5A, NW12/2003G126

Crystal structures of mouse autocrine motility factor complexed with various carbohydrate phosphate inhibitors

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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.

Partial amino acid sequencing of mouse AMF (mAMF) 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 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 crystal structure of the inhibitor-free open form and those of various lengths of inhibitor bound closed forms of mAMF at high resolution (1.4-1.8 Å). This series enabled us to provide the first comprehensive report of the structure-activity relationships of carbohydrate phosphate inhibitors with respect to the inhibition of the cytokine activity of AMF.

Experimental

Crystallization and X-ray data collection

The expression and purification of recombinant mAMF were performed as described elsewhere [2]. The data collection for inhibitor-free mAMF 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 space group was $P2_1$ and the unit-cell dimensions were a = 69.97 Å, b = 115.88 Å, c = 73.27 Å, and β = 101.76°. The current best diffraction data from an inhibitor-free mAMF crystal were collected up to 1.8 Å resolution. Data from the crystals of the inhibitor complexes were collected by procedures similar to those described above.

Structure determination

Initial phase determination for inhibitor-free mAMF was done by the molecular replacement technique using the coordinate set of inhibitor-free human AMF (PDB code 1JIQ [1]) as a search model. Crystallographic refinement was performed using the program REFMAC. The refined structure of inhibitor inhibitor-free mAMF was used for the structure determination of the inhibitor complexes by the difference Fourier method.

Results and Discussion

We have determined the high-resolution crystal structures of the inhibitor-free form and the eight types of inhibitor [phosphate, erythrose 4-phosphate (E4P), arabinose 5-phosphate (A5P), sorbitol 6-phosphate (S6P), 6-phosphogluconic acid (6PGA), fructose 6-phosphate (F6P), glucose 6-phosphate (G6P), or mannose 6-phosphate (M6P)] complexes of mouse AMF. The inhibitory activities of the six-carbon sugars (G6P, F6P, M6P, and 6PGA) were found to be significantly higher than those of the four or five-carbon sugars (E4P or A5P). A structural comparison revealed that a water-mediated hydrogen bond between one end of the inhibitor and a rigid portion of the protein surface in the shorter-chain inhibitor (E4P) complex is replaced by a direct hydrogen bond in the longer-chain inhibitor (6PGA) complex. Thus, to obtain a new compound with higher inhibitory activities against AMF, water molecules at the inhibitor-binding site of AMF should be replaced by a functional group of inhibitors in order to introduce direct interactions with the protein surface [3].

References

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