motif, while the C-terminal domain shows a typical restriction endonuclease fold. By structural comparison and mutational analysis we showed that the active site of SdaI is located at the C-terminal domain and exhibits a new variation of the canonical PD...(D/E)XK active site motif. Mutational analysis of the residues from the predicted recognition helix of the wHTH motif suggests that SdaI determinants of sequence specificity are clustered at the N-terminal domain. The modular architecture of SdaI, wherein one domain mediates DNA binding while the other domain is predicted to catalyze hydrolysis, distinguishes SdaI from the previously characterized restriction enzymes interacting with symmetric recognition sequences.

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MS04 P08

C1-inhibitor structure reveals a novel mechanism of heparin potentiation Veronika Harmat[a,b], László Beinrohr[c] , József Dobó[c], Zsolt Lőrincz[c], Péter Gál[c], Gábor Náray-Szabó[a,b], Péter Závodszky[c] *[a]Structural Biology and Chemistry Laboratory, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary. [b]Protein Modelling Group, Eötvös Loránd University, Hungarian Academy of Sciences, Budapest, Hungary. [c]Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, Budapest,Hungary.* E-mail: harmatv@ludens.elte.hu

Keywords: immune regulation, serine-protease inhibitors, electrostatic interactions

C1-inhibitor (C1-inh), a member of the serpin family of serine-protease inhibitors, is the major downregulator of inflammatory processes in blood plasma. Genetic deficiency of C1-inh results in hereditary angioedema, a dominantly inheritable, potentially lethal disease. Anti-inflammatory activity of heparin a naturally occurring glycosaminoglycan is realized through increasing inhibitory activity of C1-inh.
Activities of many serpins are modulated by ligand binding. Heparin enhances the inhibitory effect of antithrombin by "bridging" the two proteins. Allostery also plays an important role in the heparin activation of antithrombin. Polyanion potentiation of protein C inhibitor against protein C by the "co-occupation" mechanism is also based on migration of proteins along the heparin chain. These mechanisms fail to explain the effect of heparin on C1-inh activity.
We crystallized the serpin domain of C1-inh using the hanging drop method. Collection of diffraction data was carried out at 100 K on beamline X11 of EMBL-outstation at DESY, Hamburg, Germany. The structure was determined using molecular replacement and refined to 2.35 Å resolution.
The structure represents a novel latent serpin form with 7-stranded sheet A and a truncated disordered N-terminal domain, which explains functional consequences of the numerous naturally occurring mutations. Unique conformation of the C-terminal tail indicates its unexpected role as a barrier in the active-latent transition.
On the basis of surface charge pattern, heparin affinity measurements and docking of a heparin disaccharide, a heparin binding site is proposed in the contact area of the serpin-proteinase encounter complex. We show how polyanions change selectivity of C1-inh by a novel "sandwich" mechanism, explaining earlier reaction kinetic and mutagenesis data. These results provide the explanation of heparin's antiinflammatory activity and may help improving therapeutic C1-inh preparations in treatment of common inflammatory diseases, such as organ transplant rejection and heart attack.

MS04 P09

Crystal structures of flax rust avirulence proteins AvrL567-A and AvrL567–D. Gregor Guncar[a,b,1,2], Ching-I A. Wang[a,1], Jade K. Forwood[a,b], Trazel Teh[a], Ann-Maree Catanzariti[d], Gregory J. Lawrence[c], Horst Joachim Schirra[b], Peter A. Anderson[e], Jeffrey G. Ellis[c], Peter N. Dodds[c] and Bostjan Kobe[a,b,f]. *[a]School of Molecular and Microbial Sciences, University of Queensland(UQ), Brisbane, Australia [b]IMB, UQ, Brisbane, Australia [c]Division of Plant Industry, CSIRO, Canberra, Australia [d]Department of Plant and Microbial Biology, University of California, Berkeley, USA [e]SBS, Flinders University of South Australia, Adelaide, Australia [f]SRC for Functional and Applied Genomics, UQ, Brisbane, Australia [1]These authors contributed equally to this work. [2]On leave from Josef Stefan Institute, Ljubljana, Slovenia.*. E-mail: g.guncar@uq.edu.au

Keywords: avirulence, plant resistance, SAD

Plants have evolved a versatile multi-layered defence system to fight pathogens. The gene-for-gene model of plant disease resistance involves direct or indirect recognition of pathogen avirulence (Avr) proteins by plant resistance (R) proteins. Flax rust (*Melampsora lini*) AvrL567 avirulence proteins and the corresponding flax (*Linum usitatissimum*) L5, L6 and L7 resistance proteins interact directly. We determined the three-dimensional structures of two members of the AvrL567 family, AvrL567-A and AvrL567-D. The structure of AvrL567-A was solved by single-wavelength anomalous dispersion (SAD) technique, using the dataset collected on the conventional in-house Cu X-ray generator, taking advantage of a bound Co^{2+} ion. The structure of AvrL567-D was solved by molecular replacement, using the structure of AvrL567-A as a search model. The structures of both proteins are very similar and reveal a β-sandwich fold with no close known structural homologues. The polymorphic residues in the AvrL567 family map to the surface of the protein and polymorphisms in residues associated with recognition differences lead to significant changes in surface chemical properties. Analysis of single amino acid substitutions in AvrL567 proteins confirm the role of individual residues in conferring differences in recognition and suggest that recognition specificity of the L proteins for the AvrL567 family members results from the cumulative effects of multiple amino acid contacts. The structure also provides insights into possible pathogen-associated functions of AvrL567 proteins, with nucleic acid binding activity demonstrated *in vitro*, suggesting a possible role in influencing host gene expression during infection. Our studies provide some of the first structural information on avirulence proteins that

bind directly to the corresponding resistance proteins, allowing an examination of the molecular basis of the interaction with the resistance proteins as a step towards designing new resistance specificities.

