micro meter to 10 micro meter. Until now a protein crystal is picked up manually from a crystallization droplet using a cryoloop and is mounted on a goniometer head. However it seems to be impossible to manipulate the protein microcrystal by hands, because the protein microcrystals are very small and fragile against a shock. So we are developing an automatic microcrystal pick-up system. In the system, we applied optical tweezers to manipulate the fragile protein crystal. It was reported that the optical tweezers at the near-infrared region traps and manipulates a cell without critical photodamage. We tried two kinds of optics for optical tweezers. One had a condensing lens, the other had a lensed fiber probe, which focused the laser on the object. The optical tweezers with the condensing lens succeeded in trapping of protein crystals with the size in 100 micro meter range. X-ray measurement of the trapped crystals indicated that laser trap with 1064 nm wavelength hardly affected the result of X-ray structural analysis. On the other hand, the optical tweezers with the two lensed fiber probe could manipulate the protein microcrystal with lower emission power. The lensed fiber probe was smaller than former, which had an advantage to be operated in the crystallization droplets at various crystallization plates.

Keywords: protein microcrystal, optical tweezers, crystal manipulation

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A new understanding of radiation damage at cryogenic temperatures

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Radiation damage is one of the remaining bottlenecks in structural biology. In the literature many aspects of radiation damage are very well described, however the underlying mechanism responsible for the loss of diffracting power of the crystals remained unknown so far. To get a deeper insight into the processes involved we have investigated radiation damage by combining traditional X-ray data collection with small angle X-ray scattering over a broader temperature range from 5 K to 160 K. All experiments were carried out at the protein crystallography beamline X06SA at the Swiss light source (SLS) equipped with a Pilatus 6M pixel detector. For all crystals individual increase or decay rates for different quality parameters with dose were determined and analyzed as a function of temperature. Interestingly a unexpected temperature dependence for all these parameters was observed. We could identify an optimal data collection temperature, where radiation damage is reduced by about 30% compared to data collection at 100 K, were most experiments are performed today. To gain information about the length scale of the disorder phenomena we performed small angle X-ray scattering experiments on cubic insulin crystals at the same experimental conditions and temperatures as used for the data collections. Analysis of the diffuse scattering signal lead us to two different mechanisms responsible for the loss of diffracting power. We could identify hydrogen gas, formed during irradiation of biological samples with X-rays, as the main damaging agent at cryogenic temperatures. Our findings are in good agreement with observations from cryo-electron microscopy and explain the basic mechanism of radiation damage at cryogenic temperatures for the first time in a conclusive manner.

Keywords: radiation damage, cryocooled crystallography, small-angle scattering

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Crystal structure of the peptidoglycan recognition protein at 1.8 Å resolution

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The mammalian peptidoglycan recognition protein-S (PGRP-S) binds to peptidoglycans (PGNs) which are essential components of the cell wall of bacteria. The crystals of PGRP-S belong to orthorhombic space group *I*222 with a = 87.0Å, b = 01.7Åand c = 162.3Å having four crystallographically independent molecules in the asymmetric unit. The structure has been determined with molecular replacement method and refined to an R_{cryst} and R_{free} factors of 0.225 and 0.247 respectively. Overall, the structures of all the four molecules are identical. The folding of PGRP-S consists of a central β-sheet with five β-strands, four parallel and one antiparallel and three α-helices. This protein fold provides two functional sites. The first of these is the PGN-binding site, located on the groove that opens on the surface in the direction opposite to the location of the N-terminus. The second site is implicated to be involved in the binding of non-PGN molecules, it also include putative N-terminal segment residues, (1-14) and helix α2 in the extended binding. The structure reveals a novel arrangement of PGRP-S molecules in which two pairs of molecules associate to form two independent dimers. The first dimer is formed by two molecules with N-terminal segment at the interface in which non-PGN binding site is completely buried whereas the PGN- binding sites of two participating molecules are fully exposed at the opposite ends of the dimer. In the second dimer formed by another set of two molecules in which the PGN binding sites are buried at the interface while the non-PGN binding sites are located at the opposite surfaces of the dimer. This form of dimeric arrangement is unique and seems to be aimed at enhancing the capability of the protein against invading bacteria.

