

Structure and function of non-catalytic modules involved in plant cell hydrolysis: exogenous enzymes to improve the nutritive value of a *Lupinus albus* based diet for piglets

Abstract

Modular glycoside hydrolases that attack recalcitrant polymers generally contain noncatalytic carbohydrate-binding modules (CBMs), which play a critical role in the action of these enzymes by directing the appended catalytic domains onto the surface of their substrates. Here we report the biochemical and structural properties of two CBMs. The CmCBM6-2, located at the C-terminus of *Cellvibrio mixtus* endoglucanase 5B was shown to contain two binding sites that display differences in ligand specificity, supporting the view that distinct binding clefts with different specificities can contribute to the variation in ligand recognition displayed by family 6 CBMs. In addition, the family 11 CBM (CtCBM11) from *Clostridium thermocellum* bifunctional lichenase-cellulase enzyme Lic26A-Cel5E, demonstrated a preference for mixed linked glucans and contains a single ligand-binding site that displays affinity for both β -1,4- and β -1,3-1,4- mixed linked glucans. This enzyme is part of the cellulosome which represents an efficient multi-enzyme complex of cellulases and hemicellulases for the hydrolysis of cellulose. Cellulosome assembly results from the interaction of cohesin with dockerin modules present in the catalytic units. Here we report the crystal structure of the Type II cohesin (CohII) from *C. thermocellum* cell surface anchoring protein SdbA. The structure of CohII is very similar to the Type I cohesin and the dockerin binding site is likely to be conserved in the two proteins. Finally, in this study the effect of supplementing a lupin based diet for piglets with α -galactosidases was evaluated. Enzyme supplementation had no significant effect on the apparent ileal digestibility of crude protein and true ileal digestibility of amino acids, but the ileal digestibility of NDF (neutral detergent fibre) was increased, which suggests a residual hemicellulase activity in the α -galactosidases used.

Keywords: Carbohydrate-binding modules; *C. mixtus*; *C. thermocellum*; Cohesin Type II; *Lupinus albus*; α -galactosidases.