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Crystal structure analyses of mouse autocrine motility factor

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Introduction

Autocrine motility factor (AMF) was identified originally by its ability to stimulate directional motility (chemotaxis) and random motility (chemokinetics) of the AMF-producing tumor cells. The AMF stimulates cell motility via a receptor-mediated signaling pathway involving 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, another role of AMF has been revealed: it stimulates AMF-producing tumor cell motility in an autocrine manner, and it acts as a paracrine factor to vein endothelial cells to induce angiogenesis with cell motility stimulation and may facilitate metastasis through these effects during the metastasis phase.

Partial amino acid sequencing of mouse AMF and full-length cDNA cloning of human AMF have identified that the AMF is genetically identical with extracellular cytokines neuroleukin and maturation factor and, interestingly, with an intracellular enzyme phosphoglucose isomerase. The PGI is a key enzyme in glycolysis and gluconeogenesis that catalyses the second step of glycolysis, the interconversion of glucose 6-phosphate (G6P) and fructose 6-phosphate (F6P). Site-directed mutagenesis studies at the sugar binding sites of human AMF/PGI have resulted in abridging cytokine activity of mutant AMFs suggesting that the regions for the enzymatic function overlap those for the cytokine function [1].

Several crystal structures of mammalian (human, rabbit, and pig) AMF/PGIs complexed with sulfate ion occupying the binding site of the substrate phosphate and with carbohydrate phosphate inhibitors are available, but high-resolution structure of AMF/PGI complexed with carbohydrate phosphate inhibitor is not available. In the absence of the high-resolution structure of AMF/PGI complexed with an inhibitor, a detailed picture of the AMF-inhibitor interactions including water molecules, which is essential for lead compound design of more effective AMF inhibitors, is not clear.

Here we report the crystallization and preliminary X-ray crystallographic studies of mouse AMF (mAMF). The crystals diffract at least 1.8 Å resolution and are suitable for X-ray structure analyses at high resolution.

Experimental

Crystallization

The expression and purification of recombinant mAMF will be described elsewhere [2]. Crystallization was carried out at 293 K by the hanging-drop vapour diffusion method. In the best case, a droplet was prepared by mixing equal volumes (2.0 + 2.0 μl) of the protein solution (6 mg/ml) and the reservoir solution (500 μl) containing 26 % (w/v) polyethylene glycol mean molecular weight of 8,000, 200 mM sodium acetate and 20 % (v/v) glycerol in 100 mM cacodylate buffer at pH 6.5. Plate-shaped crystals with typical dimensions of approximately 0.3 x 0.3 x 0.01 mm^3 could be grown in 2 weeks [2].

X-ray data collection

The data collection was performed by rotation method at 100 K using an ADSC Q210 CCD detector with synchrotron radiation (λ = 1.00 Å at beam line NW12 of the Advanced Ring of the Photon Factory). The Laue group was found to be 2/*m* and the unit-cell dimensions were *a* = 69.97 Å, *b* = 115.88 Å, *c* = 73.27 Å, and β = 101.76°. Only reflections with *k* = 2n were observed along the (0 *k* 0) axis, indicating a monoclinic space group $P2_1$. An assumption of two subunits (one dimer) per asymmetric unit leads to an empirically acceptable V_M value of 2.32 $Å^3$/Da, corresponding to a solvent content of 47 %. The current best diffraction data from an mAMF crystal were collected up to 1.8 Å resolution.

Results and Discussion

Initial phase determination was done by the molecular replacement technique using the coordinate set of inhibitor-free human AMF (PDB code 1JIQ [1]), having 90 % amino acid sequence identity with mAMF, as a search model. Further model building and refinement at 1.8 Å resolution are currently in progress.

References

[1] N. Tanaka et al., J. Mol. Biol. 318, 985 (2002).
[2] N. Naba et al., in preparation.

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