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Host recognition of bacteriohphage K1F: EndoNF in complex with helical polysialic acid

Eike Ch Schulz[1], Katharina Stummeyer[2], Achim Dickmanns[1], Rita Gerardy-Schahn[2], Ralf Ficner[1]
[1]University of Goettingen, Department for molecular structural biology, Justus von Liebig Weg 11, Goettingen, Niedersachsen, D-37077, Germany, [2]Hannover Medical School MHH, Department for Cellular Chemistry, OE4330, Carl-Neuberg-Strasse1, D-30625 Hannover Germany, E-mail：eschulz1@gwdg.de

Alpha-2,8-linked polysialic acid (polySia) is an important mediator of cellular motility and functional plasticity in the vertebrate brain and has implications in tumor metastasis. PolySia is also a common cell wall modification of pathogenic prokaryotes like Escherichia coli K1 and Neisseria meningitides serogroup B that cause meningitis and severe sepsis in humans. The only known source for enzymes that specifically degrade polySia are E. coli K1 specific bacteriophages. They possess endosialidases as host specificity determining tailspike proteins required to digest the bacterial polySia capsule during infection. We now determined several crystal structures of active site mutants of an endosialidase cloned from bacteriophage K1F (endoNF) in complex with oligomeric sialic acid. The structures have been refined to resolutions up to 1.5 Å. A well defined electron density map of oligomeric sialic acid could be observed for three binding sites, one of which is located in the active site cleft. The complex structure confirms the helical conformation of polySia and supports the model of a substrate assisted catalytic mechanism.

Keywords: bacteriophage, polysialic acid, endoNF

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Structure of main protease from a global infectious human coronavirus, HCoV-HKU1

Qi Zhao
TsingHua Univ., School of Medicine, new lifescience building 201# Tsinghua Univ Beijing China, Beijing, Beijing, 100089, China, E-mail：zhaoqi@xtal.tsinghua.edu.cn

Human coronavirus HKU1 (HCoV-HKU1) is a new coronavirus that was first identified in in Hong Kong in 2005. Infection by HCoV-HKU1 occurs worldwide and causes syndromes such as common cold, bronchitis and pneumonia. The coronavirus main protease (Mpro), which is a key enzyme in viral replication via a cascade of proteolytic processing of replicase polyproteins, has been identified as an attractive target for rational drug design. In this study, we report the structure of HCoV-HKU1 Mpro in complex with a synthetic compound N3. The structure of HCoV-HKU1 serves as a model for group 2a coronaviruses, which are distinct from group 2b coronaviruses such as SARS-CoV. This structure and enzyme activity assays also support the relative conservation at the P1 position based on genome sequencing. This complex structure also provided clues of substrate binding mode at P3 position which was thought to be solvent-exposed.

Keywords: coronavirus, main protease, HCoV-HKU1

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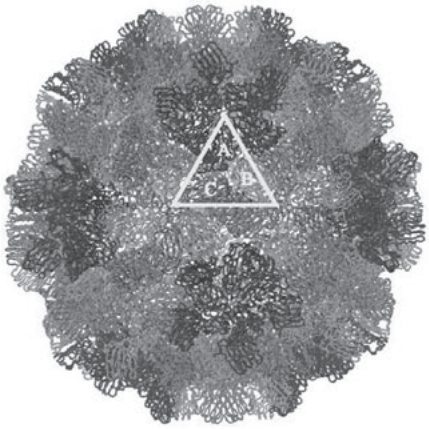
The structure of melon necrotic spot virus determined at 2.8Å resolution

Yasunobu Wada[1], Hideaki Tanaka[1], Eiki Yamashita[1], Tamaki Ichiki-Uehara[2], Toshihiro Omura[2], Tomitake Tsukihara[1]
[1]Osaka University, Institute for protein research, 3-2, Yamadaoka, Suita, Osaka, Osaka, 565-0871, Japan, [2]National Agricultural Research Center, 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan, E-mail：nobu-w@eos.ocn.ne.jp