MS04 P10

Crystal structure of the heterodimeric restriction endonuclease R.BspD6I. Galina Kachalova [a,b], Eugeny Rogulin[b], Alfiya Yunusova[b] , Rimma Artyukh[b], Tatyana Perevyazova[b], Ludmila Zheleznaya[b], Nickolay Matvienko[c],Hans D. Bartunik[a] [a]*Max-Planck Unit for Structural Molecular Biology, Hamburg, Germany,* [b]*Institute of Theoretical and Experimental Biophysics, Pushchino, Russian Federation,* [c]*Institute of Protein Research, Pushchino , RussianFederation*
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Keywords: endonuclease, nicking, DNA binding

R.BspD6I represents a new type of restriction endonucleases. The heterodimeric enzyme is characterized by a number of unique features such as (i) site- and strand-specific nicking activity of the large subunit in the absence of small subunit [2], (ii) recognition of the pseudosymmetric DNA sequence 5'-GAGTC-3'/5'-GACTC-3' by the large subunit at a position remote from the cutting site, (iii) a complete lack both of endonuclease activity and DNA-binding capability of the small subunit alone, (iv) grossly different molecular weights of the two subunits (71kDa and 20 kDa, respectively). We solved the structure of the large subunit of R.BspD6I [1] at 1.84Å resolution and the structure of the small subunit at 1.5Å resolution. The crystal structure of the large subunit represents the first known 3D structure of an endonuclease with site- and strand-specific nicking activity, thus providing a basis for investigating the nicking mechanism on a molecular level.
All X-ray diffraction data were measured on the beamline BW6/DORIS. The structure of the large subunit (604 a.a.) was solved on the basis of MAD phasing at the Br K edge. Br sites were identified with SHELXD/SHELXE [3]. The quality of the initial density map was sufficient for automatic tracing of most of the protein chain with ARP-wARP[4], completed in manual building steps using O [5] . The final model was refined with SHELXL-97 to a R-factor of 20% (Rfree = 25.4%) at 1.84 Å resolution. Recognition, linker, and cleavage domains were identified as separate folds of the large subunit. The probable functions of the domains were derived from an analysis of their topologies.
The structure of the small subunit (186 a.a.) was determined by molecular replacement with MOLREP[6] using the cleavage domain of the large subunit as a search model. No similarity to any other protein sequence present in the PDB was detected. The structural model of the small subunit was built with ARP-wARP and refined with REFMAC[7]. We derived docking models of the heterodimeric enzyme and of the complex with DNA. This provided a basis for suggesting a structural mechanism of the endonuclease function of R.BspD6I.

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MS04 P11

Structural studies on RIPs elucidates the differences in their action on polynucleotides Simona Fermani[a], Giovanna Tosi[a], Giuseppe Falini[a], Valentina Farini[b], Letizia Polito[b], Luigi Barbieri[b], Andrea Bolognesi[b], [a]*Department of Chemistry "G. Ciamician".* [b]*Department of Pathology, Alma Mater Studiourum, University of Bologna, Italy.* E-mail: simona.fermani@unibo.it

Keywords: ribosome-inactivating proteins, crystal structure, adenosine glycosylase activity

In a variety of higher plants (mostly Angiospermae, both mono-and dicotyledons) have been isolated toxins able to inhibit protein synthesis and to induce cell death. These toxins, called Ribosome Inactivating Proteins (RIPs), are localized in different parts of the plant [1]. RIPs are also found in some fungi and bacteria.
RIPs were found to remove a single adenine from rRNA [2], thus being denominated rRNA N-glycosidases (rRNA N-glycohydrolases, EC 3.2.2.22). This adenine lies in a GAGA tetraloop, called sarcin/ricin loop, being the site of action of these two toxins, highly conserved in ribosomes of bacteria, plants and animals. The cleavage of that specific N-glycosidic bond irreversibly damages ribosomes. It was also ascertained that all RIPs deadenylate other polynucleotides, as RNAs from different sources, poly(A), DNA, and even poly (ADP_ribosyl)ated proteins [3]. RIPs show different degrees of homology in their amino-acid sequence, similar chemico-physical properties and seem to have identical enzymatic activity. The role of RIPs in nature is an important and challenging question since RIPs are one of the most abundant enzymes in plants and they appear to be widely conserved in the different plant species. RIPs have important application in agriculture and mainly in medicine: immunotoxins, toxin-antibody conjugates, have been used in the therapy against cancer, parasitic and autoimmune diseases.
Here, we present the three-dimensional structure of three type 1 RIPs, dianthin 30 [4] bouganin and lychnin [5,6]. Their polynucleotide adenosine glycosylase activity was also determined together with the other known RIPs: PAP-R, momordin I, saporin-S6 and ricin chain A.
The experiments on various substrates showed that saporin-S6 amongst the tested RIPs, released the highest number of adenine molecules from rat ribosomes and polyadenine, while its efficiency is similar to dianthin 30, bouganin and PAP-R on herring sperm DNA.
The structural comparison between considered RIPs in the catalytic site and in the region surrounding the active cleft, gave some hints to the understanding of the different behaviours of these enzymes in the deadenylation of various substrates

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