Carbohydrate Polymers **21** (1993) 147–149

Properties of the binding domain of glucoamylase

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This paper reviews some of the work from the AFRC Institute of Food Research laboratory on the binding properties of the starch binding domain in glucoamylase from *Aspergillus niger*.

INTRODUCTION

Glucoamylase from *Aspergillus niger* is a widely used enzyme in many areas of cereal starch utilisation. It releases glucose from the non-reducing ends of starch and related oligo- and polysaccharides, and can hydrolyse α1,4, α1,6 and α1,3 linkages between adjacent glucose residues. Glucoamylase consists of two functionally distinct domains for catalysis and binding, separated by an O-glycosylated linker region of about 40 amino acids (Svensson *et al.*, 1983). The latter is rich in serine and threonine residues, glycosylated with predominantly one, two or three mannose residues (Gunnarsson *et al.*, 1984).

The binding domain enhances the rate of hydrolysis (by the catalytic domain) of starch granules. This paper reviews the work from this laboratory on the isolated starch binding domain, and on the role of the O-glycosylated linker containing 10 or 38 amino acid residues.

STARCH BINDING DOMAIN PRODUCTION

The C-terminal sequence of glucoamylase is shown in Fig. 1 (adapted from Svensson *et al.*, 1983). The asterisks indicate residues which are glycosylated, and the underlined residues constitute the linker peptide. The residues 512 to 616 are the starch binding domain.

Proteinase type VII (E.C. 3.4.21.4) was used to generate several products from intact glucoamylase 1. After proteolysis and purification of products by ion exchange chromatography (mono Q), gel filtration (Sephacryl S-200) and a repeat ion exchange step (mono Q), the peptides shown in Fig. 2 were obtained (Belshaw & Williamson, 1990; Williamson *et al.*, 1992).

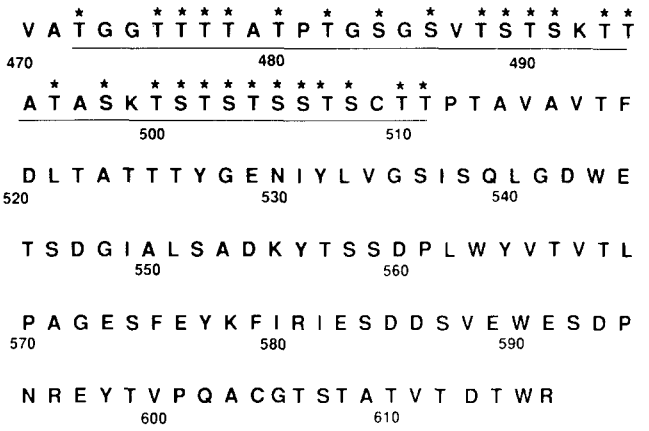

Fig. 1. C-terminal sequence of glucoamylase.

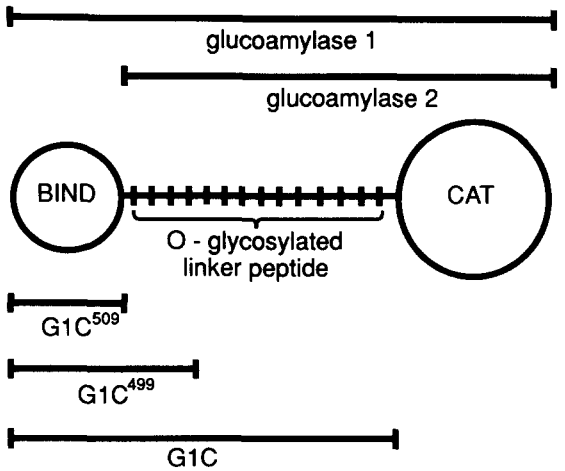

Fig. 2. Peptides obtained from glucoamylase. Glucoamylase 1 and 2 are found in culture supernatants of *Aspergillus niger*. G1C is residues 471–616, $G1C^{499}$ represents residues 499–616, and $G1C^{509}$ is residues 509–616. The representation is diagrammatic, although it is likely that the two domains are discrete and spatially separate (Williamson *et al.*, 1992).

