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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MS04 P12

Ribosomal protein L1 in complex with the rRNA and mRNA: regulation of L1 translation. Alexey Nikulin[a], Svetlana Tishchenko[a], Natalia Nevskaya[a], Ekaterina Nikonova[a], Vladislav Kljashtorny[a], Oleg Nikonov[a], Sergei Volchkov[a,b], Wolfgang Piendle[c], Maria Garber[a], Stanislav Nikonov[a] [a]*Institute of Protein Research RAS, Pushchino, Moscow Region, 142290, Russia.* [b]*Institute of Cell Biophysics RAS, Pushchino, Moscow Region, 142290, Russia,* [c]*Institute for Medical Chemistry and Biochemistry, A-6020 Innsbruck, Austria.*
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Keywords: RNA-protein complexes, RNA-protein interactions, ribosome structure and function

A typical example of regulation on the level of translation is coordinated synthesis of ribosomal components during ribosome biogenesis. When synthesized in excess over rRNAs, some primary rRNA binding ribosomal proteins interact with their mRNA and inhibit translation of their own genes and the other genes in their operons. A serial works on the structures of complexes between ribosomal protein L1 and its targets on 23S rRNA and mRNA have been carried out by our group [1, 2, 3, 4]. The structures of the ribosomal protein L1 protuberance and two regulatory complexes have been determined and analyzed. Both binding sites on the RNAs share a conserved consensus structure, however, the protein binds to its 23S rRNA target site with at least 10-fold higher affinity than to its regulatory site on the mRNA. A structural analysis of L1-rRNA and L1-mRNA complexes revealed structurally invariant regions of the RNA-protein interfaces presumably responsible for the RNA-protein recognition. This region belongs exclusively to domain I of the protein. We conclude that domain II of the protein does not contribute to the RNA recognition but increased the stability of the ribosomal complexes in comparison with the L1-mRNA one. To confirm experimentally that domain I of the L1 protein recognizes targets for the protein on the both RNA molecules, truncated L1 variant has been prepared and RNA-binding ability of the isolated domain I was tested. This work was supported by the Russian Academy of Sciences, the Russian Foundation for Basic Research (grants №№04-04-49634, 03-04-48327) and the PCB RAS program. The research of M.G. was supported in part by International Research Scholar's award from HHMI.

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MS04 P13

The Electron Transfer Complex Rubredoxin – Rubredoxin Reductase from *P. aeruginosa*. Gregor Gregor Hagelüken[a], Dirk W. Heinz[b], Burkhard Tümmler[c], Wolf-Dieter Schubert[a] [a]*Molecular Host Pathogen Interactions,* [c]*Structural Biology, Helmholtz Centre for Infection Research, Braunschweig,* [c] *Hannover Medical School, Hannover, Germany.*
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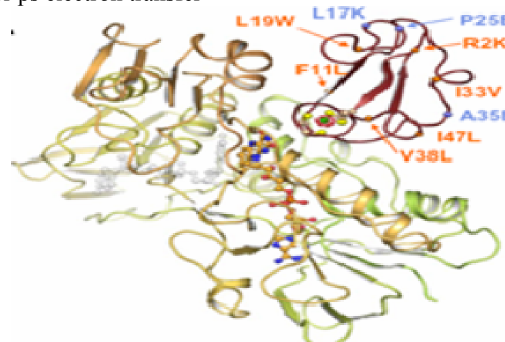
Keywords: Protein-Protein Recognition, Electron Transfer, Pathogenic Bacteria

Crude oil spills represent a major ecological threat due to the chemical inertness of the constituent n-alkanes. The Gram-negative bacterium *Pseudomonas aeruginosa* is one of the few bacterial species able to metabolize such compounds. Three chromosomal genes, *rubB*, *rubA1* and *rubA2* coding for an NAD(P)H:rubredoxin oxidoreductase (RdxR) and two rubredoxins (Rdxs) are required for this ability. They constitute an electron transport (ET) pathway shuttling reducing equivalents from carbon metabolism to the membrane-bound alkane hydroxylases AlkB1 and AlkB2. The RdxR/Rdx system is also crucial as part of the oxidative stress response in archaea or anaerobic bacteria, and has been analyzed in detail as a model system for ET processes. We have solved the structure of RdxR of *P. aeruginosa* both alone and in complex with Rdx, without the need for crosslinking at 2.3 and 2.4 Å resolution, respectively.
RdxR consists of two cofactor-binding domains and a C-terminal domain essential for the specific recognition of Rdx. Only a small number of favorable interactions govern mutual recognition of RdxR and Rdx, corroborating the transient nature of the complex. The shortest distance between the redox centers is 7.5 Å, allowing for ET rates in the picosecond range.

Fig. 1 The complex of Rubredoxin:NADH oxidoreductase (orange-green) with rubredoxin (red). The distance from FAD of RdxR to the Fe^{3+} of rubredoxin is 7.5 Å allowing for ps electron transfer



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