STABILITY OF THE NATIVE AND IMMOBILIZED INULINASE TO VARIOUS DENATURIZING AGENTS

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Today enzyme inulinase (2,l-β-D-fructanfructanohydrolase, EC 3.2.1.7), which splits off fructose residues from the nonreducing end of the molecule of inuline, is of great interest in connection with the possibility of its using in the production of fructose from inuline and inuline-containing materials. When using inulinase for reducing inuline-containing materials, it has received the product-95% fructose syrup, which doesn't demand of special purified methods. Another direction of using of this enzyme is the direct fermentation of inuline into ethanol.

Endoinulinase Aspergillus awamori has been purified by ammonium sulfate precipitation, gel-chomatograhy on sephadex G-100, SDS-PAGE electrophoresis. The immobilization of inulinase by ion exchange AV-26 and AV-17-2P has been made by adsorption and glutaraldehyde methods. The effect of UV-radiation and carbamide on the stability of native and immobilized enzyme has been investigated. DRT-400 lamp has been used in UV irradiation.

It has been determined that UV irradiation in doses 75.5-151.0 J/m^2 leads to the inactivation of soluble inulinase both immobilized inulinase preparations preserve 96 % of catalytic activity in dose 151.0 J/m^{2}min. Doses 906-1400 J/m^2 cause disulfide bonds degradation and the photolysis of catalytic site as a result of amino acid radicals formation. We have observed an increase oh the adsorptively immobilized inulinase stability at the 302 J/m^2. Covalently bonded protein preserves hydrolytic activity in high doses (1300 J/m^2). Thus, covalent immobilization provides high stability for the inulinase to UV irradiation. The type of a way of binding influences significantly on the heterogeneous enzyme preparations stability.

It is shown, that the thermo stability of adsorb immobilized inulinase, bound with anionite AV-26, increases in comparison with native inulinase: the immobilized enzyme has the max catalytic activity at temperature 70°C. For immobilized and native enzymes optimums pH are practically the same, only there is a wider range of meanings pH from 4.5 to 5.0. Activity of the native preparates is preserving completely the thermo stability of covalent bound inulinase is more higher than at the adsorb immobilization. So, after heating to 100°C inulinase, immobilized by the chemical method, shows 19% of the catalytic activity of the native enzyme. The fixation of the triple structure by the multipoint interaction between active groups of the carrier and of the protein takes place after immobilization of the enzyme on anionites. It was shown, that the immobilization leads to the increasing of the activation energy (E_{act}), ΔH of the hydrolysis reaction of inulinase in comparison with diffusion difficulties of the high molecular substrate during the approaching to bounding and catalytic groups of the active center. The negative value ΔS for the hydrolysis reaction of inulinase, realized by the native enzyme, means that the breaking up of the polymeric substrate proceeds with high speed and is characterized by the high order. After the immobilization of inulinase ΔS of the enzyme breaking up of inulinase decreases, apparently at the expense of the direct interaction on of the enzyme with the substrate.

It is shown, that the incubation of soluble inulinase with carbamide in concentration 8 mol/L leads to the total denaturation of the enzyme, and its activity isn't registered. After the interaction of immobilized inulinase with the solution of carbamide in concentration 8 mol/L for 60 min with constant mixing the enzyme showed the catalytic activity (30% of activity of immobilized unmodified inulinase).

Thus, the stability of inulinase in relation to denaturizing agents has been shown to increase with the immobilization of ion exchange. The character of binding with the matrix affects greatly the stability of immobilized enzyme to physical factors.

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LOW-FREQUENCY NEUROMUSCULAR ELECTRICAL STIMULATION TRAINING OF HUMAN SKELETAL MUSCLES IN CONDITIONS OF GRAVITATIONAL UNLOADING

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A number of studies have indicated that sudden exposure to microgravity environment causes a decrease in the tone of the skeletal muscles [Kakurin et al., 1971b; Kozlovskaya et al., 1984], reduction of muscle strength [Cherepakhin & Pervushin, 1970; Kakurin et al., 1971a; Mitarai et al., 1980; Grigor'yeva & Kozlovskaya, 1985; Koryak, 1998; 2002], perceptual and coordination disorders in the neuromuscular systems [Ross et al.,