

STUDIES ON IMMOBILIZED GLUCOSE OXIDASE BY DIETHYLAMINOETHYL CELLULOSE COMPLEXES

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ABSTRACT

The properties of immobilized glucose oxidase (GOD) by the complexes of diethylaminoethyl cellulose (DEAEC) with different polymers, such as polymethylacrylic acid (PMAA), polyacrylic acid (PAA), polystyrene sulfonic acid (PSSA), polyvinylalcohol (PVA), polyethylene oxide (PEO) and styrene-maleic acid copolymer (PSMA) were investigated. The activity of immobilized GOD was obviously influenced by the component of the DEAEC complexes. The relative activity of the immobilized GOD reached to maximum and over 90% of the native GOD, when the DEAEC-PMAA DEAEC-PAA complexes were used as a carrier with the molar ratio of DEAEC and polyacid of about one. Michaelis constants (K_m) of the immobilized enzymes of DEAEC-GOD-PMAA and DEAEC-GOD-PAA were determined to be 1.25 and 1.00, respectively. Moreover, the immobilized GOD has a good storage stability and cyclic life.

INTRODUCTION

Large-scale industrial application of several thousands kinds of enzymes hitherto known have been hampered by their high cost and instability. Enzymes for industrial and analytical use may have increased if they can be successfully immobilized in an active state. Diethylaminoethyl cellulose is already a useful support for such enzymes as invertase^[1], amino acid acylase^[2] and glucoamylase^[3]. Recent developments in enzyme technology have shown that the enzyme can be immobilized on an insoluble carrier and still retain significant activity. A wide range of methods has been proposed for the immobilization of enzyme^[4], for example, covalent binding of enzymes to polymeric supports, covalent crosslinking of enzymes with native polymers or synthetic macromolecule, absorption on insoluble matrices, physical entrapment within crosslinked polymer-matrices or within microcapsule, hollow fibers and spun fibers. Recently a novel immobilization method of enzymes by polymer-polymer complexes have been reported^[5,6]. This method has an advantage of being simple, stable and of high catalytic activity because the conformation of the native enzymes in the polymer complexes have not been changed basically. Moreover the binding between the DEAEC and PMAA, and between polymer carrier and enzymes is hydrogen bonded. In this paper, the immobilized glucose oxidase by the polymer complexes of DEAEC having a definite shape with different polymers were investigated.

EXPERIMENTAL

1. Reagents and instrumentation

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All reagents used were reagent grade. Diethylaminoethyl cellulose (cellulose DE52) capacity: approx. 1.0 meq/g, content of *N* is 1.90%, molecular weight of PMAA and PAA is 20,000 and 69,000, respectively. GOD was purchased from SIGMA chemical company. UV-Visible spectra were taken on UVIDEC-320 made in Japan.

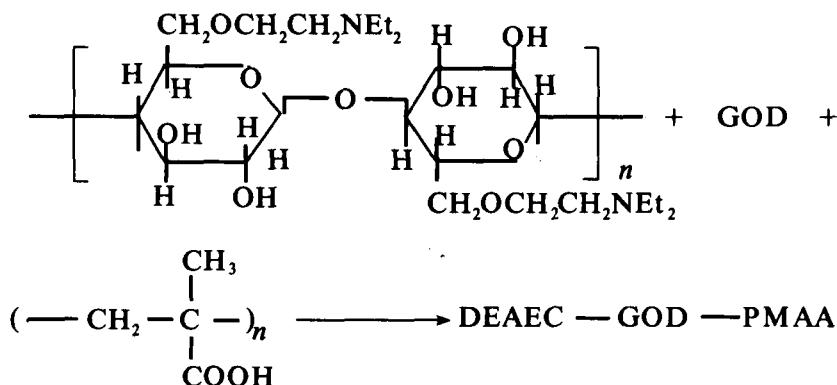
2. The enzyme reaction

Oxidation of glucose: According to the literature the reaction may be expressed as the following scheme:



3. Preparation of immobilized enzymes

Preparation of DEAC-GOD-PMAA is as follows:



0.5 g of DEAC was suspended in 1 ml of deionized water GOD solution (2mg/ml) at 5 °C for 10 hrs., then 1.68 ml 0.4M PMAA solution was added, and kept standing overnight in the refrigerator. The above immobilized enzymes were washed with water and then dried.

