Combination of classic biochemical methods and atomic-force microscopy in studying of hydrolytic enzymes structure

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Quaternary structure of protein is an association when new biological properties appear which are unusual in tertiary structure. Many hydrolytic proteins have quaternary structure and consist of even protomers number.

Object of investigations was a lipase obtained from *Rhizopus japonicus* 1403. Catalytic activity was determined by spectrophotometric method with the use of rhodamine 6G, amount of protein was assayed by Lawry method. Apparent molecular masses were discovered by gel filtration at Sephadex G 150 and electrophoresis by Davies. Combination of these methods revealed that lipase molecule has dimeric structure and contains two catalytically active subunits with molecular mass of 46±2 kD. Activity of each monomer is significantly lower than dimer's one what indicates significant contribution to triglycerides hydrolysis regulation mechanisms.

It has been established that IR spectrum of subunits does not change significantly that indicates an absence of structural damage during protomers separating.

To determine a character of lipase subunit interactions it was carried out a covalent binding of enzyme protomers with glutaric aldehyde. Functional groups of glutaric aldehyde react with amino groups of lysine on the molecular surface. Therefore, there is a high probability of transversal bonds appearance in the area of subunit contacts. Catalytic activity assay of immobilized lipase displayed that it was decreased by 73 %. This allows assuming that two protein globules in open conformations take part in lipase dimerization.

Interactions between protomers have hydrophobic nature.

Lipase surface character was observed by acid-base titration method.

Visualization of lipase quaternary structure was carried out by atomic-force microscopy. This method allows to obtain an image of immobilized on mica lipase surface, and this permits to conclude an important role of quaternary structure in lipase catalytic activity regulation.