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MECHANISMS OF THE ADSORPTION IMMOBILIZATION OF INULINASE ON ION-EXCHANGERS AV-17-2P AND KU-2 MATRICES

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Аннотация. Изучены условия и механизмы иммобилизации инулиназы на носителях (фрагменты матриц ионообменных смол КУ-2 и АВ-17-2П), определены значения аффинностей связывания. Расчетные данные показывают, что при формировании комплекса инулиназы с матрицей смолы КУ-2 значительную роль играют ван-дер-ваальсовы взаимодействия и водородные связи, тогда как анионит АВ-17-2П не образует водородных связей с инулиназой, а формирует только ван-дер-ваальсовы взаимодействия.

Несмотря на значительное совпадение аминокислотных остатков в сайте связывания комплекса фермент-носитель, которое для КУ-2 составляет 60% от общего числа аминокислот, участвующих в формировании связей при иммобилизации, а для АВ-17-2П – 47% от их числа, механизмы процесса адсорбции инулиназы на положительных и отрицательно заряженных матрицах лигандов значительно отличаются друг от друга.

Ключевые слова: компьютерное моделирование, инулиназа, иммобилизация, КУ-2, АВ-17-2П

Abstract. We study the conditions and mechanisms of immobilization of inulinase with immobilization carriers (fragments of matrices of ion-exchanger KU-2 and AV-17-2P) and find their binding affinities. The calculated data show a significant role played by van der Waals interaction and hydrogen bonds in forming complexes of inulinase with a matrix of KU-2 resin, while AV-17-2P doesn't form hydrogen binding with inulinase, but forms only van der Waals interaction.

It is established that, despite considerable coincidence of the amino acid in contact site of enzyme-carrier complex (which constitutes 60% of total number of the amino acids participating in the immobilization bond for KU-2 and 47% for AV-17-2P), mechanisms of adsorption of inulinase on positive and negatively charged ligand matrices significantly differ from each other.

Keywords: computer modeling, inulinase, immobilization, KU-2, AV-17-2P

Today the development of the preparation of food for diabetic and preventive purposes is becoming increasingly relevant. There is increased interest in fructose, which may be used in the diets of patients with diabetes. Fructose is generally obtained from starch by a multi-step process, which includes the cleavage of starch into glucose, the treatment of glucose with glucosoiomerase, and the chromatographic separation of fructose and glucose. The preparation of fructose from an inulin-containing raw material, especially from Jerusalem artichoke, is a promising direction. The inulin content in the tubers of Jerusalem artichoke reaches 20–25%, while the syrup contains at least 70% fructose.

Inulinase (2,1-β-D-fructanfructano hydrolase, EC 3.2.1.7) which cleaves inulin and other fructose-containing polymers into fructose, is widespread among higher plants and microorganisms and can be used for industrial production of fructose from vegetable raw materials. However, the use of enzymes is limited for at least two reasons.

First, they are unstable during storage and during various, especially heating, treatments. Second,

the repeated use of enzymes is difficult due to the complexity of their separation from the reagents and reaction products. In addition, it is difficult to predict the behavior of the enzyme and the reaction kinetics in industrial fermenters. These difficulties can be overcome by using enzyme preparations immobilized on insoluble carriers.

Researches pay special attention to the problems of selecting and modifying carriers, developing immobilization methods, studying the kinetic aspects of catalysis by heterogeneous enzyme preparations [1-12]. In our laboratory, the adsorption and covalent methods of inulinase immobilization on a number of synthetic cation exchangers and anion exchangers have been empirically developed, allowing up to 80-85% of the native enzyme activity [13, 14]. However, despite many practical developments in the field of carbohydrases immobilization, the molecular mechanisms of this process have not been fully studied. There are only data of IR-spectroscopy and electron microscopy of enzyme-carrier complexes, which do not allow to make a complete picture of the types, number and length of bonds and interactions within the complex of the immobilized enzyme preparation and the carrier matrix. The results of X-ray diffraction analysis and molecular docking are presented in the open press only for complexes of carbohydrases and their low-molecular ligands. In this connection, the aim of the work was to reveal the mechanism of the adsorption immobilization process of inulinase on polymer matrices of ion-exchange resins KU-2 and AV-17-2P: calculation of the enzyme affinity values with respect to the carriers for immobilization, determination of the number, types and lengths of bonds and interactions between the inulinase molecule and carrier.

