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

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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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