FUNCTIONAL STUDY OF THREE STARCH BINDING DOMAINS TANDEMLY ARRANGED

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Introduction. α-amylases are glycoside-hydrolases that catalyze the hydrolysis of internal α-1, 4 glycosidic bonds in starch and glycogen, generating smaller oligosaccharides (1). The α-amylase of *Lactobacillus amylovorus* has a structure that consists of a catalytic domain (CD) and a carboxy-terminal starch-binding domain with 5 identical carbohydrate binding modules (CBM family 26) in tandem (2). Each repeat acts as an independent fixing module with an additive or synergic effect between the units (3). This cooperativity phenomena, has never been described for amylases, although it has been observed in some cellulases and chitinases. However, based on the adsorption constants, it has been proposed that the amylase binds the starch granule with a lower number of modules (3).
To test this hypothesis we will compare the starch adsorption capacity of three and five CBMs fused in two chimeric proteins, the green fluorescent protein and the catalytic domain of the same amylase (Figure 1).

Fig.1 Proteins constructed to evaluate adsorption capacity. The first one represents the native amylase.

Methods. Catalytic domain was obtained by PCR from the whole amylase gene and clone into CloneJET vector [Thermo Scientific]. Green fluorescent protein was previously cloned in pQE31 (Qiagen) [4] Three starch binding domains from family CBM26 of *Lactobacillus amylovorus* α-amylase previously cloned into pQE31 vector were fused to the own amylase catalytic domain, and to the *gfp* (Green Fluorescent Protein) by complementarity of a *BamH*I site. Constructions were verified by restriction analysis and sequencing. The resultant proteins (GFP-CBM26x3 & CD-CBM26x3) will be expressed in *Escherichia coli* XL-10. Expression products will be purified from bacterial extract by immobilized metal affinity chromatography and by affinity to beta-cyclodextrin, and the proteins adsorption capacity to granular starch will be characterized and compare the to the whole amylase and the GFP-CBM26x5, previously constructed (4).

Results. Affinities for starch of the chimeras with 5CBM were analyzed, both proteins adsorbed to starch similarly; *Kad*=0.46 for GFP-CBM26x5 and *Kad*=0.54 for the amylase (Figure 2).

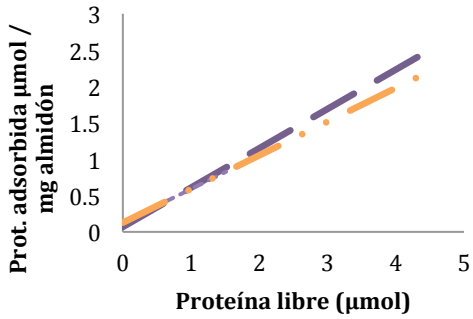

Fig. 2 Adsorption isotherms of the GFP-CBM26x5 fusion (○) and the whole alpha-amylase (○).

In the case of the protein fusions with 3CBM, the expression conditions for the chimeras are currently under investigation.
Clearly, the protein constructed with 5CBM efficiently bind to the starch granule. The presence of tandem modules is likely an evolutionary adaptation designed to allow degradation of raw starch by improving lactobacillus α-amylase binding to the starch granule contained by vegetal material.

Conclusions. The starch adsorption capability can be transfer to proteins not related to starch hydrolysis or hydrolysis.
The minimum number of CBM required for adsorption is still under investigation.

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