The Identification of CXCR1 Residues Critical for Interleukin-8 Binding

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Introduction

Interleukin-8 (IL-8) is a proinflammatory cytokine that targets two G-coupled protein receptors, CXCR1 and CXCR2, resulting in a diverse array of signaling events linked to many cancers (*1*). IL-8 is a high affinity ligand for both receptors, however, CXCR1 is more specific for IL-8 as opposed to CXCR2. Several have identified the binding interface of IL-8 that is necessary for engaging model peptides derived from the N-terminus of the CXCR1 receptor (*2*). However, the explicit residues critical for IL-8 engagement on the CXCR1 side have yet to be fully characterized at atomic resolution. Thus, here we have used a previously established recombinant peptide system in our lab to probe a labeled CXCR1 N-terminal peptide (CXCR1pep) in the context of IL-8 and, thereby, identify critical residues on the CXCR1 side.

Experimental

The sequence encoding the N-terminal residues of human CXCR1 that corresponds to residue 9-29 (MWDFDDLNFTGMPPADEDYSP, referred to as CXCR1pep) was cloned and purified utilizing our previously described recombinant system (*3*). Standard VNMR BioPack sequences were used to assign the peptide in its free form, which included a CBCAcoHN and HNCACB. Using 15N,13C-labeled CXCR1pep, both 15N-edited and 13C-edited heteronuclear-single quantum coherence (HSQC) spectra were collected during titrations with increasing concentrations of IL-8 at 600 MHz. Unlabeled human IL-8 was expressed and purified as previously described (3).

Results and Discussion

To begin determining the underlying interactions that dictate IL-8 engagement with its CXCR1 receptor, we performed chemical shift perturbations on a 15N,13C-labeled peptide derived from the N- terminal region of CXCR1. Both 15N- and 13C-HSQC experiments were utilized to probe the labeled peptide with the addition of recombinant IL-8 (Fig. 1). Titration experiments indicate that both the amide and Cα chemical shift perturbations for CXCR1pep Asp13 exhibit the largest changes upon addition of IL-8, which is consistent with a dominant electrostatic interaction. Although several electrostatic interactions have been predicted, this relatively straightforward study now allows for the dissection of specific residues that contributed to the CXCR1/IL-8 interaction.

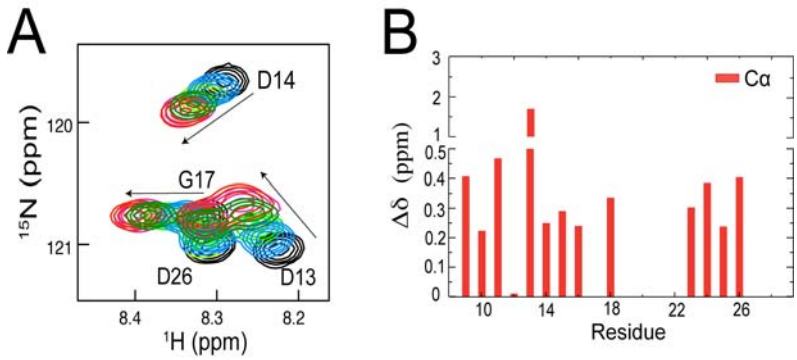

Figure 1. A) 15N-HSQC titration experiments with 0.5 mM 15N,13C-labeled CXCR1pep and increasing concentrations of IL-8. B) Cα chemical shift changes between the free CXCR1pep and fully bound CXCR1pep/IL-8 complex.

Conclusions

Our data suggest that CXCR1 Asp13 underlies the electrostatic interaction with the IL-8 ligand.

Acknowledgements

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References

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