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

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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.

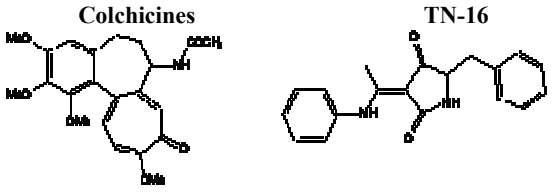

Fig.1. Structural formulae of colchicines and TB-16

[1] Ravelli R.B.G., et al., *Nature*, 2004, 428, 198

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.