IL-8 Does Not Dissociate Upon Binding to its CXCR1 N-Terminus

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Introduction

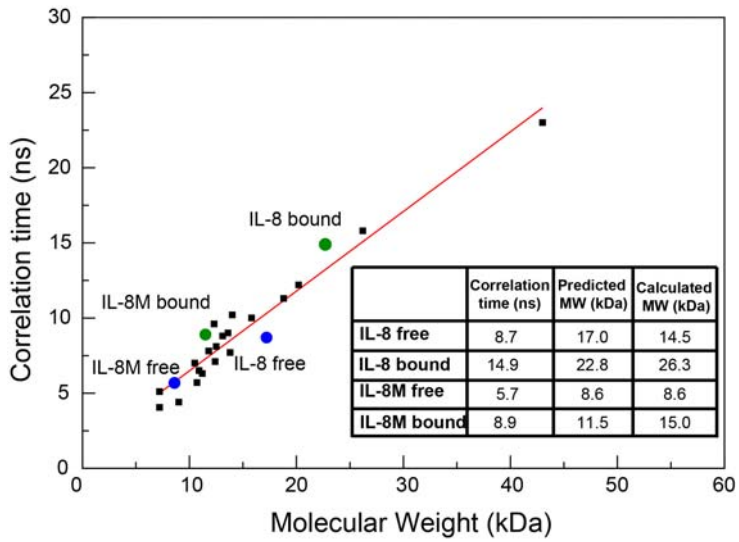
Interleukin-8 (IL-8) is a well known proinflammatory cytokine that targets two G-coupled protein receptors, which include CXCR1 and CXCR2[1]. While the primary receptor for IL-8 is the flexible N-terminus of CXCR1, there have been reports suggesting that the IL-8 dimer dissociates upon binding[2]. Moreover, based on biophysical experiments that interrogated the IL-8 interaction with the N-terminal region of CXCR1, this dissociation was also reported[3]. However, using relaxation experiments conducted here on both wild-type IL-8 and a monomeric mutant of IL-8 we show that upon IL-8 binding to the CXCR1 N-terminal peptide (CXCR1pep) that there is no dissociation.

Experimental

The sequence encoding the N-terminal residues of CXCR1 that corresponds to residue 9-29 (MWDFDDLNFTGMPPADEDYSP, referred to as CXCR1pep) along with both wild-type IL-8 and a mutant form that was unable to form a tight dimer (called IL-8M). Spectroscopy was performed on a solutions 600 MHz instrument at the NHMFL. Standard VNMR BioPack sequences were used to collect both R1 and R2 relaxation rates for both forms of IL-8 in their free and bound states.

Results and Discussion

We took advantage of the fact that both longitudinal (R1) and transverse (R2) relaxation can be utilized to estimate the correlation time of a macromolecule and that that this, in turn, is related to the molecular weight of the macromolecule. To this end, we measured R1/R2 relaxation rates for four species, which included IL-8, IL-8/CXCR1pep, IL-8M, IL-8M/CXCR1pep. Using correlation times extracted from each of these data sets, IL-8 bound to the CXCR1pep was found to be closer to the predicted molecular weight of a dimer.



	Correlation time (ns)	Predicted MW (kDa)	Calculated MW (kDa)
IL-8 free	8.7	17.0	14.5
IL-8 bound	14.9	22.8	26.3
IL-8M free	5.7	8.6	8.6
IL-8M bound	8.9	11.5	15.0

Figure 1. IL-8 does not dissociate to a monomeric form upon binding to CXCR1pep. The molecular weights of both dimeric IL-8 (IL-8) and monomeric IL-8 (IL-8M) are plotted in their free forms and bound forms. R2 and R1 relaxation rates were used to calculate the correlation times.

Conclusions

While subsequent conformational changes could occur upon IL-8 engaging its CXCR1 receptor, there is no evidence to suggest that the IL-8 dimer must dissociate in order to bind the N-terminal region of CXCR1, as we have recently published[4].

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