SORPTION OF GLUCOAMYLASE FROM ASP. AWAMORI VAR X-100/D-27 ON ITS OWN CELL WALL

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Glucoamylases (E^C.3.2.i.3) catalyse hydrolysis of glucosidic α 1-4, α 1—6 bonds in starch, amylose, glycogen and maltooligosaccharides. These enzymes, produced mainly by fungi are presented in cultural medium by some forms, different in molecular masses and sorption on insoluble starch. For glucoamylase from Asp. awamori var X-100/D-27 it was shown that the main form of this enzyme, secreted by fungi, had the additional substrate binding centre with a very hogh affinity for cyclodextrins. During fungigrowth some more enzyme forms appear which have the same activity concerning maltose and short maltooligosaccharides as the main form but the low affinity for starch and glycogen. Elucidating the role of this centre we have shown that glucoamylase had the hogh affinity to its own cell wall, and this affinity was determined mainly by the additional centre.

The electron micrograph of a thin section of fungi giphae shows that the enzyme is specifically situated in the cell wall.This picture is nicely reproduced for the cells obtained at different stages of the growth from 24 hours to 72 hours i.e. for all the period of active glucoamylase secretion. The antibodies against the main form denaturated by guanidin-HCl are used in these experiments. Experiments with sorbents from the cell wall have shown that the main GA form delayed on column, but the reduced forms obtained from the main one by acid proteinase treatment, fell through it. Maltose does not wash off the main form from the column, 0.5—1 *M* NaCl also is not effective for desorption, but β-cyclodextrin completely desorbates the enzyme. Apparently the main enzyme form is bound with the cell wall by additional substrate-binding.

What is the physiological sense of the second center of the enzyme? Usually the enzyme catalysis is believed to rise with the growth of affinity between a substrate and an enzyme. Our data suggest an idea that the role of the second centre is just in binding of the enzyme with the cell wall. It is possible that growth of the enzyme concentration near fungi wall leads to enhancing of glucose flow into the cell.

Evidently such a situation is especially profitable at the low level of density of the cells. With the growth of micelial mass and the increase of intercell contacts this advantage diminishes. Fungi growth correlates with acidification of cultural medium and acid proteinase begins to transform the main enzyme form into the reduced one, which has not an affinity to the cell wall and transits into a solution. Note that the processing takes place at pH 3. Fungi biomass is 50—60% of maximum just for such a pH value of cultural medium.