The structure of melon necrotic spot virus (MNSV) was determined at 2.8 Å resolution. Although MNSV is classified into the genus Carmovirus of the family Tombusviridae, the three-dimensional structure of MNSV showed a higher degree of similarity to tomato bushy stunt virus (TBSV), which belongs to the genus Tombusvirus, than to carnation mottle virus (CMtV), turnip crinkle virus (TCV) or cowpea mottle virus (CPMtV) from the genus Carmovirus. Thus, the classification of the family Tombusviridae at the genus level conflicts with the patterns of similarity among coat-protein structures. MNSV is one of the viruses belonging to the genera Tombusvirus or Carmovirus that are naturally transmitted in the soil by zoospores of fungal vectors. The X-ray structure of MNSV provides us with a representative structure of viruses transmitted by fungi.



Keywords: virus, tinbusviridae, coat protein

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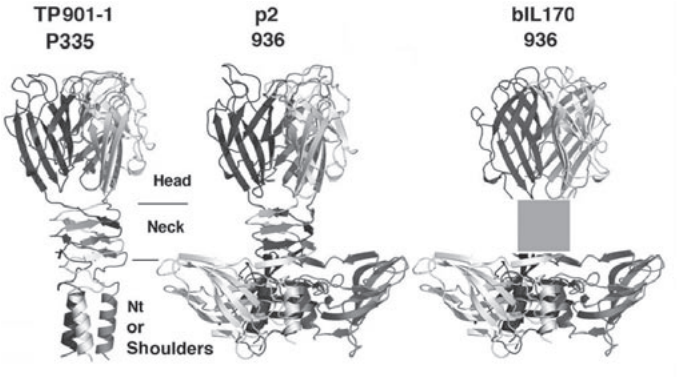
Modular structure of receptor binding proteins from *Lactococcus lactis* phages

Christian Cambillau[1], Silvia Spinelli[1], Stephanie Blangy[1], Mariella Tegoni[1], Sylvain Moineau[2], Denise M Tremblay[1,2], Valerie Campanacci[1]
[1]CNRS-Universities of Marseille, AFMB, Campus Luminy, 163 Av de Luminy, case 932, MARSEILLE CEDEX 09, PACA, 13288, France, [2]Departement de Biochimie et de Microbiologie, Faculte des Sciences et de Genie, Universite Laval, Quebec City, Quebec, Canada, G1K 7P4, E-mail：christian.cambillau@afmb.univ-mrs.fr

Lactococcus lactis is a Gram positive bacterium, used by the dairy industry for the manufacture of fermented milk products. The double-stranded DNA bacteriophage p2 infects L. lactis strains by using a receptor-binding protein (RBP) located at the tip of its tail. The crystal structure of phage p2 RBP, reveals a homo-trimeric protein formed of three domains: the shoulders, a beta-sandwich attached to the phage; the neck, an interlaced beta-prism, and the receptor recognition head, a 7 stranded beta-barrel. The complex of RBP with a neutralizing llama VHH domain identified the area binding to the bacterial receptor. It is able to bind various saccharides present in the bacterial receptor. The structural similarity between the head domain

and those of adenoviruses or reoviruses, which invade mammalian cells, suggests that these viruses, despite evolutionary distant targets and the different chemical nature of their genomes (DNA vs RNA), might have a common ancestral gene. Results on RBPs of other lactococcal phages (bIL170 and TP901-1) will also be presented, which further illustrate the modular nature of these proteins in the viral world, and their insertion in the viral baseplate.



Keywords: receptor binding protein, bacteriophage, *Lactococcus lactis*

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Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain

Chwan-Deng Hsiao, Chun-Yuan Chen, Chung-ke Chang, Tai-huang Huang
Academia Sinica, Institite of Molecular Biology, No. 128, Academia Rd. sec. 2, Nankang, Taipei, N/A, 115, Taiwan, E-mail：hsiao@gate.sinica.edu.tw

