by the bacterium. Purification from the supernatants of the crude bacterial extracts by a combination of ultrafiltration, chromatography on EDTA-modified DOWEX-1x2 anion exchanger and size exclusion chromatography (Superdex 200) leads to separation of the 62.7 kDa enzyme. N-terminal sequencing of the first 30 residues confirms the enzyme to be a chitinase with the closest sequence homologue a chitinase D–like enzyme from *Bacillus thuringiensis* serovar *finitimus* YBT-020 [3], even if the overall domain organisation differs and has not been observed as yet.

Quality of the 62.7 kDa exochitinase samples has reached the level suitable for structural studies. The first crystallisation experiments and further sequencing and characterisation have been performed.

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Keywords: enzyme, structure-function studies, chitinase

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Structural insights into autoactivation mechanism of p21-activated protein kinase

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The p21-activated kinases (PAKs) are serine/threonine protein kinases defined by their interaction with the small G proteins, and play important roles in diverse cellular processes including cytoskeletal dynamics, growth/apoptotic signal transduction and regulation of transcription factors. All PAKs contain an N-terminal regulatory domain and a C-terminal kinase domain. Full activation of PAKs requires autophosphorylation of a critical threonine/serine (Thr423 in PAK1) located in the activation loop of kinase domain. A large body of experimental evidence shows that phosphorylation of PAK1 Thr423 is a trans-autophosphorylation reaction that is wholly dependent on dimerization of PAK1 (i.e. between two identical kinase molecules). Here, we report the crystal structures of phosphorylated and unphosphorylated PAK1 kinase domain. The phosphorylated PAK1 kinase domain has the conformation typical of all active protein kinases. Interestingly, the structure of unphosphorylated PAK1 kinase domain reveals an unusual dimeric arrangement expected in an authentic enzyme-substrate complex, in which the activation loop of the putative 'substrate' is projected into the active site of 'enzyme'. The 'enzyme' is bound to AMP-PNP and has an active conformation, whereas 'substrate' is empty and adopts an inactive conformation. Thus, the structure of asymmetric homodimer mimics a trans-autophosphorylation complex, and suggests that the unphosphorylated PAK1 could dynamically adopt both the active and inactive conformations in solution.

Keywords: p21-activated kinase, pre-existing equilibrium, trans-autophosphorylation

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Site directed mutagenesis to the rescue: unravelling conformational changes of Inositol 1,3,4,5,6 pentakisphosphate 2-kinase

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Inositol polyphosphates are a wide group of second messengers, involved in key cellular events. In particular, Inositol Pentakisphosphate 2-Kinase (IP5 2-K) converts inositol pentakisphosphate (IP5) to inositol hexakisphosphate or phytic acid (IP6). IP5 2-K is the unique enzyme that phosphorylates the axial position (2-OH) of the inositide ring. IP6 is present in all eukaryote cells and plays an essential role in processes such as lymphocyte development or apoptosis. In addition, IP6 tends to accumulate in plant seeds, what may have detrimental effects in human health and environment. Briefly, phytic acid is a potent chelator agent, contributing to malnutrition in populations where the diet is grain-based. We are undertaken structural studies by X-ray Crystallography to understand the mechanism underlying the function and regulation of this key enzyme in cell biology.

We have very recently crystallized and solved the first structure of an IP5 2-K from *A. thaliana*, in complex with substrates (IP5, IP5 plus AMP-PNP) and products (IP6, IP6 plus ADP) [1], [2]. The enzyme presents an αβfold, being divided in two lobes, a N lobe, conserved from protein kinases, and a C-terminal lobe. The N lobe and some residues from the C lobe are implicated in the nucleotide binding Also, a big part of this C-terminal lobe (the CIP-lobe) forms a novel structural region to bind the inositol phosphate. Despite all this knowledge, many obscure aspects remains around the catalytic mechanism and conformational changes of the enzyme. In order to elucidate this questions, and after a deep analysis of existing structural information, we are combining X-ray crystallography with other techniques, principally site directed mutagenesis. This approach has been very successful to improve the crystallizability of our samples as well as to gain insights into the no bonded (apo) and AMP-PNP bonded forms of IP5 2-K. All these findings represent an important tool to design inhibitors for the enzyme, what have potential applications in biomedicine and animal feed staff industry, for example in designing crops with low phytate levels.

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Structural basis of methylglucose lipopolysaccharide biosynthesis in mycobacteria

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