heart failure. The EST gene was cloned and expressed in *E. coli*; the recombinant protein is a non-disulfide linked homotrimer with a monomer molecular weight of 33 kDa in both solution and crystalline state, indicating that these ESTs function as trimers. EST hydrolyzed DL-MATI to produce DAT with the degree of conversion of 49.5 % and an enantiometric excess value 97.2 % at optimum pH 8~10 and temperature 57~67 ^{o}C. The crystal structure of EST has been determined by X-ray diffraction to a resolution 1.6 Å, confirming that EST is a member of the α/β hydrolase fold superfamily of enzymes and includes a catalytic triad Ser97, Asp227 and His256. The active site is located approximately in the middle of the molecule at the end of a pocket ~12 Å deep. The EST-DL-MATI complex structure has also been determined and shows that the oxyanion hole can form by peptide NH groups of Thr98 and Trp31 that form hydrogen bonds with the carbonyl oxygen of the DL-MATI. The EST can hydrolyze methyl ester group without affecting the acetylthiol ester moiety in DL-MATI. The examination of substrate specificity of EST toward other linear esters revealed that the enzyme showed specific activity toward methyl esters and that it recognized the configuration at the C-2 position. The knowledge of substrate specificity, molecular recognition and structure of a substrate-binding site of EST is useful for enzymatic engineering for broader ester substrates.

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MS05 P04

How structural features of MsrBs shed lights to the catalytic mechanism. Fanomezana M. Ranaivoson[a], Fabrice Neiers[b], Sandrine Boschi-Muller[b], Guy Branlant[b], André Aubry[a] and Frédérique Favier[a]
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Keywords: catalytic mechanisms, substrate binding, methionine sulfoxide reductase

The thioether side-chain of methionine residues oxidizes easily to sulfoxide *in vivo*, especially under oxidative stress conditions. To maintain the cellular viability, organisms possess methionine sulfoxide (MetSO) reductases (Msrs), enzymes devoted to the repair of such oxidized proteins. To efficiently reduce the two possible stereoisomers of the sulfoxide function, two classes of Msrs have evolved: MsrA, specific to Met-*S*-SO, and MsrB specific to Met-*R*-SO [1]. The characterized catalytic mechanism for both MsrA and MsrB is based on the reactivity of two or three cysteine (Cys) residues and can be divided according to two major steps: a reductase step in which the substrate is reduced, and a recycling step needed by the enzyme to recover its initial active state [2]. The first step ends with the oxidation of the enzyme to a sulfenic acid intermediate. In the second step, the reacting Cys form an internal disulfide bond which necessitates the intervention of a reducing partner such as thioredoxin to terminate the recycling process. In the case of MsrBs, the known structure from *Neisseria gonorrhoeae* [3] shows a spatial disposition of the active site apparently optimized for the execution of both the first and the second steps. But the analysis of this structure combined with sequence alignments reveal that in other MsrBs where the reacting Cys are distributed differently, structural differences may appear in particular to allow the disulfide bond formation during the second step.
Here will be presented a series of crystallographic structures of MsrBs from two different organisms, retracing some steps of the catalytic mechanism. From the observation of these structures, some of the structural origins of the efficiency of the catalysis will be discussed, with regard to the deductions made with the structure from *N. gonorrhoeae*. In addition, the comparison between the two organisms of the current study will highlight the differences in the conformational behavior of the two MsrBs during the mechanism, due to the relative positioning of their Cys.

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MS05 P05

Yellow lupine pathogenesis-related protein as a reservoir for cytokinins Humberto Fernandes*[1], Anna Bujacz[2], Oliwia Pasternak[1], Grzegorz Bujacz[1,2], Michal Sikorski[1], Mariusz Jaskólski[1,3] [1]*Center for Biocrystallographic Research, Inst. of Bioorg. Chem., Pol. Acad. Sci., Poznan, Poland.* [2]*Technical Univ. of Lodz, Poland.* [3]*Faculty of Chemistry, A. Mickiewicz Univ., Poznan, Poland.* E-mail: humberto@man.poznan.pl

Keywords: plant pathogenesis-related proteins, plant hormones, cytokinins

Plants, forced to grow in harsh conditions and exposed to pathogenic activity, have developed several means of defense. Besides establishing a physical barrier by strengthening their cell wall, plants also produce antibiotic compounds called phytoalexins and accelerate cell death to suppress the spread of infection. Furthermore, the expression of a number of proteins is induced by various types of pathogens, or by chemicals such as ethylene or salicylic acid. These proteins, designated PR (pathogenesis-related), are grouped into seventeen classes according to their biological activity and sequence homology. PR proteins of class 10, which are coded by multigene families, are small (17 kDa), slightly acidic, and cytosolic. The main feature of their three-dimensional structure is a seven-stranded antiparallel β-sheet surrounding a long C-terminal α-helix, with a large cavity created between these two structural elements. Although PR-10 proteins are abundant in plants, their physiological role still remains unknown. One hypothesis implicates PR-10 proteins in cytokinin binding. Cytokinins act as versatile hormones in regulation of plant development and growth. Two groups of cytokinins are known, adenine derivatives, such as zeatin, which are of natural origin, and synthetic urea derivatives, such as CPPU or DPU. Our crystallographic data obtained for a zeatin complex of a yellow lupine PR-10 protein have demonstrated the protein's unusual ability to bind as many as three zeatin ligands in the internal cavity. The high data resolution and excellent quality of the electron density maps allowed modeling of the zeatin molecules with atomic accuracy. Recently, the same PR-10 protein has been crystallized in complex with the urea-type cytokinins, DPU and CPPU.