Coronavirus nucleocapsid proteins are basic proteins that encapsulate viral genomic RNA to form part of the virus structure. The nucleocapsid protein of SARS-CoV is highly antigenic and associated with several host-cell interactions. Our previous studies using nuclear magnetic resonance revealed the domain organization of the SARS-CoV nucleocapsid protein. RNA has been shown to bind to the N-terminal domain (NTD), although recently the C-terminal half of the protein has also been implicated in RNA binding. Here, we report that the C-terminal domain (CTD), spanning residues 248－365 (NP248-365), had stronger nucleic-acid-binding activity than the NTD. To determine the molecular basis of this activity, we have also solved the crystal structure of the NP248-365 region. Residues 248－280 form a positively charged groove similar to that found in the infectious bronchitis virus (IBV) nucleocapsid protein. Furthermore, the positively charged surface area is larger in the SARS-CoV construct than in the IBV. Interactions between residues 248-280 and the rest of the molecule also stabilize the formation of an octamer in the asymmetric unit. Packing of the octamers in the crystal forms two parallel, basic helical grooves, which may be oligonucleotide attachment sites, and suggests a mechanism for helical RNA packaging in the virus.

Keywords: severe acute respiratory syndrome coronavirus, nucleocapsid, viral packaging

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Crystallization and structure determination of recombinant hepatitis E virus-like particle

Naoyuki Miyazaki[1,2,3], Che-Yen Wang[2,3], Akifumi Higashiura[1], Atsushi Nakagawa[1], Tian-Cheng Li[4], Naokazu Takeda[4], Li Xing[2,3], Tsukihara Tomitake[1], Tatsuo Miyamura[4], R. Holland Cheng[2,3]
[1]Institute for Protein Research, Laboratory of Protein Synthesis and Expression, 3-2 Yamadaoka, Suita, Osaka, 565-0871, Japan, [2]Molecular and Cellular Biology, University of California, Davis, CA 95616, USA, [3]Department of Biosciences at Novum, Karolinska Institute, Halsovagen 7, 14157 Huddinge, Sweden, [4]Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan, E-mail：naomiyazaki@protein.osaka-u.ac.jp

Hepatitis E virus (HEV) causes water-borne epidemics with a substantial mortality rate in pregnant women. It accounts for the major part of enterically transmitted hepatitis infection worldwide. The major structural protein is derived from open reading frame 2 of the viral genome, which forms the virus-like particles (VLPs) as expressed in insect cells. Single crystals of HEV-VLPs obtained by the hanging-drop vapor-diffusion methods at 293 K diffracted X-rays to 3.8 Å resolution, and the atomic structure of the HEV-VLP has been determined by phase extension from a low-resolution electron microscopy structure. The structure of the capsid protein of HEV has three domains, which exhibit similarities to the caliciviruses, agents of acute nonbacterial gastroenteritis.

Keywords: virus structure, virus coat proteins, viral structure and function

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The N-terminal induced-fit loops of capsid protein of Rice dwarf virus stabilize capsid of the virus

Hisashi Naitow[1], Naoyuki Miyazaki[2], Tomitake Tsukihara[2], Atsushi Nakagawa[2], Toshihiro Omura[3], Nobuo Kamiya[4]
[1]RIKEN Harima Institute / RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho,, Sayo-gun,, Hyogo, 679-5148, Japan, [2]Institute for Protein Research, Osaka University, [3]National Agricultural Research Center, [4]Material Science & Chemistry, Graduate School of Science, Osaka City University, E-mail：naitow@spring8.or.jp

Induced-fit loops were observed in the N-terminal region of the P8 outer-capsid protein of Rice dwarf virus. In thirteen independent P8 molecules, each of the loops had a different conformation. These induced-fit loops appeared to stabilize and strengthen the binding between the respective P8 outer-capsid proteins and the P3 core-capsid protein, with the heterologous bindings, in the double-layered capsid, having disparate symmetry. Among five kinds of P8 trimer, all the P8 proteins in the P-trimers that were located at the five-fold axes exhibited some disorder in the N-terminal region. This observation, together with the total binding energy between the P-trimer and vertical P3 and horizontal P8 was lowest among the trimers, suggests that it would be this trimer that would be released when one of the outer-capsid trimers is shed from the core particle to mediate the biological activity of the virus. In figure, the thirteen