Carbohydrate Polymers 0144-8617/93/$06.00

STARCH BINDING PROPERTIES

The properties of the three peptides containing the binding domain were examined. G1C, G1C^{499} and G1C^{509} all bound to granular corn starch. Using the equation

$$q_{ad}/q = \frac{K_p \cdot q_{max}}{1 + K_p \cdot q}$$

where q_{ad} is the amount of protein adsorbed, q is the free protein in solution, K_p is a constant and q_{max} is the maximum amount of proteina dsorbed by the starch, the value of K_p (an apparent association constant) was calculated. From the equation $\Delta G^{app} = -RT \ln K_p$, where ΔG^{app} is the apparent free energy change, R is the gas constant and T is the temperature, free energy values for the interactions were calculated (Table 1).

The results show that the affinity shows a small increase with an increase in O-glycosylated linker. The total linker, however, contributes only ~1 kJ/mol to the interaction with starch, compared to 25 kJ/mol for the binding domain alone. The amount of total protein bound to the granules at saturation is identical for all three peptides. Therefore, the length of O-glycosylation does not affect the stoichiometry of adsorption, which is probably a monolayer of protein molecules by surface area calculations.

G1C^{499} was deglycosylated using α-mannosidase. Only 8% of the sugars remained, as determined by the phenol-sulphuric acid method (Dubois *et al.*, 1956). This peptide did not show saturation characteristics. Instead, the binding isotherm showed no evidence that saturation was reached even at 4 × K_d for glycosylated G1C^{499} (Fig. 3).

Since the protein did not aggregate in solution as shown by gel filtration on a TSK G3000 SW column, the protein must associate on the starch granule to form a layer several protein molecules thick. This result may help explain why glucoamylase 1 also appears not to reach saturation on binding to starch (Dalmia & Nikolov, 1991). The presence of the catalytic domain in the latter may encourage aggregation on the starch surface to produce a layering effect of several protein molecules. On removal of the catalytic domain, as for G1C, G1C^{499} or G1C^{509}, the protein only forms a monolayer with no aggregation. Deglycosylation reverses this effect. Thus the O-glycosylation helps to maintain the minimum binding stoichiometry, i.e. a protein monolayer.

CONCLUSIONS

1. The binding domain binds to corn starch granules in a monolayer which shows clear saturation.

Table 1. Interaction energy between binding domains and corn starch

Enzyme fragment	$1/K_p$ (μM)	ΔG^{app} (kJ/mol)	q_{max} (μmol protein/ mg starch)	q_{max} (μg protein/ mg starch)
G1C	12·7 ± 0·5	−26·0	1·08 ± 0·02	27
G1C^{499}	16·8 ± 0·9	−25·3	1·58 ± 0·03	27
G1C^{509}	19·6 ± 2·0	−25·0	2·14 ± 0·11	27

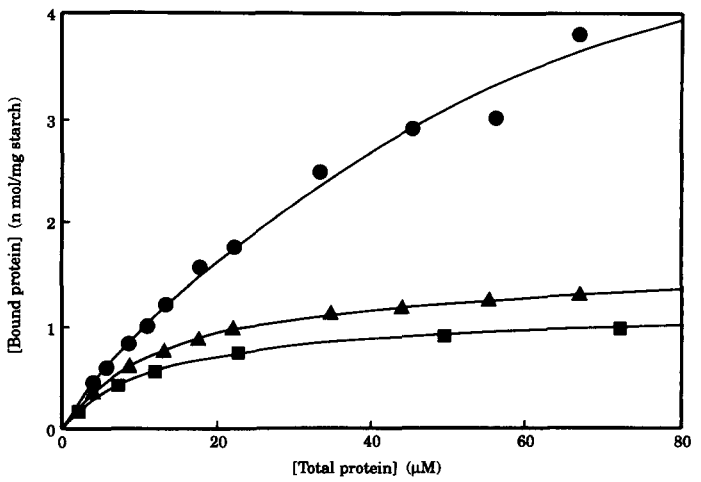

Fig. 3. Binding of GIC (■), GIC^{499} (▲) and deglycosylated GIC^{509} (●) to a suspension of corn starch granules (10 mg/ml) at 4°C, pH 4·5 in 5 mM sodium acetate. Total protein was estimated using absorbance at 280 nm (Belshaw & Williamson, 1990; Williamson *et al.*, 1992). Bound protein after equilibration was measured after centrifugation using the Lowry protein assay (Lowry *et al.*, 1951).

2. Deglycosylation of part of the linker is sufficient for the protein to form multilayers on starch granules.
3. The O-glycosylation plays a very minor role in the strength of binding to starch granules.

ACKNOWLEDGEMENT

We would like to thank the Agricultural and Food Research Council for funding.

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