The combination of X-ray crystallography and NMR method in understanding chemokine oligomerization mechanisms

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Abstract

Protein X-ray crystallography is an important method for determining protein static structure and NMR method demonstrates advantage in studying protein dynamics in solution. The combination of the two methods allow us to comprehensively characterize protein structural features. Here, we will demonstrate two cases related with chemokine oligomerization while the usage of NMR method assists the validation of the determined X-ray structures. Chemokines, acting as chemotactic cytokines, control migration in nearby responsive cells. The molecules are broadly associated with inflammation, angiogenesis and viral infection. Most chemokines enable to form different oligomers, responsible for their functional diversities. To understand the details, we study the structural basis of oligomerization mechanisms.

For chemokine CCL5, the oligomer formation is important for carbohydrate binding. We developed strategies to study the oligomerization structure. Specifically, we mixed different CCL5 mutants, which are deficient in forming oligomer, with the native CCL5 to prepare complexes with reduced oligomerization property. Under optimal ratios, we determined the crystal structures by X-ray. Combining with the evidences of NMR, we proved that the oligomerization is through more than one interaction. Two different charge-charge interactions alternatively contributed in the oligomerization process and N-terminal conserved FAY motif additionally involves in protein precipitation.

For chemokine CXCL4, it is a chemokine with anti-angiogenic property. CXCL4L1, a CXCL4 variant with three-residue difference in the C-terminal helix, showed higher potency of anti-angiogenesis. In the X-ray crystallography, the two chemokines shared structural similarity of forming a tetramer. To understand how the sequence difference derives the functional discrepancy, we used NMR to characterize their solution properties. CXCL4L1 but not CXCL4 with great tendency to dissociate into monomers and the monomers act as the minimal active unit for activating its membrane receptor. Considering that CXCL4 behaves as a stable tetramer, the result explains why CXCL4L1 contains better biological activity.

Keywords: Chemokine, Oligomer, Oligomerization, GPCR

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