A Study of Polyion-Enzyme Complexes: Quaternized Poly(vinyl Dimethylaminobenzal)-Glucose Oxidase-Potassium Poly (acrolein Bissulfate) Complex

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Synopsis

This report has mentioned a study that includes preparation of a polyion-enzyme complex consisting of quaternized polyvinyl dimethylaminobenzal (QPVDAB), potassium polyacrolein bissulfate (KPABS), and glucose oxidase (GOD), and evaluation of relationships between properties and structure of the complex are discussed.

INTRODUCTION

In the 1960s, Michaels, Bailey, Liquori, and others first developed a variety of intermacromolecular complexes.[1-3] These complexes can be divided into the following four classes on the basis of the main interactions: polyelectrolyte complexes based on polyanion and polycation; hydrogen-bonding complexes; stereocomplexes based on isotactic and syndiotactic polymers; and charge transfer complexes based on electron accepting and electron donating.[4] Good transport properties, ion selectivity, water and oxygen sorption, and compatibility to living mass are their main characteristics. Thus they can be used as ion-conducting materials, oxygen-permeating membranes, living materials, and so forth. Intermacromolecular complexes have great potential in developing and using these special functional materials. In recent years, immobilization of living species (enzymes, cells, and so on) by intermacromolecular complexes has been developed.[5-8] Recently, we immobilized glucose oxidase (GOD) by two oppositely charged polyions. One of them is quaternized polyvinyl dimethylaminobenzal (QPVDMAB) (I). The other is potassium polyacrolein bissulfate (KPABS) (II). Both the polycation and polyanion combine with GOD, forming a QPVDMAB-GOD-KPABS complex. The main advantage of the complex includes high specific activity, long storage life, and reproducibility of enzymatic reaction. Experiments have shown that the complex is due to Coulomb forces and covalent bonds.

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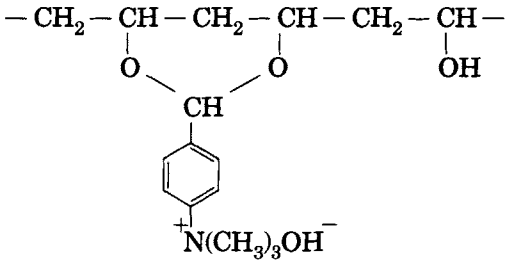
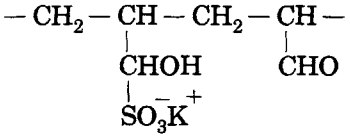


(I)



(II)

EXPERIMENT

Preparation of QPDMAB

p-Dimethylaminobenzaldehyde was added to methyl iodide as reactant and reaction medium, forming quaternary ammonium, *p*-dimethylaminobenzaldehyde, a light yellow crystalline compound. The product thus obtained was added to an aqueous solution of polyvinyl alcohol in the presence of hydrogen chloride and then was neutralized with potassium hydroxide, forming a QPVDMAB solution. The resultant product was obtained by means of salting out.

Preparation of KPABS

Polyacrolein was prepared using solution polymerization in the presence of water as medium and potassium persulfate. The polyacrolein thus obtained as a white powder was suspended in water, and then potassium bissulfate was added to the suspension. The mixture was heated to form a viscous transparent liquid. Ethyl alcohol was added, yielding a KPABS precipitate.

Preparation of QPVDMAB-GOD-KPABS

A given weight of glucose oxidase was added to the aqueous solution of QPVDMAB. After shaking for several hours at room temperature, an equivalent weight of the KPABS (aqueous solution) was dropped slowly into the above solution under stirring. The reaction lasted several hours, resulting in a QPVDMAB-GOD-KPABS complex (immobilized enzyme), a flocculent white solid. At the end, the solid coimplex was washed with water, immersed in phosphate buffer (0.2 *M*, pH 6.0) for 24 h, dried at room temperature, and ground to 60 mesh of grain. The specific activity of the QPVDMAB-GOD-KPABS complex (immobilized enzyme) was measured to be 71 U/g

(wet complex) using an approach based on the determination of oxygen consumption in the enzymatic reaction system.

RESULTS AND DISCUSSION

Structure of the QPVDMAB-GOD-KPABS

The conductivity curve of the GOD and QPVDMAB solution is shown in Fig. 1. The curve shows an abrupt discontinuity at GOD-QPVDMAB = 40:60 (v/v). This indicates that salt linkages are formed between $-COO^-$ groups on the GOD molecules and $-N^+(CH_3)_3$ groups on the QPVDMAB molecules, generating a QPVDMAB-GOD complex.

The conductivity curve of the KPABS and the QPVDMAB solution is shown in Fig. 2. This curve also shows a discontinuity when the polycation completely neutralizes the polyanion at KPABS-QPVDMAB = 60:40 (v/v). It indicates that the salt linkages are formed between $-SO_3^-$ on the KPABS and $-N^+(CH_3)_3$ groups on the QPVDMAB, resulting in a QPVDMAB-KPABS complex.

