV –CCL19 structure determination. BT, CV –Pulse field gradient NMR analysis. LW, CV –Heteronuclear NOE analysis. FD, DL, CM, PW, CV –Chemical shift mapping onto CCL19. GC, DL, MF, DZ, CV –Competitive binding as-say. AB, YB, GC, FD, SE, MF, DG, SG, DL, EL, VL, AM, CM, TM, MO, AP, HP, AR, SS, BT, PW, DZ –Protein purification. EK, MS –Cell migration assay.CV –wrotethe manuscript.

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The BMRB ID for CCL19 chemical shift assignments is 19960. The PDB ID for the CCL19 structure is 2MP1.

antigen presenting dendritic cells and naïve T-cells to secondary lymphoid organs (SLO)[2]. CCR7 expression in cancer is also associated with metastasis to lymph nodes expressing CCL19 and CCL21[3, 4]. CCL19 differs from CCL21 in that it lacks a thirty-four residue polybasic C-terminus. Hence, CCL21 is thought to form a stationary, glycosaminoglycan bound chemoattractant gradient while CCL19 is believed to form a soluble chemoattractant gradient[5, 6].

While the chemokine receptors CCR5 and CXCR4, which can serve as coreceptors, are most often associated with HIV-1 infection, CCR7 along with CCL19 are involved in the establishment of latently infected resting T-cells[7, 8]. Activation of CCR7 by CCL19 followed by HIV-1 infection of resting T-cells leads latent infection through increased nuclear localization and integration but not reproduction of infectious HIV-1 virus[7, 8]. Latently infected resting T-cells are one reason why current highly active antiretroviral therapies, which can bring viral loads to near zero, do not eradicate HIV-1[7–9].

Chemokines, like CCL19, are thought to activate their receptors through a two site-two step binding and activation model[10, 11]. The chemokine receptor N-terminus binds the chemokine domain first while the chemokine N-terminus subsequently binds the chemokine receptor leading to activation and cellular migration. Hence, peptides corresponding to a chemokine receptor's N-terminus have served as a model for studying the site one interaction between the chemokine and the receptor's N-terminus. Additionally, chemokine receptor N-termini contain numerous aspartic and gluctamic acids along with tyrosines that are posttranslationally modified to sulfotyrosine; this receptor posttranslational modification increases affinity for chemokine ligands[12, 13].

P-selectin glycoprotein ligand-1 (PSGL-1) is expressed on leukocytes and has an extracellular mucin like domain with an acidic N-terminus that also contains three tyrosines that can be posttranslationally modified to sulfotyrosine[14]. PSGL-1 is involved in chemokine mediated leukocyte recruitment through binding to endothelial selectins, which mediates leukocyte rolling and tethering, the initial step in the leukocyte extravasation cascade[14]. This interaction is primarily mediated through selectin binding to a branched O-glycan found in the N-terminus of PSGL-1[14]. This glycosylation is absent in resting T-cells[14]. Other posttranslational modifications also occur in the N-terminus of PSGL-1, including the previously mentioned sulfotyrosine residues. Sulfotyrosines in N-termini of chemokine receptors increase affinity for chemokine ligands[13].

Veerman *et al.* showed that resting T-cells coexpressing CCR7 and PSGL-1 had increased chemotaxis towards CCL19 and CCL21 and an enhanced recruitment to secondary lymphoid organs[15]. The enhancement was not the result of PSGL-1 binding to its canonical selectin ligands and promoting rolling[15]. Rather, the boost was due to a direct interaction between the N-terminus of PSGL-1 and CCL21 or a presumed, but yet to be observed, interaction between CCL19 and PSGL-1[15]. Veerman *et al.* hypothesized that direct interaction of PSGL-1 with CCL19 or CCL21 may promote increased presentation of CCL19 and CCL21 to CCR7 or that these chemokines may induce PSGL-1 to signal in a fashion that increases migratory responses[15]. In an attempt to determine if CCL19 binds to PSGL-1 and address Veerman *et al.*'s hypothesis, we solved the solution structure of CCL19

and used chemical shift mapping to investigate its binding to the N-terminus of either CCR7 or PSGL-1.

CCL19 was produced using a procedure adapted from Lu et al.[16] The ^{15}N-^{1}H HSQC spectrum of [U-^{15}N] CCL19 was homogenous with distinct peaks throughout, suggesting folding (Fig. S1A). Additionally, CCL19 induced directed migration of bone marrow derived dendritic cells *in vitro* confirming proper folding and functionality of the recombinant CCL19 (Fig. S1B)[17, 18]. CCL19 chemical shift assignments, structure determination and chemical shift mapping experiments followed the procedure described previously for CCL21 and other proteins[19]. A detailed description of all methods can be found in the supporting information.