MATERIALS AND METHODS

As the model of the enzyme that became the target for docking, in the Protein Data Bank (PDB) data bank, we selected the structure of inulinase from *Aspergillus ficuum* (molecule code: 3SC7), which was obtained by X-ray diffraction in 2011 with a resolution of 1.5Å, which is well suited for computer calculations by the flexible docking method [15].

For these calculations, this model was "purified" from water molecules and other compounds. It is important to note that the inulinase sample was crystallized along with several organic ligands, including D-mannose, which is an enzyme activator [16]. The known location of this sugar on the enzyme surface allowed us to calibrate the package for docking (Autodock VINA) with a specific example of the mannose–inulinase complex. Before the numerical calculations, we placed charges on the enzyme surface and chose the cell for docking the calculations, the dimensions of which were 68x58x60 Å. The center of the enzyme molecule (which was considered in a rigid form) was at a point with coordinates of x = 37.044, y = –36.997, and z = –5.365. The same data for the target were used for the subsequent calculations of the chosen set of ligands.

According to the docking results, the location of the interaction of the computer-simulated ligand predicted by the program was concordant with the experimentally established localization of mannose. The affinity of this interaction was –4.5 kcal/mol.

For polymer, a chain consisting of five monomers was chosen. Each ligand in the docking calculations had the maximal conformational freedom: rotation of the functional groups around all single bonds was allowed. The charges in the ligand molecule were placed automatically using the MGLTools 1.5.6.rc3 package.

Inulinases from *Kluyveromyces marxianus* and *Helianthus tuberosus* were the objects for the experiments, the homogenous fractions of which were obtained in the laboratory of biophysics and biotechnology of Voronezh State University [17].

Inulin MP Biomedicals isolated from chicory was used as a substrate (molecular weight of the order of 5,000 daltons). Synthetic carriers for immobilization used ion-exchange resins KU-2 and AV-17-2P, a detailed description of which was given in [13, 18]. Hydrolysis of the substrate was carried out in a thermostat-controlled glass reactor at 70 °C and pH 4.5 [16].

The content of the protein in the samples was evaluated by the Lowry method [19]. The catalytic activity of the enzyme was measured spectrophotometrically by the resorcinol method [16]. For the unit of catalytic activity of inulinase, an amount of enzyme was taken that catalyses the production of 1 μM fructose per 1 minute.

RESULTS AND DISCUSSION

The localization of matrix binding sites for inulinase immobilization revealed by us in calculations explains the extremely low level of catalytic activity of the enzyme when bound to a support: the sites of sorption of inulinase do not affect the active center of the enzyme, and even, perhaps, to some extent, orient the macromolecule, more effectively exposing its active center to the substrate.

The calculated values of the affinity of the carriers with respect to inulinase and the experimentally determined percentage of the retention of the enzyme activity during immobilization are presented in the table.

Earlier we established the direct participation of the sulfogroup in the process of inulinase sorption on the KU-2 matrix. A definite contribution to the formation of the enzyme-carrier complex is made by hydrogen bonds [20]. Calculations showed that the sulfogroups of the KU-2 cationite form two hydrogen bonds with the Asn 42 (3.06 and 3.22 Å) residue and one hydrogen bond with the Gly 68 residue (3.05 Å). In addition, a series of van der Waals interactions are formed between the sulfogroups and the carrier carbon matrix and the Trp 40, Glu 43, Asn 61, Val 66, Trp 67, Glu 97, Phe 99, Tyr 128, Arg 175, Asn 176, Glu 233, Val 318, Gly 323 (Fig. 1).

