The Structure of Melanoma Inhibitory Activity Protein

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INTRODUCTION

Melanoma inhibitory activity (MIA) is a 12 kDa secreted protein that is normally expressed in cartilage but is also produced by malignant melanoma [1, 2]. It has been reported to have effects on both cell growth and adhesion, and it may play a role in melanoma metastasis. Historically, it was identified as a factor secreted from melanoma cells that inhibited their growth *in vitro* [3]. Later studies indicated a correlation between increased plasma levels of MIA and a more advanced metastatic disease state[4]. It may be MIA's ability to inhibit the attachment of melanoma cells to the extracellular matrix that aids in the formation of metastases [5]. In cartilage, MIA's expression begins upon chondrogenesis and continues through development and in mature chondrocytes, suggesting that MIA is fundamental to the cartilage cell phenotype [6]. Also, MIA's expression is inhibited *in vitro* concurrently with repression of cartilage cell phenotype when retinoic acid is added to cartilage cells, hence, MIA is also known as cartilage derived retinoic acid sensitive protein (CD-RAP) [2].

MIA has been identified in several species (rat, mouse, bovine, and human) and is a member of a new family of proteins. Recently, two new paralogs were discovered, and a fourth putative paralog can be predicted from genomic sequences. In addition, MIA has low sequence homology to SH3 domains. Unlike SH3 domains, MIA has short N- and C- terminal extensions of approximately twenty amino acids to the SH3 domain which contain three of its four cysteines, also unique to MIA. Intrigued by the possibility of a secreted molecule that is related to SH3 domains but functions as an independent module, we undertook a full structural determination. Here, we describe the 1.4 Å crystal structure.

EXPERIMENTAL

Diffraction data was collected on one native crystal at both the Advanced Light Source (ALS) at Lawrence Berkeley Laboratory on beamline 5.0.2 and at the Stanford Synchrotron Radiation Laboratory (SSRL) on beamline 9-2. Diffraction data was also collected on a crystal of selenomethionine containing protein at SSRL on beamline 9-2 at three wavelengths, and phases were determined using multiwave anomalous diffraction [MAD] methods. All data were integrated with MOSFLM [7] and scaled with SCALA [8] through use of the automation program ELVES (J.M. Holton, manuscript in preparation). The overall R_{merge} for the selenium data was 6.7% to 1.4 Å. Seven selenium sites were found by the program SOLVE [9], and phases were refined with MLPHARE [8].The overall figure of merit was 0.697 to 1.7 Å. Solvent flattening and histogram mapping [10] produced a clearly interpretable electron density map within hours of data collection. Most of the backbone and side chains were traced automatically using ARP/wARP [11]. The structure has been refined to 1.4 Å, with an R_{cryst} of 20.7 and an R_{free} of 23.1.

RESULTS

MIA is a small single-domain globular protein containing an SH3 subdomain. The SH3 subdomain has N- and C- terminal extensions of about twenty amino acids each which add additional structural elements, forming a novel fold. MIA consists of seven β-strands, two short segments of 3_{10} helix, and several loops. Five of its strands make up the portion of the molecule that has an SH3 domain fold; two three stranded β-sheets pack at approximately right angles to eachother, and an 18-residue hairpin-like loop, analogous to the Src family of SH3 domains' RT-loop is formed between strands β2 and β3. The N- and C-terminal extensions to the SH3 subdomain form two additional β-strands which add onto the basic SH3 domain fold. This is the first structure solved of a secreted protein containing an SH3 subdomain.

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This work was supported by grants awarded to T.M.H. from the National Institutes of Health and the PEW Scholars Program in the Biomedical Sciences, and a National Institutes of Health training grant to J.C.L.

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