CCL19 displays the typical chemokine fold (Fig. 1A). With thirty three constraints per residue and a root mean squared deviation of 0.49 Å for backbone atoms, the ensemble of 20 lowest energy structures (Fig. 1B) shows good agreement with the exception of the N and C termini. These are unstructured based on heteronuclear NOE values (Fig. S2). Structural statistics can be found in supplemental table 1. Pulsed field gradient NMR[20] measured a translational diffusion coefficient of 1.42×10^{-6} cm^2/s for CCL19 (8.8 kDa), which is consistent with that measured for the known monomer ubiquitin (8.6 kDa), 1.43×10^{-6} cm^2/s, suggesting CCL19 is monomeric.

Titrations of [U-^{15}N] CCL19 with peptides corresponding to the N-terminus of CCR7 (residues 2–30 with a C24A mutation to prevent oxidative dimer formation, no posttranslational modifications) or mature PSGL-1 (residues 2–15, no posttranslational modifications) were monitored using ^{15}N-^{1}H HSQC spectra. Backbone amide chemical shift perturbations were plotted versus CCL19 residues (Fig. S3) and residues with significant change were mapped onto the CCL19 structure (Fig. 2A, 2B, and S3). CCR7 and PSGL-1 induced overlapping chemical shift perturbations in the N-loop, third β-strand, and along the α-helix indicating a possibility for competitive binding to CCL19 (Fig. 2).

In mature CCR7 and PSGL-1, the N-terminal amino acid is glutamine, which may either spontaneously or through enzymatic catalysis form pyroglutamate. CCL19 residues with chemical shift perturbations were nearly identical when titrated with CCR7 or PSGL-1 N-terminal peptides containing or lacking this posttranslational modification (Fig. S3). Similarly, pyroglutamate (pGlu) did not dramatically alter CCL19's affinity for either peptide. The dissociation constant (K_d) for the CCR7 2–30 C24A was 12 ± 13 M while the K_d for CCR7 pGlu1–30 C24A was 52 ± 36 M (Fig. S3). The lack of an effect of pGlu was even more apparent for the N-terminus of PSGL-1. PSGL-1 2–15 had a K_d of 18 ± 15 μM while PSGL-1 pGlu1–15 had a K_d of 20 ± 17 M (Fig. S3). We hypothesize other posttranslational modification that are not present in the peptides used here, like sulfation of tyrosines or glycosylation of the N-termini of CCR7 or PSGL-1 may alter affinity[12, 13, 15]. However, these modifications are not required to investigate whether or not the N-termini of CCR7 or PSGL-1 compete for binding to CCL19.

To confirm competitive binding [U^{15}N] CCR7 2–30 C24A or [U-^{15}N] PSGL-1 2–15 N-terminal peptide was titrated with unlabeled CCL19 followed by titration with PSGL-1 or

CCR7 peptide, respectively. Peaks in either labeled peptide showed perturbations upon CCL19 addition indicating binding (Fig. 2C, left; Fig. S4, left). Upon addition of the unlabeled peptide perturbations reversed confirming direct competition (Fig. 2C, right; Fig. S4, right).

Here we show binding of CCL19 to the N-terminus of PSGL-1 and present the solution structure of CCL19. Observing a direct interaction between CCL19 and the PSGL-1 N-terminus confirms Veerman *et al.'s* suspicions of such an interaction and further supports their hypothesis that PSGL-1 binding directly to CCL19 enhances chemotaxis and recruitment of resting T-cells to the SLOs[15]. Additionally, we show competition between the N-termini of CCR7 and PSGL-1 for binding to CCL19. This competition suggests any increased presentation of CCL19 by PSGL-1 to CCR7 would be more complex than a simple coreceptor model where CCL19 simultaneously binds PSGL-1 and CCR7. Direct competition between these N-termini for CCL19 suggests investigating the possibility of CCL19 signaling through PSGL-1, originally proposed by Veerman *et al.* as a possible mechanism for enhancing resting T-cell chemotaxis and recruitment, may be warranted[15]. Other explanations are possible; PSGL-1, which is highly expressed on T-cells, could serve to buffer the local concentration of CCL19. For instance, Matin *et al.* describe interleukin 7 (IL-7) sinks in mice that rapidly absorb injected, exogenous IL-7[21]. However, neutralizing antibodies preloaded with IL-7 show increased cytokine activity when injected into mice, presumably because the antibody buffers the free IL-7 concentration[21]. PSGL-1 binding to CCL19 may protect CCL19 from potential CCL19 "sinks" such as the atypical chemokine receptors to which CCL19 binds[22, 23]. At the same time, this binding would increase the local concentration of CCL19 at the surface of the T-cell making activation of CCR7 more likely when CCL19 dissociates from PSGL-1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CCR7	CC chemokine receptor 7
CCL19	CC chemokine ligand 19
CCL19	CC chemokine ligand 21
PSGL-1	P-Selectin glycoprotein ligand-1
HSQC	Heteronuclear sin-gle quantum coherence

