A 2.0 Å Structure of GMI, a Member of the Fungal Immunomodulatory Protein Family from Ganoderma Microsporum

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The higher fungi and mushrooms (Basidiomyceta) have been widely adapted as part of traditional medicine system as well as daily dietary in Far East Asia region, including Taiwan, Japan, Korea and China, to enhance body defense capability and promote health. Studies, at least using the extracts from golden needle mushroom, Flammulina velutipes, suggest the potent of anti-tumor, anti-leukemic cells, anti-viral, anti-fungal and immunomodulatory activity (Otagiri, Ohkuma et al. 1983; Wasser and Weis 1999; Wang and Ng 2000; Fukushima, Ohashi et al. 2001; Ng, Ngai et al. 2006).

Since the identification of LZ-8 (Kino, Yamashita et al. 1989), from Ganoderma lucidum (靈芝, 或稱赤芝) as a fungal immunomodulatory protein (FIP), efforts have been made to find more proteins with similar potent. So far, at least five more FIPs have been identified, including FIP-fve (Flammulina veltipes, 金針菇), FIP-vvo (Volvariella volvacea, 草菇), FIP-gts (Ganoderma tsugae, 松杉靈芝), FIP-gja (Ganoderma sinensis, 紫芝), LZ-8, and GMI (Ganoderma microsporum,小孢子靈芝). Those FIPs compose of 110-114 amino acids and both Histine and Cysteine are absence among them.

Understanding the functional domain that serves as a immunomodulator in FIPs potentially has both scientific and commercial significant. Here we report a 2.0Å structure of GMI protein, a protein recently successfully cloned from Ganoderma microsporum and over-expressed using Pichia pastoris developed by Dr. Huang's lab. Previously, the structure of FIP-fve with atomic resolution (Paaventhan, Joseph et al. 2003) was resolved, and it appears to be a dimmer with non-covalent interactions through the helices in the N-terminus. Each monomer consists of an N-terminal alpha-helix followed by a fibronectin III fold. It is also concluded that the formation of dimmer is critical for the biological activity of FIPs.

In this study, we report a 2.0Å structure of GMI protein, determined by X-ray diffraction and phasing with FIP-fve (PDB id: 1OSY) as molecule replacement template. As shown in Figure 1, GMI structure unveils two interesting facts: first, GMI appears as a tetramer instead of dimmer. The rich non-covalent and hydrophobic interactions through the interface of the interlocking helices in the N-terminus form a dimmer-dimmer arrangement (Figure 2). Secondly, the conformation and arrangement of loops at the neighbor of residues 64 and 105 of GMI appear to be significantly different from those observed in the corresponding region of FIP-fve structure. Indicating the importance in determination of specificity and functionality.

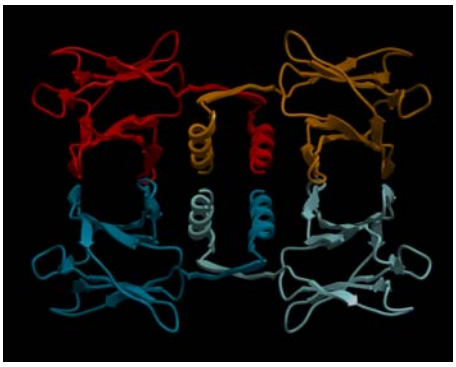

Figure 1. Structure of GMI resolved at 2.0Å. Instead of a dimmer, the GMI protein appears to exist as a tetramer form.

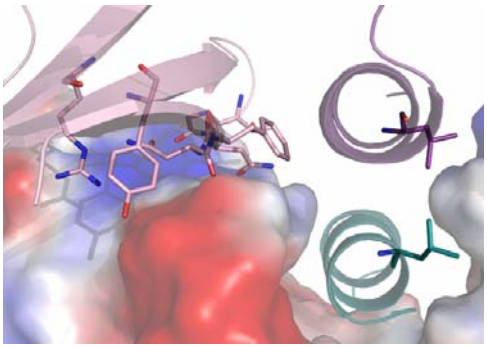

Figure 2. nteractions between dimer-dimer interface

According to the results of sequence alignment, those two loops happen to locate at the regions that show the lowest sequence homology and therefore the correlation and significance of this observation is worthwhile a further investigation.

GMI protein appears to exist as tetramer architecture, and this is supported by the fact that a high molecule weight band close to the size of tetramer of GMI can be observed in the protein gel. Since GMI protein is more heat sensitive than LZ-8 and it loses its biological activity even when temperature is restored (personal communications). Comparison of the structure of GMI to LZ-8 might be able to give us a hint regarding how to produce an even more stable and potent protein by carefully engineering the protein structure.