GLUCOSE OXIDAZE PREPARATIONS DERIVED FROM *PENICILLIUM* FUNGI FOR BIOCATALYTIC SYSTEMS

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Glucose oxidase (EC 1.1.3.4) (GOX) is widely used in food, chemical industries and medicine. Significant GOX application trend is generation of derived biosensors and biofuel cells. Earlier highly active producers of extracellular GOX *Penicillium adametzii* and *P. funiculosum* were isolated at laboratory of enzymes, Institute of Microbiology, National Academy of Science, Belarus and methods for producing GOX with different purity grade were elaborated.

Aim of this study is to obtain and characterize purified glucose oxidase by *Penicillium* fungi to be further used in biocatalytic systems.

Purification of GOX from *P. adametzii* and *P. funiculosum* was carried out by the schemes comprising combination of concentration by ultrafiltration of fungal cultural filtrate (membranes with nominal minimal mass retention limit 10 kDA), gel-filtration (Sephacryl S-300) and ion-exchange chromatography (DEAE ceramic). Derived enzymes were homogeneus, characterized by high specific activity 224-238 U/mg protein, GOX activity constituted 1289.8-1317.2 U/ml.

Spectrophotometric and spectrofluorometric analyses have shown that GOX spectra are typical for flavoproteins. The absorption spectrum peaks were recorded at 380 and 455 nm, an excitation spectrum – 373 and 447 nm, and an emission spectrum – 530 and 562 nm.

Secondary structure of GOX produced by *P. adametzii* and *P. funiculosum* was deciphered. It was shown that both enzymes were characterized by presence of structural fragments with diverse conformation (α-helix, β-sheet, irregular sequences) and minimum of molar ellipticity at 212 and 216 nm.

Despite the fact that the studied enzymes show 99 % similarity in amino acid composition, they differ considerably in physical-chemical properties.

Optimal conditions for enzyme action were found in temperature range 55 -70 °C *(P. adametzii),* 55 - 60 °C *(P. funiculosum)* and pH range 4.5-7.5 and 5.0-8.0, respectively. Distinctions in thermal and pH stability of fungal GOX were revealed. Incubation of *P. adametzii* enzyme at 50°C during 1 hour resulted in 18% loss of activity, in contrast to 45 % reduction for *P. funiculosum* GOX. The enzymes of P. *adametzii* and *P. funiculosum* retained stability at pH 5.0 and pH 4.9-9.0, respectively. GOX of *P. funiculosum* was distinguished by higher stability in alkaline pH zone. After 24 h exposure to pH 11.0 and 12.0 *P. funiculosum* enzyme preserved 62 % and 57 % of initial activity.

The buffer system for expression of maximal enzyme activity was defined (0.01 M phosphate buffer, pH 7.0).

Parameters of GOX interaction with ferrocene and its derivatives (ferrocenecarboxylic acid, ferrocenecarboxylaldehyde, methylferrocene-methanol) were investigated. It was found that in comparison with ferrocene-modified enzyme, affinity of GOX modified with its derivatives to β-D-glucose increased by 1.5-2.1 times (Km=9.4-13.3 mM). The best GOX catalytic characteristics resulted from methylferrocene-methanol modification. The obtained findings were corroborated by fluorometric FAD/ $FADH_2$ studies.

Currently research is under way at the Centre of Nanotechnology and Materials Science - NanoTechnas, Faculty of Chemistry, Vilnius University to generate biofuel cell with improved characteristics incorporating above - described GOX.

This work was financially supported by Foundation of Basic Studues, Belarus republic (project B11LIT-012).