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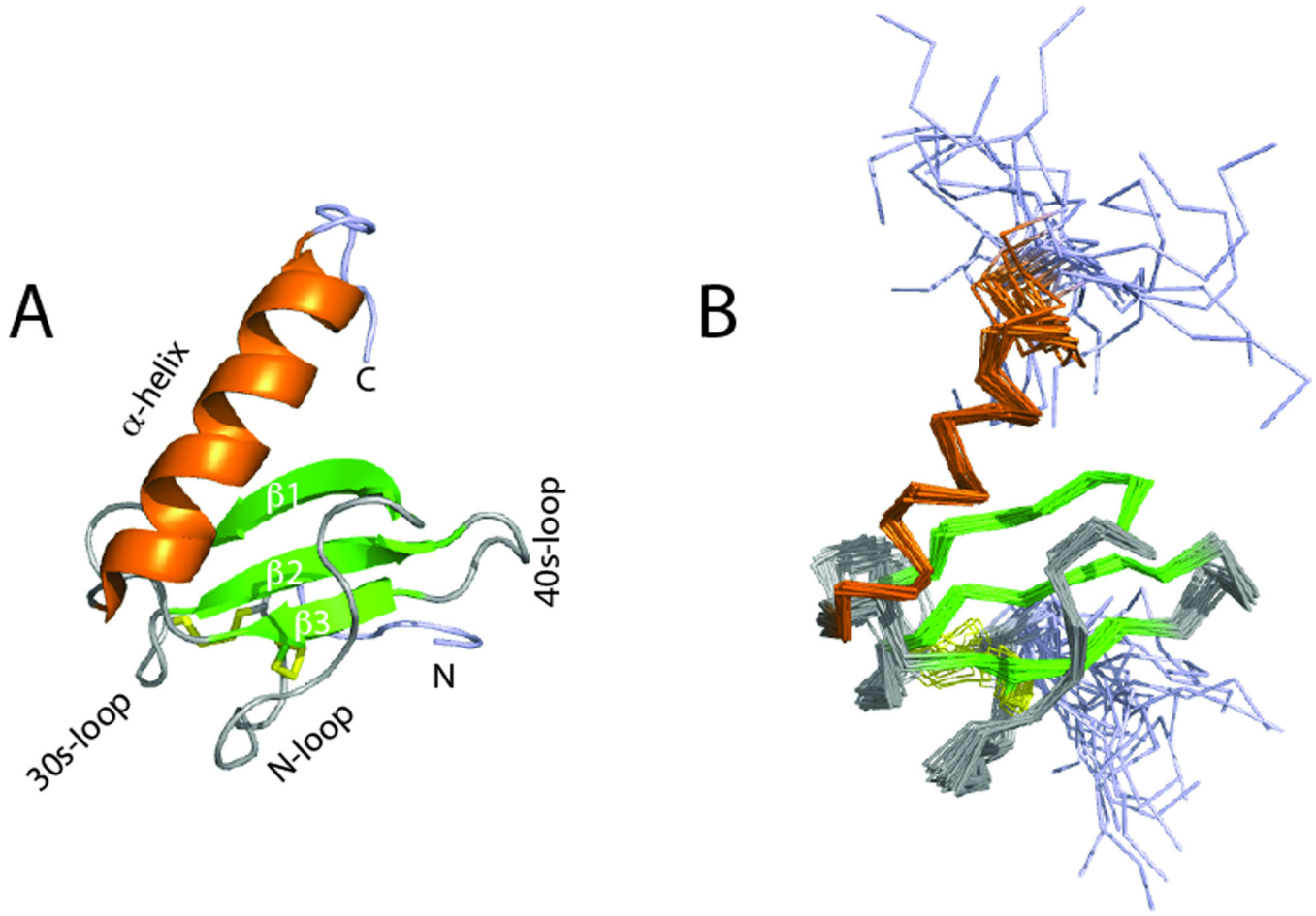

Figure 1.

Solution structure of CCL19. (A) Lowest energy conformer of CCL19. CCL19 has a canonical chemokine fold consisting of a flexible N-terminus and N-loop followed by an antiparallel three-stranded β-sheet, a C-terminal α-helix, and a short flexible C-terminus. Conserved disulfide bonds are shown in yellow. (B) Ensemble of 20 CCL19 structures.