4. Methods for the determination of the activity of GOD

This was performed according to the literature⁷. Glucose solution, GOD, KI, $(\text{NH}_4)_2\text{MoO}_4$ solution, o-tolidine alcohol solution, was measured at 620 nm.

RESULTS AND DISCUSSION

The natural polymer, cellulose, can be made in a fixed shape. The cellulose containing functional group, diethylaminoethyl cellulose was chosen for the carrier. The glucose oxidase was immobilized by the interpolymer complexes of the diethylaminoethyl cellulose with polymers containing different functional groups, such as PMAA, PAA, PSSA, PVA, PEO and PSMA. The immobilized enzymes by the polymer complexes have a sandwich structure⁸. The activity of the immobilized GOD on different kinds of polymers in DEAC complexes is shown in Table 1. From the Table, it is apparent that the activity of the immobilized enzymes, was remarkably influenced by the kind of the polymers which complexed with DEAC. The order of the activity of the immobilized GOD is as follows: PAA > PMAA > PSMA > PSSA > PVA > PEO. It is attributable to the differences in the interaction, stereoscopic effect, hydrophilicity and hydrophobicity of the various DEAC complexes. The acid

group of PAA, PMAA, PSMA and PSSA may have a more stronger interaction

Table 1 The activity of the immobilized GOD on different kinds of polymers in DEAEC complexes

Kind of polymer	PAA	PMAA	PMA	PSSA	PVA	PEO
Activity (%)	64.1	52.5	15.9	3.0	2.0	1.4

with amino group in DEAEC. However, the interaction between the DEAEC with PVA and PEO is weaker. For the same addition content of enzyme, the immobilized enzyme amount is increased with the increasing interaction between the polymer complex, therefore the activity and stability of the immobilized enzymes are also increased. The sulfonic acid group in the PSSA broke the GOD due to the over strong acidity. The polymer containing benzene rings adjacent to the GOD increases the stereoscopic effect and hydrophobicity of the environment of the GOD. It is unfavorable to the permeation of the substrates and products, so that the activity of the GOD has been affected. PAA and PMAA have a fitting interaction

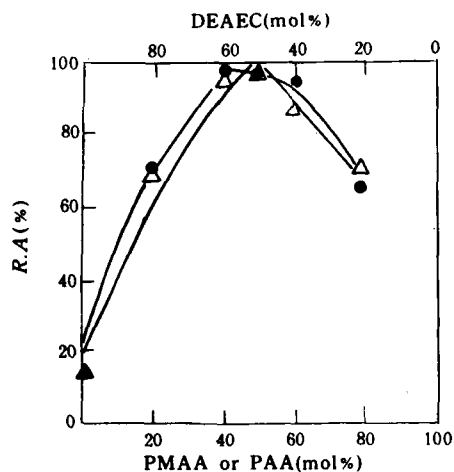


Fig. 1 Relationship between the relative activity and the component of the DEAEC complexes for the DEAEC-GOD-PAA (—●—) and DEAEC-GOD-PMAA (—△—) immobilized enzymes.

with GOD and DEAEC, and a good hydrophilicity (the hydrophilicity of PAA is better than that of PMAA). Therefore, the complexes of DEAEC-PAA and DEAEC-PMAA are very effective for the immobilizing GOD for chosen as carriers.

The relationships between the relative activity of immobilized GOD and the component of the polymer complexes in the DEAEC-GOD-PAA and DEAEC-GOD-PMAA immobilized enzymes were shown in Fig. 1. It is apparent that the maximum relative activity is observed at about 1:1 molar ratio of polymeric acid to DEAEC, in which the interaction between the polyacid and DEAEC is the best. It agreed closely with that obtained by the above-mentioned experimental results.

