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Structure of the N-terminal spectrin repeat of the plakin domain of BP230

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The bullous pemphigoid autoantigen 1 (BPAG1) is a member of the plakin family of cytolinker proteins that associate with and cross-link components of the various cytoskeletal systems [1]. The epithelial isoform of BPAG1, known as BPAG1e or BP230, is a component of hemidesmosomes (HDs) that are multiprotein complexes that mediate firm adhesion of epithelial cells to the underlying basement membrane [2]. In the HDs, BP230 links the integrin α6β4 (a laminin receptor) to the cytokeratin system. BP230 contains in its N-terminal region a plakin domain (a sequence of ~ 1000 residues conserved in the plakin family) formed by eight Spectrin Repeats (SR2 to SR9) and a SH3 domain inserted in the SR5. Upstream of the SR2 there is a 55-residues long isoform-specific tail. The N-terminal region of BP230 harbors binding site for the cytoplasmic region of the integrin β4 subunit [3].

Here we report the crystal structure of the SR2 of BP230. The structure was solved by molecular replacement and was refined against data to 2.0 Å resolution. The structure shows the characteristic SR fold, which consists of three α-helices connected by short loops. The helices are organized in a bundle with a left-handed twist that is stabilized by coiled coil interactions. The SR2 of BP230 shares a 31% sequence identity with the equivalent region of plectin, which is another member of the plakin family present in HDs. Most of the conserved residues are located in the core of the helical bundle; thus, the polypeptide backbones of the SR2 of BP230 and plectin are almost identical. Nonetheless, there are significant differences in the solvent accessible surfaces between BP230 and plectin. Finally, we have used circular dichroism and limited proteolysis to analyze the structure of the N-terminal tail of BP230. Our data suggest that the N-terminal tail is an intrinsically disordered region.

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Crystal structure of PK10, unique characteristics of a MAPK from *Leishmania major*

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Ser/Thr protein kinases (STPKs) are enzymes involved in signal transduction pathways. They act as "molecular switches", showing at least 2 conformations: the active and inactive states. We aim to understand the molecular mechanisms involved in STPKs' catalytic regulation in *Leishmania spp.*, a protozoan parasite that causes human leishmaniasis, a neglected tropical disease. Our work has focused in LmaPK10 a 'mitogen activated protein kinase' (MAPK) associated with differentiation control during the parasite's life cycle. LmaPK10 is phosphorylated in the amastigote stage, when the parasite enters the host macrophages[1]. Extensive attempts to generate LmaPK10 knock-out parasites failed, suggesting that the protein is essential for parasite's survival (induced complementation approaches are underway). LmaPK10 is thus a potential drug target within a rational design strategy. LmaPK10 also shows interesting sequence features, distinguishing it from human MAPKs and probably involved in catalytic regulation according to their key positions. Recombinant LmaPK10 was expressed in *E. coli* and purified to homogeneity. *In vitro* kinase activity was assayed in presence of ^{32}ATP using surrogate substrates, detecting weak auto- and substrate phosphorylation. LC/MS analysis allowed to define the autophosphorylated peptides, primarily within the activation loop, but also beyond. Crystallization conditions were found employing the full-length protein, as well as a protease-resistant C-terminal truncated construct (PK10ΔC, lacking the final 43 amino acids). PK10ΔC diffracted X rays to high resolution ($SGP4_32_12$) allowing its structure to be solved by molecular replacement using human p38 as search probe (*3HV3*) and refined to 1.9 Å resolution.

To the best of our knowledge, PK10ΔC (pdb 3PG1) represents the first trypanosomatid MAPK structure to be reported. The protein crystallized in a non-phosphorylated conformation and structural analysis allowed us to identify regions and residues that are unique to this enzyme, in particular an N-terminal helix replacing conserved ß-strands, and more importantly, an inserted ß-hairpin just before the N-terminal lobe αC helix, important for catalytic regulation. PK10ΔC appears to be trapped in a conformation that shares features with both the fully active and inactive states [2], owing to mutations in key positions otherwise absolutely conserved in STPKs. Affinity quantitation by fluorescence allowed us to determine dissociation constants for ATP, and other nucleotide substrate derivatives, as well as binding constants for the human p38-specific inhibitor SB203580. We have recently solved the structure of PK10ΔC in complex with SB203580, revealing detailed patterns of the binding pocket, with a full structural analysis currently underway. We envisage to obtain the phosphorylated, expectedly active, conformation of LmaPK10 in order to perform biochemical and crystallographic analyses of the regulatory conformational switch, clearly relevant within the rational drug discovery strategy. Acknowledgments: LEISHDRUG , PEDECIBA, ANII

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Crystal structures of adenomatous polyposis coli and its complex with Sam68

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The adenomatous polyposis coli (APC) tumor suppressor protein is commonly mutated in colorectal tumors. The best-known function