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Crystal structure of human osteomodulin.

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We have determined for the first time the crystal structure of human osteomodulin, achieving a resolution of 2.17 Å.

1 Introduction

Osteomodulin (OMD), is a member of the class II small leucine repeat protein (SLRP), regulating the diameter and shape of collagen fibrils [1]. The mechanism by which OMD and other SRLPs is unknown, particularly at the molecular level. To build a bottom up approach in the elucidation of its mechanism, we sought to determine the crystal structure of human OMD.

2 Experiment

Human OMD (excluding residues 1–20 belonging to the signal peptide) labeled with a FLAG-tag at the C-terminus was cloned into pFastbac1 [2]. Bacmid was made upon transformation of the pFastbac vector in DH10Bac cells. Sf9 cells (Invitrogen) infected with the baculovirus containing the sequence of OMD were incubated at 27 °C for 3 days. Cells were centrifuged and the supernatant collected. The soluble OMD protein was purified from the supernatant using an anti-FLAG M2 affinity chromatography column, followed by size exclusion chromatography.

Purified OMD at 9.9 mg/ml was subjected to sparse matrix crystallization in an Oryx8 system (Douglas Instruments) with commercially available kits (Hampton Research) at 20 °C. Protein crystals were identified, and subjected to optimization to improve the quality and size of the crystal. The best crystals were obtained in a mother liquor containing 200 mM ammonium phosphate and 24% PEG 3350. The crystals reached full-size within three weeks, after which they were harvested, immersed in mother liquor containing 40% PEG 3350, and frozen in liquid N_2 until data collection at the photon factory.

Data collection was carried out at beamline AR-NE3A of the Photon Factory (Tsukuba, Japan) using a wavelength of 1.000 Å at a temperature of 100 K. The structure was determined by the method of molecular replacement with the program PHASER36 using the coordinates of the protein decorin (PDB entry code 1XKU) [3]. The coordinate and structure factors have been deposited in the Protein data bank under accession code 5YQ5.

3 Results and Discussion

The structure of human OMD showed the typical curved solenoid fold of SLRPs (Fig. 1). Importantly, the analysis of crystal packing suggested that OMD is a monomeric protein, a departure from what is observed for other members of this family of proteins. The structure revealed at least four N-glycosylation sites at residues Asn113, Asn187, Asn242, and Asn316.

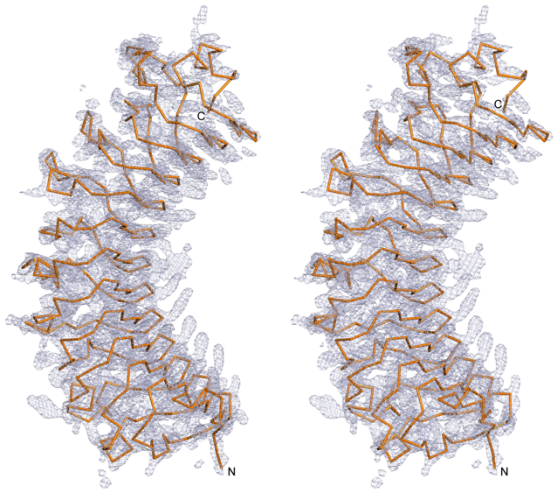

Fig. 1: Stereo view of OMD. The sigma-A weighted electron density map (2fo-fc) is contoured to 1.5 σ [2].

This crystal structure of OMD is a milestone in the molecular understanding of this protein, since it has provided the basis to rationalize biophysical and biochemical data [2].

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