The relationship between the activity of enzyme and pH was shown in Fig. 2, comparing with the native enzyme GOD, the relationship between enzyme activity

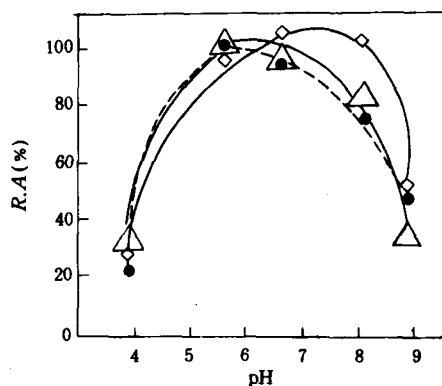


Fig. 2 Relationship between the relative activity and pH for GOD (—●—), DEAEC-GOD-PAA (—◇—), DEAEC-GOD-PMAA (—△—)

and pH-activity curves for DEAEC-GOD-PMAA was nearly unchanged, however the optimum pH for DEAEC-GOD-PAA was spread in a wide range (from 5.8 to 8.0) It indicates that the complex of the DEAEC and PAA, which has a good hydrophilicity, provides a microenvironment containing weak acidity and alkalinity, and can play a role of buffer for external pH.

As a general rule, the relationship between temperature and reaction rate obey Arrhenius equation

$$K = A \exp (-Ea/RT)$$

where K is a reaction rate parameter, T an absolute temperature, Ea an activation energy, A and R , a constant. However, the enzymic reaction obeys Arrhenius equation only at lower temperature due to metamorphosis of the enzyme at high temperature, so that the enzymic reaction have an optimum reaction temperature. The relationship between activity and temperature was shown in Fig.3. It indicates that the thermal stability of the immobilized enzymes, DEAEC-GOD-PMAA, DEAEC-GOD-PAA, is higher than that of native enzyme GOD and may be attributed to the polymer complex provided marched protection against the enzyme.

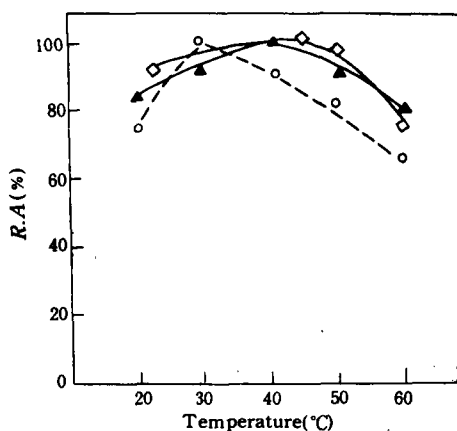


Fig.3 Relationship between the relative activity and temperature for GOD (—○—), DEAEC-GOD-PAA (—◇—), DEAEC-GOD-PMAA (—▲—)

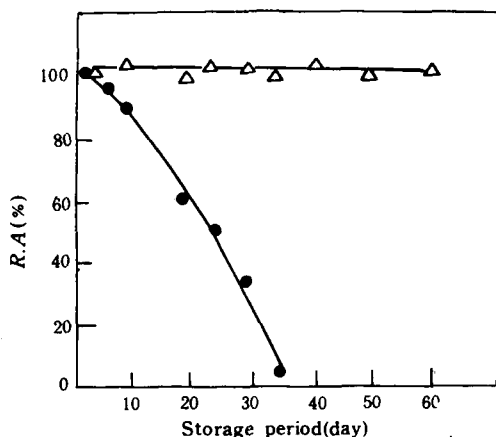


Fig.4 The storage stability of the GOD (—●—), DEAEC-GOD-PAA and DEAEC-GOD-PMAA (—△—)

The storage stability of the native and immobilized GOD was shown in Fig.4. It can be seen that GOD keeps only its half activity after stored for 25 days, however the activity of the immobilized GOD, DEAEC-PAA and DEAEC-PMAA have no change after stored at 25 °C for 50 days.

The native enzymes are difficult to be separated from substrates and products, in general, they can be used once only. However, the immobilized enzymes are easy to be separated from substrates and products, and can be reused. The stability for recycling use of the immobilized enzymes, DEAEC-GOD-PMAA and DEAEC-GOD-PAA was shown in Fig.5. The two immobilized enzymes can be reused 13 and 8 times, respectively, without any appreciable loss of the relative activity.

