USING COMPUTER SIMULATION FOR INVESTIGATION OF GLUCOAMYLASE QUATERNARY STRUCTURE

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The investigation of the quaternary structure of hydrolytic enzymes is becoming more important as a result of the wide application of hydrolase in industry, analytical practice and medicine.

For this reason, we investigated features of the supramolecular organization of the enzyme glucoamylase from molds *Aspergillus awamori*.

The catalytic activity of glucoamylase was measured by the glucose oxidize method. The total protein level in the solution was determined by Lowry method. For studying the quaternary structure of the enzyme we used the solution of sodium dodecyl sulfate (10^{-5} mol/l). The separation of subunits was performed by the method of gel chromatography on Sephadex G-200 with subsequent determination of their molecular weight by the formula (1).

To study the stoichiometry of interaction between subunits, we used the service Gramm-X and applied programs Maestro 9.6 and Mole 2. It was found – with the help of the method of gel-chromatography - that glucoamylase has a quaternary structure. It consists of two identical subunits with a mass of 53.6 kDa each, that have the same catalytic activity as the dimmer molecule. It is known from the literature that post-translational folding of simple proteins having low molecular weight, - corresponding to the mechanism of nucleation and growth - occurs on an "all or nothing"principle. However, the formation of quaternary structure of proteins having significant molecular weight (100 amino acids) is typically characterized by the presence of a kinetic intermediate state. For this reason, for the study of the dissociation of subunits of glucoamylase we used a free internet service of molecular modeling called Gramm-X. The obtained models were studied using programs Maestro 9.6 and Mole 2.

It is shown that in 4 out of 10 cases of the examined interactions association process occurs with the union of the two cavities of the active center of the globules. In five cases, the cavities remain independent and retain their shape, as in the free enzyme. It is found that small cavities in the location of the interaction between globules change their volume and shape. The amino acid residues involved in the interaction of the two subunits in the formation of dimmeric structures of the enzyme have been identified. Interaction model of four globules gives the same change in internal structure, as if there were two globules.

When two dimmers interact there might be an elongation of one cavity of the active centers of one of the globules that may reach $\sim 9400A^3$.

M = 6,698-0,987/Ve/Vo where Ve – volume of output Vo – volume of the column (1)