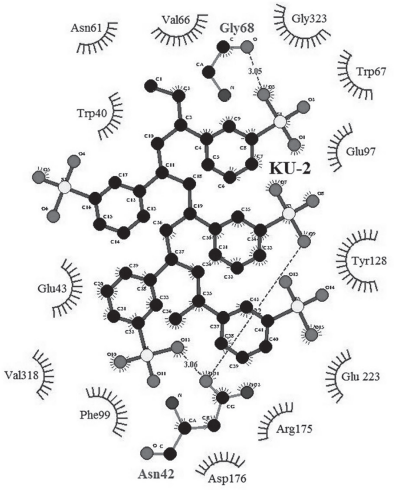


Fig. 1. The bonds and interactions between the inulinase molecule and the matrix KU-2 (dashed lines denote hydrogen bonds)

Between the inulinase molecule and the AV-17-2P matrix, only van der Waals interactions are formed, with both carbon atoms and nitrogen atoms participating in their formation from the side of the carrier, and the side of the protein – the amino acid residues Trp 40, Asn 42, Glu 43, Gln 59, Trp 67, Glu 97, Phe 99, Tyr 128, Thr 130, Gln 133, Val 66, Glu 233, Val 234, Thr 255, Asn 265, Asp 298, Asn 320, Gly 323, Lys 332 (Fig. 2).

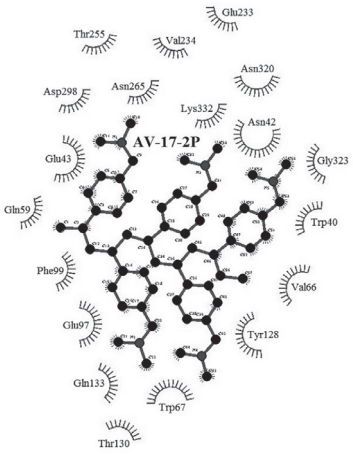


Fig. 2. Interactions between an inulinase molecule and the AV-17-2P matrix

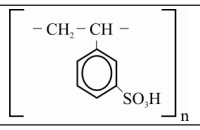
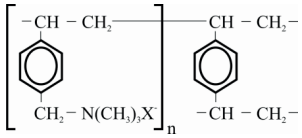
It should be noted that many amino acid residues on the surface of inulinase (in particular, Trp 40, Asn 42, Gln 43, Trp 67, Glu 97, Phe 99, Tyr 128, Gly 233, Gly 323) form a contact region when the enzyme is sorbed both on the cation KU-2 (9 coinciding with the anion exchanger of amino acid residues from 15 residues in the composition of the contact region) and on the anionite AV-17-2P (9 coinciding with the cation exchange of amino acid residues from 19 residues within the contact area).

CONCLUSIONS

In the process of the study on the inulinase enzyme model, as well as the models of its ligands – matrix fragments for immobilization of KU-2 and AV-17 2P, the value affinities of the binding of the enzyme with the carrier were determined.

Table.

Values of affinity and percent preservation of inulinase activity after immobilization

Carrier	Structure of monomer unit	Affinity, kcal/mol	Preservation of inulinase activity, %	
			from *Kluyveromyces marxianus* [13, 16]	from *Helianthus tuberosus* [18]
KU-2, cation exchanger		–7.3	61.7	80.4
AV-17-2P, anion exchanger		–6.8	75.5	22.5

It was found that, despite the significant coincidence of amino acid residues in the contact region of the enzyme-carrier complex, which for cation-exchange resin KU-2 is 60% of the total number of amino acids involved in the formation of bonds during immobilization, and for anionite – 47% of their number, the mechanisms of the adsorption process of inulinase on matrices of positively and negatively charged ligands is different from each other.

The materials presented in the article can be used for a deeper study of the molecular mechanisms of the inulinase interaction with the matrix of a number of synthetic ion exchangers; they must be taken into account when developing new methods for evaluating and regulating the structural state of protein molecules under conditions of their immobilization on various types of carriers.

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