The Michaelis constant K_m is an important kinetic parameter in the enzymic chemistry and can be estimated from the lineweaver-Burk Plot. It expresses the

concentration of the substrate at half maximum reaction rate and the disassociation force of the complex of the enzyme and substrate. The lineweaver-Burk curves of the native enzyme and immobilized enzymes were shown in Fig. 6. From the cross section for the straight line on the horizontal axis ($-1/K_m$). The K_m was estimated to be 0.69, 1.00, 1.20, for GOD, DEAE-GOD-PAA and DEAE-GOD-PMAA, respectively. It can be seen that the K_m of the immobilized GOD was higher than that of GOD. The changes in the catalytic behavior of the enzyme were attributed to the heterogeneous nature of the local enzymic environment, in which the concentration of substrates, products and other cofactors may be different from that in the solution. The K_m of the PAA complexes is nearer to native enzyme due to the good hydrophilicity.

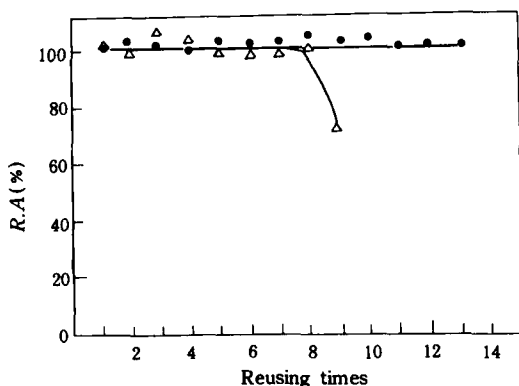


Fig.5 The reusing stability of immobilized enzymes. DEAE-GOD-PMAA (—●—), and DEAE-GOD-PAA (—△—)

The effect of the immobilized enzymes has been investigated mainly by comparing the activity of the immobilized enzyme with that of the native enzyme in solution. In most cases the activity of the immobilized enzyme is lower. The ratio of the immobilized enzyme activity over the activity of the free enzyme is called immobilization efficiency (η):

$$\eta = \frac{\text{activity (immobilized)}}{\text{activity (free)}}$$

η is generally lower than 1. The decrease in activity was attributed to the conformational changes in the structure of the active center of the enzyme molecule and to the shielding effect of the matrix, which makes the active center less accessible to substrates. The η of the immobilized GOD, DEAE-GOD-PMAA and DEAE-GOD-PAA was estimated to be 90% and 99%, respectively. So such high η value indicated that the GOD enzyme active center is basically unchanged and this method immobilized with complexes of DEAE-PMAA and DEAE-PAA is an excellent technique for immobilizing GOD enzyme.

In conclusion, the immobilization efficiency of the immobilized GOD enzyme with the complexes of the DEAE-PMAA and DEAE-PAA is as high as over 90%. The immobilized GOD enzymes, DEAE-GOD-PMAA and DEAE-GOD-PAA can be used in a wider pH range, with increasing thermal

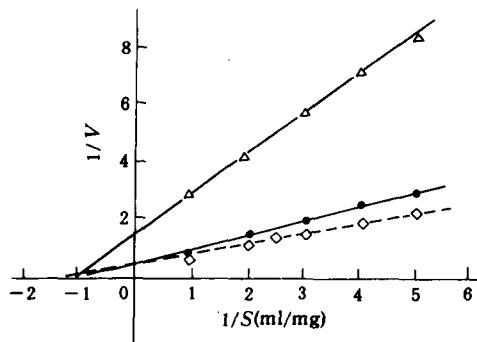


Fig.6 Lineweaver-Burk Curves of native enzyme GOD (—◇—), immobilized enzymes, DEAE-GOD-PMAA (—●—) and DEAE-GOD-PAA (—△—)

stability and having a good activity, storage stability and reusing stability. Moreover, the immobilized enzymes have a good shape, which is advantageous to the column reactor. All of these exhibit that the immobilized GOD has a promising prospect for application.

Keywords:

Immobilized glucose oxidase; Diethylaminoethyl cellulose; Polymer complex

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