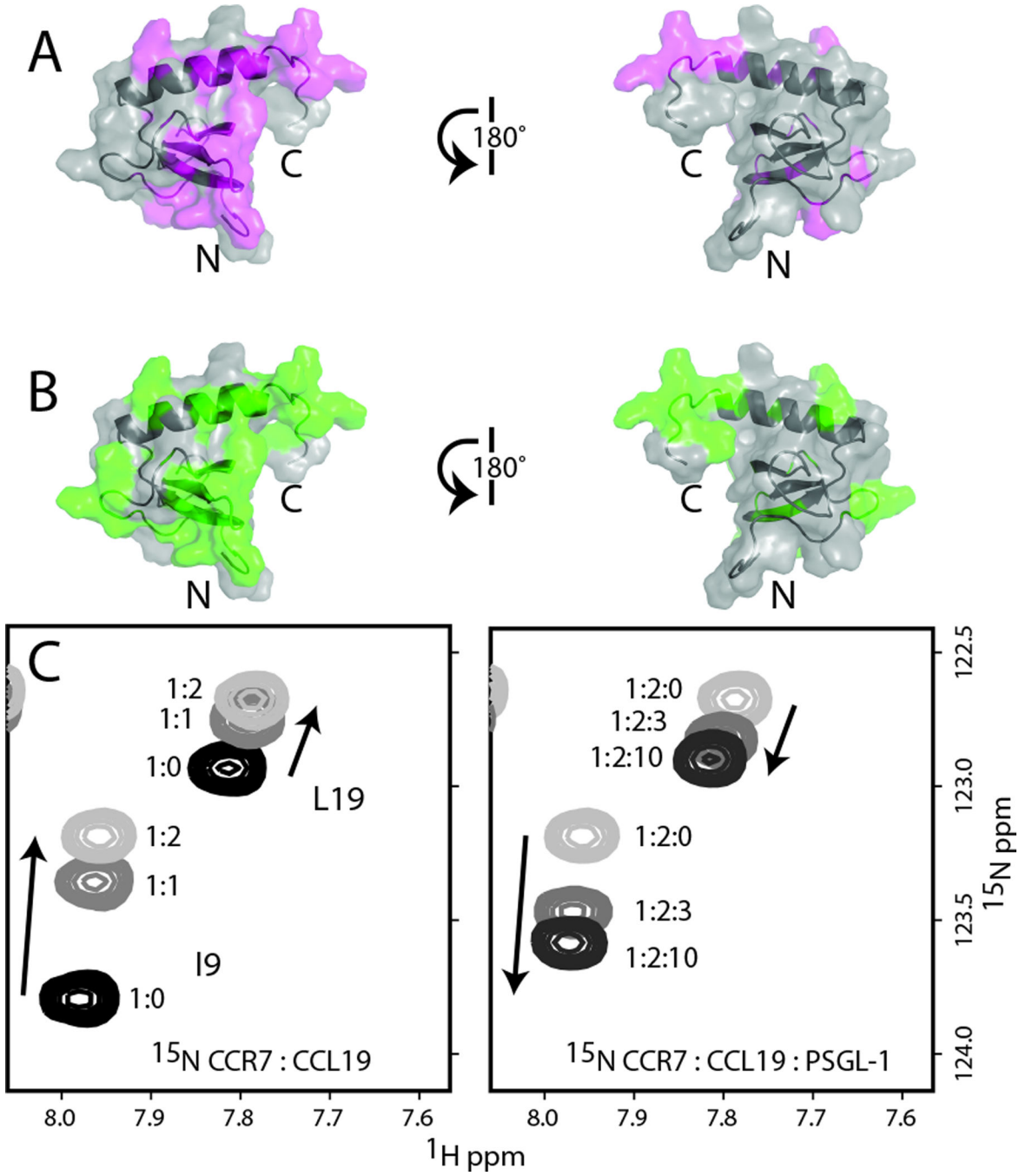

Figure 2.

CCL19's binding site for the N-termini of CCR7 and PSGL-1 overlap and binding is competitive. (A) Structure of CCL19 with perturbations from the CCR7 N-terminus highlighted in magenta. (B) Perturbations from the N-terminus of PSGL-1 are mapped onto the structure of CCL19 in green. (C) A portion of an ^{15}N-^{1}H HSQC spectra showing the titration of [U-^{15}N] CCR7 2–30 C24A with CCL19 (left) followed by titration with PSGL-1

2–15 (right). Upon addition of PSGL-1 2–15 peaks from CCR7 return toward their original position or unbound state. Molar ratios are as indicated in the figure.