process occurs in the native mixture between two dimeric forms, MxM (swapped) and M=M (unswapped), held together by the additional constraints of two inter-chain disulphide bridges. This peculiarity offers the opportunity to study the effects of selected mutations at the O-interface on the M=M / MxM equilibrium, within a substantially invariant quaternary assembly. Two variants, having Pro 19 (P19A) or the whole sequence of the hinge peptide 16-22 replaced by the corresponding residues of RNase A (BS-hinge-A), show equilibrium and kinetic parameters of the swapping similar to those of the parent protein. On the contrary, mutation of L28 (L28Q, P19A/L28Q) significantly affects the swapping processes. The X-ray structures of the MxM forms of P19A and BS-hinge-A, and the MxM and M=M forms of the double mutant P19A/L28Q have been determined. The structural effects of the mutations are discussed on the basis of the MxM/M=M equilibrium data measured in solution. The relative insensitivity of the swapping tendency to the substitutions in the hinge region, and in particular to the replacement P19A, contrasts with the results obtained for other swapped proteins and can be rationalized in terms of the unique features of the seminal enzyme. On the other hand, the substitution in position 28 points to a crucial role of the interface residues in the swapping of BS-RNase.

Keywords: swapping , ribonuclease mutations, structural analysis

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Molecular Mechanisms of Thrombin-Mediated Fibrin Assembly

Igor Pechik[a], S. Yakovlev[b], G.L. Gilliland[a], L. Medved[b], [a]*University of Maryland Biotechnology Institute, Rockville, MD, U.S.A.* [b]*University of Maryland School of Medicine, Baltimore, MD, U.S.A.* E-mail: pechik@umbi.umd.edu

Conversion of fibrinogen into fibrin (fibrin assembly) is mediated by thrombin, which cleaves two pairs of fibrinopeptides, fpA and fpB, from fibrinogen Aα and Bβ chains, respectively. Fibrin assembly occurs in two stages starting with the removal of fpA, which triggers formation of two-stranded protofibrils, followed by the removal of fpB, which enhances lateral association of protofibrils into thicker fibers. Non-substrate interaction of thrombin with the central part of fibrin(ogen) seems to play an important role in these processes. To establish the structural basis for the sequential cleavage of fpA and fpB, we crystallized thrombin in complex with a fragment corresponding to the central part of fibrin(ogen), and solved the structure of this complex at 3.65 Å resolution [1]. Next, we modeled possible arrangements of fibrinopeptides-containing NH_2-terminal portions of the Aα and Bβ chains, which were not identified in the electron density map. The resulting structure reveals that thrombin binds fibrinogen in a way that promotes positioning of the Aα chains in its active site cleft. This explains the preferential removal of fpA at the first stage of fibrin assembly. Subsequent modeling of a protofibril by docking the structure of the dimeric fibrin-derived D-D fragment into the complex suggests that the fpB-containing portions in such structure should interact with D-D to restrict their positions to the vicinity of the active site cleft. In agreement, surface plasmon resonance experiments reveal that these portions indeed interact with D-D. These provide the rationale for the accelerated removal of fpB upon the formation of protofibrils.

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Keywords: protein complex structure, fibrinogen, blood coagulation

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The Complex of the Polycomb Group Proteins Ring1B (RNF2) and Bmi1 –A Structure-function-analysis

Gretel Buchwald[a], Petra van der Stoop[b], Oliver Weichenrieder[a], Anastassis Perrakis[a], Maarten van Lohuizen[b], Titia K. Sixma[a], [a]*Department of Molecular Carcinogenesis,* [b]*Department of Molecular Genetics*, *Netherlands Cancer Institute, Amsterdam, The Netherlands.* E-mail: g.buchwald@nki.nl

The Ring finger proteins Ring1B and Bmi1 belong to the Polycomb group proteins (PcG), which form large multimeric protein complexes. These complexes covalently modify histones leading to the transcriptional repression of specific genes. Mammalian homologs of Ring1B and Bmi1 are part of the PRC1L complex (Polycomb repressive complex 1-like), which acts as an E3 ligase in the ubiquitination of histone H2A [1].

Here we present the crystal structure of the Ring1B-Bmi1 Ring domain dimer at a resolution of 2.0 Å. The structure reveals that the interaction is mainly determined by structural elements that flank the Ring motifs. Details of the structure will be presented.

An *in vitro* ubiquitination assay was used to study E3 ligase activity of the Ring1B-Bmi1 complex. Adding either the full length Ring-Ring complex or the truncated form significantly activated ubiquitination of histone H2A.

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Keywords: ubiquitin system, Ring1B (RNF2), Bmi1

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Structure of a Giant Hemoglobin of the Gutless Beard Worm *Oligobrachia mashikoi*

Nobutaka Numoto[a,b], Taro Nakagawa[c], Akiko Kita[a,d], Yuichi Sasayama[e], Yoshihiro Fukumori[c], Kunio Miki[a,b], [a]*Graduate School of Science, Kyoto University.* [b]*RIKEN Harima Institute/SPring-8.* [c]*Graduate School of Natural Science and Technology, Kanazawa University.* [d]*Research Reactor Institute, Kyoto University.* [e]*Institute of Nature and Environmental Technology, Kanazawa University, Japan.* E-mail: numoto@kuchem.kyoto-u.ac.jp

Pogonophorans and vestimentiferans obtain their nutrition solely from their symbiotic chemoautotrophic sulfur-oxidizing bacteria because they have no mouth or gut. These animals have sulfide-binding 400 kDa and/or 3500 kDa hemoglobin (Hb) which transports oxygen and sulfide simultaneously, and the symbiotic bacteria are supplied with sulfide by these giant Hbs. We have determined for the first time the crystal structure of a 400 kDa vascular Hb from pogonophoran *Oligobrachia mashikoi* at 2.85 Å resolution as an intact oligomeric form. The structure is hollow-spherical, with outer and inner diameters of about 120 Å and 50 Å, respectively, and composed of a total of 24 globins as a dimer of dodecamer. This dodecameric assemblage would be a common unit to both 400 kDa and 3500 kDa Hb. The structure of the mercury derivative used for phasing provides insights into the sulfide-binding mechanism. The mercury compounds bound to all free Cys residues that have been expected as sulfide binding sites. Some of the free Cys residues are surrounded by Phe aromatic rings, and mercury atoms come into contact with these residues in the derivative structure; it is strongly suggested that sulfur atoms bound to these sites could be stabilized by aromatic-electrostatic interactions by the surrounding Phe residues.

Keywords: proteins, hemoglobins, supramolecular assemblies

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Structural Biology of Type IV Secretion System

Laurent Terradot[1,2], Richard Bayliss[1], Clasien Oomen[1], Gordon Leonard[2], Gabriel Waksman[1], [1]*Institute of Structural Molecular Biology, Malet Street, London, WC1E 7HX, UK.* [2]*Macromolecular Crystallography Group ESRF, 6, rue Jules Horowitz, F-38043 Grenoble Cedex, France.* E-mail: terradot@esrf.fr

Type IV secretion systems (T4SSs) are machineries used for the transport of macromolecules across the bacterial cell envelopes of Gram-negative bacteria. The archetypal T4SS, the VirB/D system was first defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefasciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4) whereas T4SS in other bacteria can display homologues for only a subset of those proteins. T4SSs are highly versatile and have been found in many bacterial pathogens such as *Helicobacter pylori* (Cag PAI), *Brucella suis* (VirB/D), and *Legionella pneumophila* (Dot, Icm). The secretion machinery spans both bacterial