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The arginine repressor of the hyperthermophile Thermotoga neapolitana: crystallization and preliminary x-ray diffraction analysis

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The arginine repressor of *Thermotoga neapolitana* (ArgRTnp) is a member of the family of multifunctional bacterial arginine repressors involved in the regulation of arginine metabolism. The hyperthermophilic repressor exhibits some unique features that clearly distinguish it from the previously studied homologues [1]. Its DNA-binding activity is nearly arginine independent and shows a broad sequence specificity. In contrast to the *Escherichia coli* and *Bacillus* ArgR proteins that bind to a pair of adjacent ARG boxes, ArgRTnp makes essentially strong contacts with only one ARG box of the operator and it has the remarkable capacity to bind also to heterologous operators and single ARG box fragments. ArgRTnp was purified as a homotrimeric protein of 49 kDa that assembles into hexamers at higher protein concentrations and/or in the presence of arginine. The structure of ArgRTnp is essential to elucidate the mechanisms of molecular regulation and thermostability of this hyperthermophilic transcription regulator. ArgRTnp was crystallized, with and without its corepressor arginine, using the hanging-drop vapour-diffusion method [2]. Crystals of the aporepressor diffracted to a resolution of 2.1 Å and belong to the orthorhombic $P2_12_12_1$ space group with unit-cell parameters a = 117.7, b = 134.2 and c = 139.3 Å. Crystals of the repressor in the presence of its corepressor arginine diffracted to a resolution of 2.4 Å and belong to the same space group with similar unit-cell parameters. Attempts at molecular replacement have not been successful. In order to solve the structure of ArgRTnp a selenomethionine derivative is being prepared. Because the ArgRTnp polypeptide chain contains only one methionine, bromide soaking will also be applied for phasing.

[1] Charlier, D. (2004). *Biochem. Soc. Trans.* 32, 310-313.
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Structure and function of PKD-CBM44 and CBM30 modules of the bifunctional Clostridium thermocellum cellulase, CtCel9D-Cel44A

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The *Clostridium thermocellum* cellulosome is a highly organized multi-enzyme complex of cellulases and hemicellulases which hydrolyses plant cell wall polysaccharides. The bi-functional multi-modular cellulase *Ct*Cel9D-Cel44A is one of the largest components of *C. thermocellum* cellulosome. The enzyme contains two internal catalytic domains, belonging to glycoside hydrolase families 9 and 44, and an N-terminal family 30 Carbohydrate-Binding Module (CBM30). The C-terminus of this cellulase, comprising a polycystic kidney disease module (PKD) and a novel CBM (designated as CBM44), has been crystallized and solved by X-ray crystallography [1,2]. Both CBM44 and CBM30 display a β-sandwich fold, which contains a convex and concave face. The concave face, or cleft, of both CBMs contains a hydrophobic platform comprising three tryptophan residues that can accommodate up to five glucose residues. The orientation of these aromatic residues is such that the bound ligand would adopt the twisted conformation displayed by the cellooligosaccharides in solution. These have been shown to be key residues for ligand binding by mutagenesis experiments. For the first time, we show that CBM44 binds with equal affinity to cellulose and xyloglucan, which comprises a backbone of β-1,4-glucan decorated primarily with xylose residues, and is a key component of the plant cell wall.
The PKD domain is also made up of two β-sheets, and displays features typical of a β-sandwich fold. Biochemical data suggest that the PKD domain in *Ct*Cel9D-Cel44A does not modulate the function of CBM44 when binding to soluble and insoluble polysaccharides nor does it bind carbohydrates *per se*. This domain may function as a non-flexible spacer domain in proteins displaying highly complex molecular architectures such as *Ct*Cel9D-Cel44A. The PKD-CBM44 structure forms a dimer related by a crystallographic two-fold. It is possible that PKD modules participate in specific protein-protein interactions within the cellulosome, which may be important in orchestrating the binding of defined repertoires of plant cell wall hydrolases in each individual multi-enzyme complex.

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[2] Najmudin *et al*., J. Biol. Chem. (2006) 281: 8815-8828.