Structure of Rat Liver Vault at 3.5 Å Resolution

Vaults are barrel-shaped particles with overall dimensions of 35 × 35 × 65 nm^3 [1], and are highly conserved in a wide variety of eukaryotes. The rat liver vault is composed of three proteins: the 99-kDa major vault protein (MVP), the 193-kDa vault poly(ADP-ribose) polymerase (VPARP), and the 290-kDa telomerase-associated protein TEP1. Additionally, the complex contains a small untranslated RNA consisting of 141 bases (vRNA). Most vault particles are present in the cytoplasm, but a few localize to the nucleus. Although several functions have been proposed for vaults since their first discovery in 1986, including roles in multidrug resistance, cell signaling and innate immunity, their cellular function remains unclear. To elucidate the structure, structural organization and physiological function of this macromolecular complex, we determined the X-ray crystal structure of the rat liver vault at 3.5 Å resolution [2]. The structure analysis was carried out using beamline **BL44XU**.

The vault shell comprises a 78-mer of MVP molecules with 39-fold dihedral symmetry [3], and has a unique barrel-like shape measuring 670 Å in length and 400 Å in maximum diameter (Fig. 1). The barrel-like particle has two protruding caps, shoulders and a body with an invaginated waist. The barrel wall has a very thin, skin-like structure, and is only 15-25 Å thick. The internal cavity of the vault particle is sufficient large to enclose most objects found within the cell. The large internal cavity suggests that the vault functions as a carrier of various molecular cargoes.

The structure of the MVP monomer is depicted by a ribbon drawing in Fig. 2. The MVP monomer folds into nine structural repeat domains, a shoulder domain, a cap-helix domain, and a cap-ring domain. Each structural repeat is a small domain consisting of antiparallel strands. The shoulder domain folds into a single α/β globular domain with four-stranded antiparallel β-sheets on one side and four α-helices on the other side. The cap-helix forms a long α-helix with 42 turns and exhibits one-fourth turn of superhelical structure (Fig. 2). The cap-ring, the polypeptide segment from Gly803 to Ala845, is at the top of the cap and forms a U-shape structure; both ends of this domain are short helical structures.

There are a total of 74 side-by-side interactions between two MVPs, of which 44 interactions were included in the cap-helix domain (Fig. 3(a)), which consists of 170 residues. Although the side chain structures of the cap-ring domain have not yet been determined, the cap-ring domain is closely packed in a restricted space. Structural studies of the intersubunit interactions suggest that the side-by-side interactions within the cap of the vault particle are the primary factors in the structural organization of the vault shell. Each MVP interacts with a neighboring MVP that is related by a twofold symmetry axis. The N-terminal residues of each MVP, Met1-Glu4, form an intermolecular antiparallel β-sheet with the corresponding residues of the other protein. An ionic bond between Glu4 and Arg42 was also detected around the twofold axis. No other intermolecular interaction between two half-vaults was observed, except for the ionic bond and three hydrogen bonds in the β-sheet.

A three-dimensional structure search for similarity to the shoulder, using the DALI server, revealed that this domain is structurally similar to the core domain of stomatin from *Pyrococcus horikoshii* (PhSto^{CD})

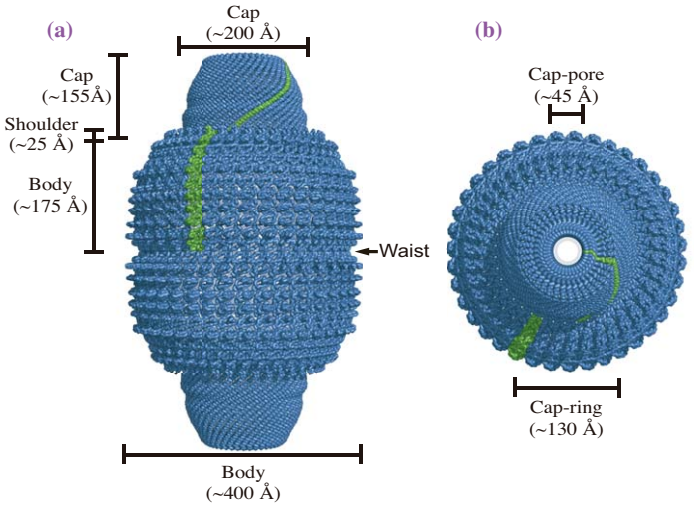

Fig. 1. Overall structure of the vault shell. One molecule of MVP is colored green, and the others are colored blue. **(a)** Side view of the ribbon representation. The whole vault shell comprises a 78-mer of MVP molecules. **(b)** Top view of the ribbon representation.

