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# Crystal structure analysis of human autocrine motility factor

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## Introduction

Autocrine motility factor (AMF) was originally identified by its ability to stimulate directional motility (chemotaxis) and random motility (chemokinetics) of the AMF-producing tumor cells. AMF stimulates cell motility and growth via a receptor-mediated signaling pathway involving morphological changes, receptor phosphorylation, a pertussis toxin-sensitive G-protein activation, inositol phosphate production, protein kinase C activation, and enhanced production of a metabolite of arachidonic acid. Recently, full-length cDNA cloning for both human and mouse AMF receptor (AMFR) genes has been reported and revealed that the AMFR is a novel type of seven transmembrane helix protein.

Primary structure studies (cDNA cloning and amino acid sequencing) have identified human AMF (558 a.a.) as genetically identical to the extracellular cytokines (neuroleukin (NLK) and maturation factor (MF)), and highly homologous to the intracellular enzyme phosphohexose isomerase (PHI). The extracellular cytokines AMF/NLK/MF were originally identified to exist as a monomer in a solution, whereas the intracellular enzyme PHI was known to exist as a dimer in a solution. Recently, crystal structures of bacterial and rabbit PHIs have been reported and showed that both bacterial and rabbit PHI molecules exist as dimer in the crystal. Although the three-dimensional structures and quaternary structures of the intracellular enzyme PHI are now established, little is known of the tertiary structure, quaternary structure and structure-function relationship of the extracellular cytokines AMF/NLK/MF.

Thus the three-dimensional structure of AMF is essential to clarify the structure-function relationship of the extracellular cytokines AMF/NLK/MF and to know how the intracellular enzyme PHI can be secreted and serve as a cytokine.

## Experimental

*Crystallization*

Expression and purification of human AMF were performed as described [1]. Crystals of human AMF were obtained by the hanging-drop vapour diffusion method, as described elsewhere [2]. Briefly, a droplet was prepared by mixing an equal volume of the protein solution containing the 8 mg/ml human AMF, 0.05 M sodium chloride, and 20% (v/v) glycerol in 0.02 M Tris buffer at pH 7.5 and the reservoir solution containing 28% (w/v) polyethylene glycol with a mean molecular weight of 8,000, 0.2 M sodium acetate, and 20% (v/v) glycerol in 0.1 M cacodylate buffer at pH 6.5. The crystals belong to an orthorhombic space group $P2_12_12_1$ with cell dimensions of $a$ = 80.77 Å, $b$ = 107.4 Å, and $c$ = 270.8 Å. Assuming four subunits (two dimers) per asymmetric unit, we obtained a $V_M$ value of 2.32 Å$^3$/Da, corresponding to a solvent content of 47%.

*X-ray data collection*

Since the crystallization conditions of human AMF described above contained 20% (v/v) glycerol in both protein and reservoir solutions, X-ray data collection could be performed at cryogenic conditions without the further addition of a cryo-protectant. Crystals in the hanging-drop were directly mounted in nylon loops and flash-frozen in a cold nitrogen gas stream at 100 K just before the data collection. The data collection was performed by a rotation method at 100 K using an ADSC Quantum4R CCD detector with synchrotron radiation ($\lambda$ = 1.00 Å at beam line 18B of the Photon Factory, Tsukuba, Japan).

## Results and Discussion

The crystal structures of the inhibitor-free open form and E4P-bound closed form of human AMF have been determined at 1.9 and 2.4 Å resolution, respectively [3]. These structures have revealed inhibitor-binding sites and conformation changes upon inhibitor binding. In addition, to examine roles of the residues in an inhibitor-binding site, we performed site-directed mutagenesis studies focusing on the cytokine activity of AMF/PHI. The crystal structure analyses and site-directed mutagenesis studies presented here support the hypothesis that there is an inhibition mechanism of AMF cytokine activity by carbohydrate phosphates for which the compound could compete for AMF binding with the carbohydrate moiety of the AMFR, which is a glycosylated seven-transmembrane helix protein.

## References

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