The structures confirm that the protein is also capable of binding these ligands in multiple copies, even though their molecular shapes and dimensions are different from zeatin. All these data suggest that PR-10 proteins can function as a reservoir for cytokinin molecules, maintaining high level of their availability and delivering them to their receptors.

MS05 P06

Interaction of tubulin with ligands that regulate its assembly in microtubules Audrey Dorléans[a], Benoît Gigant[a], Armelle Vigouroux[a] and Marcel Knossow[a], [a]*Laboratoire d'Enzymologie et Biochimie Structurales CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France.* E-mail : audrey.dorleans@lebs.cnrs-gif.fr

Keywords: tubulin, structure, drug design

Microtubules are tube-shaped polymers of αβ-tubulin heterodimers. They are key components of the cytoskeleton that are crucial in the development and maintenance of cell shape, in the transport of organelles throughout cells and in cell signalling. During mitosis they form the mitotic spindle, which is necessary for the correct partitioning of chromosomes in cell division. To fulfil these functions, microtubules alternate phases of slow growth and fast depolymerization in a process known as dynamic instability which is itself coupled to hydrolysis of the nucleotide bound to the tubulin β-subunit. In the cell, dynamic instability is controlled by regulatory proteins. In addition, it is poisoned by exogenous small molecule compounds, some of which are used as anti-cancer drugs. The X-ray crystal structure of tubulin in complex with the small molecule colchicine and with the stathmin domain of the stathmin-like protein RB3 has been determined [1] in the lab at 3.5 Å resolution. Other therapeutically aimed agents target the colchicine site and affect tubulin assembly in microtubules. We have determined the structure of TN-16 in complex with tubulin. The chemical formulae of TN-16 and colchicine are presented in Figure 1. Interestingly, although the TN-16 and colchicine binding sites overlap, they differ significantly. In particular TN-16 contacts the central β-sheet of the nucleotide binding domain of the tubulin β-subunit. Implications of the structure of the TN-16-tubulin complex for the mechanism of action of this anti-mitotic compound will be discussed.

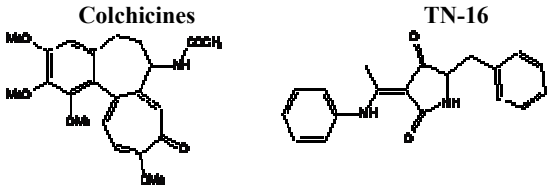

Fig.1. Structural formulae of colchicines and TB-16

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MS05 P07

Sugar-converting enzymes: new insights into structures and mechanisms Nushin Aghajari[1], Stéphanie Ravaud[1], Xavier Robert[1], Hildegaard Watzlawick[3], Birte Svensson[2], Ralf Mattes[3] & Richard Haser[1] [1]Laboratoire de BioCristallographie, Institut de Biologie et Chimie des Protéines,UMR 5086-CNRS/UCBL, IFR128 « BioSciences Lyon-Gerland » (7 Passage du Vercors, F-69367 Lyon cedex 07, France) http://www.ibcp.fr/rhaser/ [2]Biochemistry and Nutrition Group, BioCentrum-DTU, Technical University of Denmark Lyngby [3]Institut für Industrielle Genetik, Universität Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany

Recent results will be reported on enzymes from various origins (plants, bacteria…) involved in sugar recognition and processing, on the basis of their high resolution structures in the presence and absence of ligands of interest (natural substrates, inhibitors, protein partners..) and of appropriate mutants. In the case of barley, the proteome analysis of the early germination of the seeds is underway, in order to contribute to better understand the regulatory processes in seed germination and identify new genes and proteins with functions in germination. In this context we are studying the structure/function relationships of two major α-amylase isozymes produced in the aleurone layer of these seeds. These enzymes in combination with limit dextrinase, β-amylases, are of pivotal importance for starch degradation and embryo growth during seed germination. Coupled enzymatic and structural analysis using site directed mutagenesis, gene shuffling, and X-ray crystallography have provided the essential data that enables the fundamental understanding of the catalytic hydrolytic cleavage of α-1,4-linked carbohydrates, in starch and related oligosaccharides.
A number of bacterial amylases are also known in terms of detailed 3D architectures. Our contribution to the structure/function relationships of amylases from psychrophilic microorganisms led to clarify the features which control molecular adaptation, recognition of sugars and high catalytic efficiency at low temperatures.
Characterization of highly efficient sucrose isomerases have also been reported from isomaltulose-producing bacteria. The first three-dimensional structures (native and complexes) of a sucrose isomerase producing predominantly trehalulose (a nutritional sugar with high health advantages for diabetics and nondiabetics) were recently established, and help to elucidate the mechanism of isomerase action.
Finally, a number of new and unexpected insights into the action of enzymes belonging to the α-amylase and sucrose isomerase families at the molecular level will be presented. They contribute to the understanding on how these enzymes tackle the processing of the different substrates they act with in nature. Ultimately, technological processes as well may benefit from the improved insight into the conversion of starch and related sugars, and in the case of the isomerases into the industrial biosynthesis of sugars with significant health advantages.