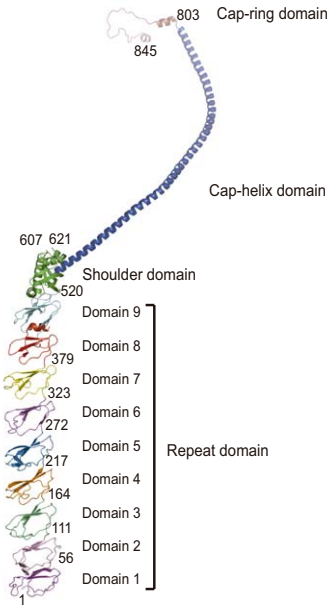

Fig. 2. Ribbon drawing of the overall fold of MVP monomer. The MVP monomer is folded into nine structural repeat domains, a shoulder domain, a cap-helix domain and a cap-ring domain. Each domain is depicted in a different color.

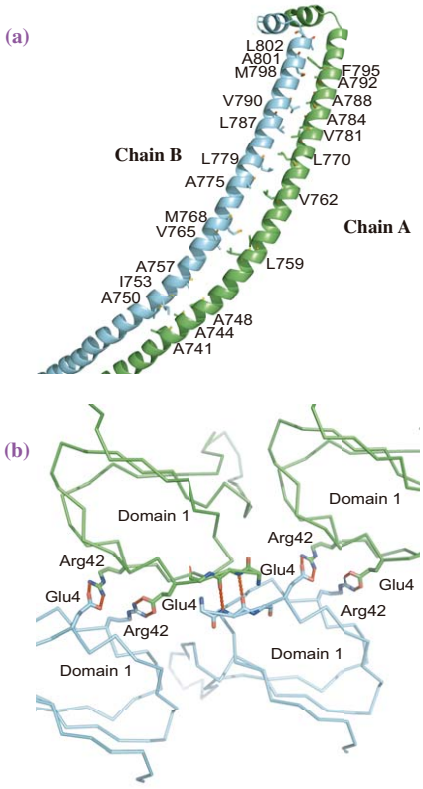

Fig. 3. **(a)** Hydrophobic interactions of the cap-helix domain. In the cap-helix domain, most of the hydrophobic residues appear at the interface between two helices, where they are able to engage in hydrophobic interactions. **(b)** Intermolecular interactions between two half-vaults. The N-terminal residues of domain 1, Met1-Glu4, form an intermolecular antiparallel β-sheet of the same residues of an MVP molecule related by a twofold rotational symmetry. Another specific interaction between two half-vaults is the ionic bond between Glu4-Arg42.

(Protein Data Bank entry 3BK6) and the flotillin-2 band-7 domain ($Flot^{BD7}$) (Protein Data Bank entry 1WIN). Three structures of the shoulder domain, $PhSto^{CD}$ and $Flot^{BD7}$, can be superposed well (Fig. 4). Human stomatin, which has 40.3% and 18.4% sequence identities with $PhSto^{CD}$ and $Flot^{BD7}$, respectively, is a major integral membrane protein of human erythrocytes. The core domain of stomatin is evolutionarily conserved, and falls within the stomatin/prohibitin/flotillin/HflK/C (SPFH) domain family [4]. Although the physiological function of stomatin is not yet clearly understood, the protein is known to be a lipid raft protein; the SPFH domain is involved in lipid raft association. The structural similarity among the shoulder domain and SPFH domain family supports the hypothesis that MVP is recruited to lipid rafts when human lung epithelial cells are infected with *Pseudomonas aeruginosa* [5].

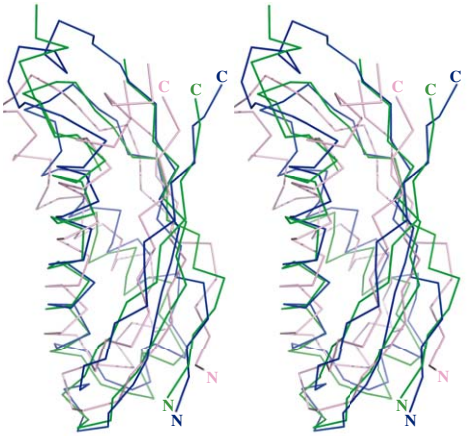

Fig. 4. Three-dimensional structure comparison of the MVP shoulder domain with $PhSto^{CD}$ and $Flot^{BD7}$. The shoulder domain (green), $PhSto^{CD}$ (blue) and $Flot^{BD7}$ (pink), are superposed well within 2.2 Å of root-mean-square deviation of equivalent Cα atoms by least-squares fitting.

Koji Kato[a], Hideaki Tanaka[b] and Tomitake Tsukihara[a,b,*]

[a] Department of Life Science, University of Hyogo
[b] Institute for Protein Research, Osaka University

*E-mail: tsuki@protein.osaka-u.ac.jp

References

[1] N.L. Kedersha *et al.*: J. Cell Biol. **110** (1990) 895.
[2] *H. Tanaka, K. Kato, E. Yamashita, T. Sumizawa, Y. Zhou, M. Yao, K. Iwasaki, M. Yoshimura and T. Tsukihara: Science **323** (2009) 384.*
[3] K. Kato *et al.*: Acta Crystallogr. D **64** (2008) 525.
[4] H. Yokoyama *et al.*: J. Mol. Biol. **376** (2008) 868.
[5] M.P. Kowalski *et al.*: Science **317** (2007) 130 (2007).