Keywords: innate immune system, pgrp, pattern recognition

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Structural insights into an affinity-based selection of virus-specific public T cell receptors

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To be protected against intracellular pathogens, vertebrates have developed an adaptive immune response based on a large number of T lymphocytes. Each T cell clone displays an Ig-like receptor (TCR) specific for antigens bound to Major Histocompatibility Complex (MHC) molecules, present at the Antigen Presenting Cell surface. Although the thymic selection generates a diverse naive repertoire

of MHC-restricted and non-autoreactive TCRs, the specific response to an antigen is characterized by the expression of a limited TCR repertoire with preferred gene segment usage. We focused our studies on the T cell response against the human beta-herpesvirus cytomegalovirus (HCMV) that infects 60 to 90% of the population and can cause life-threatening diseases in immunocompromised patients. In most individuals sharing the widespread MHC allele HLA-A*0201 (A2), HCMV-specific T cells target the same epitope NLV, derived from the viral protein pp65. While the NLV-specific TCR repertoire is heterogeneous in healthy donors, a dramatic diversity reduction and a high affinity TCR selection occur in chronic inflammation and immunodepression. To provide new insights into the structural basis of antigen recognition by T cells and generation of protective immune response against a major infectious agent in human, we have determined the crystal structure of the ternary complex between NLV, A2 and a TCR expressed by a predominant clone (RA14) derived from a rheumatoid arthritis patient. These data, with binding and activation experiments, provide evidences of constrains for preferred TCR gene usage in this viral peptide recognition. Contrasting with known structures of public TCRs, a novel antigen read-out is observed with the recognition of three peptide and one A2 specific hot spots.

Keywords: structures of T-cell receptor complexes, HLA, X-ray crystallography of proteins

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Crystal structure of a pattern recognition protein required for fungal detection in *Drosophila*

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In *Drosophila*, the synthesis of antimicrobial peptides in response to microbial infections is under the control of the Toll and Imd signaling pathways. The Toll signaling pathway responds mainly to gram-positive bacterial and fungal infection while the Imd pathway mediates the response to gram-negative bacteria. Microbial recognition upstream of Toll involves peptidoglycan recognition proteins (PGRPs) and glucan binding proteins (GNBP). The sensing of gram-positive bacteria is mediated by PGRP-SA and GNBP1 that cooperate to detect the presence of lysine-type peptidoglycan in the host. Recently it has been shown that the pattern recognition receptor GNBP3 is required for the detection of fungal cell wall components. We have solved the crystal structure of the N-terminal domain of GNBP3 at 1.8 Å by single wavelength anomalous dispersion (SAD) method using a Sm derivative. This structure together with the functional studies contributes to improve understanding of the molecular mechanisms underlying innate immune system in *Drosophila*.

Keywords: innate immunity, pattern recognition, molecular recognition

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Structure-function correlations in vertebrate defensins

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Defensins are small (3-5 kD), cysteine-rich cationic proteins, mostly recognized for their antimicrobial properties. The last several years of research have also revealed the ability of defensins to interact specifically with a range of cellular receptors, including chemokine receptors. Using X-ray crystallography, functional assays, and other biophysical methods, we attempted to correlate the biological activities of defensins with their structural characteristics. We were able to demonstrate that the N-terminal fragment of human beta-defensin is critical for productive signaling through the chemokine receptor CCR6. Specifically, we can explain why human beta-defensins 1 through 3 activate CCR6, whereas beta-defensin 4 does not. We also demonstrated that the human receptor CCR6 can be activated by non-human defensins, if the molecules contain the required structural determinants. Our data suggest that for most (possibly for all) biological activities of defensins that belong to both alpha and beta subfamilies, specific oligomerization is irrelevant. Furthermore, we have established specific roles for highly-conserved residues in human alpha-defensins. We explained the functional/structural roles of a salt bridge (Arg-Glu) or of the presence of a Gly residue, both nearly invariant in alpha-defensins. Although all alpha-defensins studied to date appear to form conserved dimers in solution, we have found the first case of a native alpha-defensin in which mutual arrangement of monomers is strikingly different from all examples reported previously. Our conclusions are supported by nearly 30 high-resolution crystal structures, accompanied by thorough biological studies in vitro, and, for selected proteins, additional studies in solution.

Keywords: defensins, chemotaxis, antimicrobial

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Molecular recognition of the natural killer cell receptors 2B4 and Ly49 with their respect ligands

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Natural killer (NK) cells eliminate virally infected and tumor cells. Among the receptors regulating NK cell function we are interested in 2B4, a member of the signaling lymphocyte activation molecule (SLAM) family that binds CD48; and the Ly49 family, regulating NK cell function by sensing major histocompatibility complex class I (MHC-I) molecules on target cells. 2B4 is the only heterophilic receptor of the SLAM family. The complex structure between the N-terminal domains of mouse 2B4 and CD48 displayed an association mode related to, yet distinct from, that of the NK-T-B-antigen dimer, suggesting a model that permits intermixing of SLAM receptors with MHC-specific receptors in the NK cell immune synapse. The crystal structures of Ly49C, Ly49G and Ly49C－H-2Kb complex, combined with mutational analysis of Ly49A, permitted a structure-based classification of Ly49s that we used to dissect the binding site into three distinct regions, each having different roles in MHC recognition. One region, located at the center of the binding site, has a similar structure across the Ly49 family and mediates conserved interactions with MHC-I that contribute most to binding. However, the preference of individual Ly49s for particular MHC-I molecules is governed by two regions that flank the central