The QPVDMAB-GOD complex reacts further with the KPABS to form a QPVDMAB-GOD-KPABS complex. In the process, a part of the $-COO^-...N^+(CH)-$ salt linkages in the complex is cleaved. The reduced $-N^+(CH_3)_3$ cation reacts with the $-SO_3^-$ anion on the KPABS, forming new $-SO_3^-N^+(CH)-$ salt linkages. This can be shown in the conductivity curve abruptly at KPABS/QPVDMAB-GOD = 52:48 (v/v) (see Fig. 3). Thus, a QPVDMAB-GOD-KPABS complex (immobilized enzyme) is formed.

In addition, there are Schiff base $-CH=N-$ groups in the complex, which are formed between amino groups on the glucose oxidase molecules and aldehyde groups on the polyacrolein.[9] It is also possible that the enzymes are entrapped in a network of the complex.

Thus, one may infer that the structure fragment of the complex may be schematized as in Fig. 4.

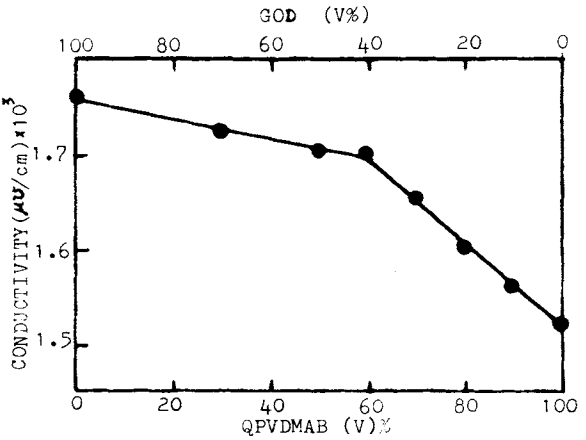


Fig. 1. Relationship between conductivity and volume ratio of GOD and QPVDMAB solution: GOD = 0.601% (g/mL); QPVDMAB = 0.506% (g/mL).

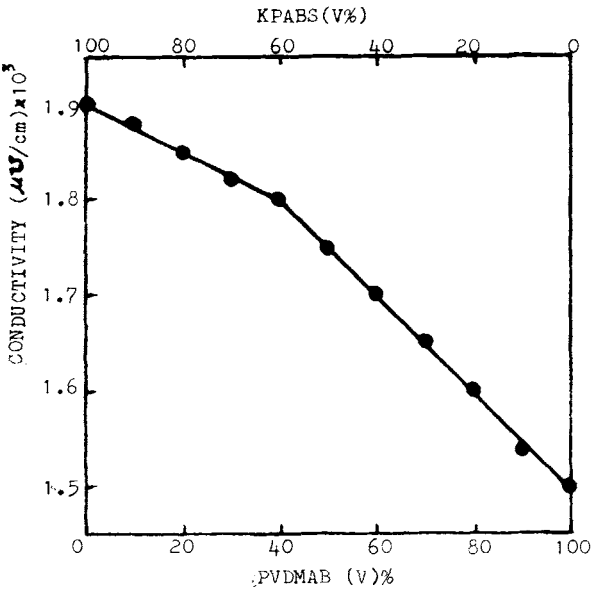


Fig. 2. Relationship between conductivity and volume ratio of KPABS and QPVDMAB solution: KPABS = 0.336% (g/mL); QPVDMAB = 0.506% (g/mL).

Properties and Structure of the Complex

The features of the polyion-enzyme complex are higher specific activity, long storage, stability, and anti-inhibitor properties. This may be due to the macromolecular salt linkages, to the network in the polyion complex, and to the microenvironment around the enzyme.

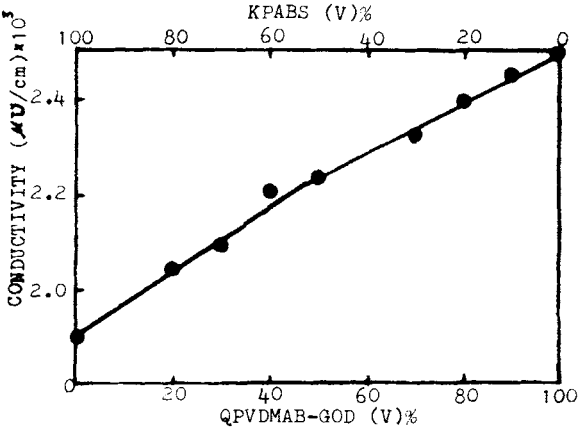


Fig. 3. Relationship between conductivity volume ratio of KPABS and QPVDMAB-GOD solution: QPVDMAB-GOD = 0.506 g QPVDMAB + 0.382 g GOD per 100 mL; KPABS = 0.336% (g/mL).

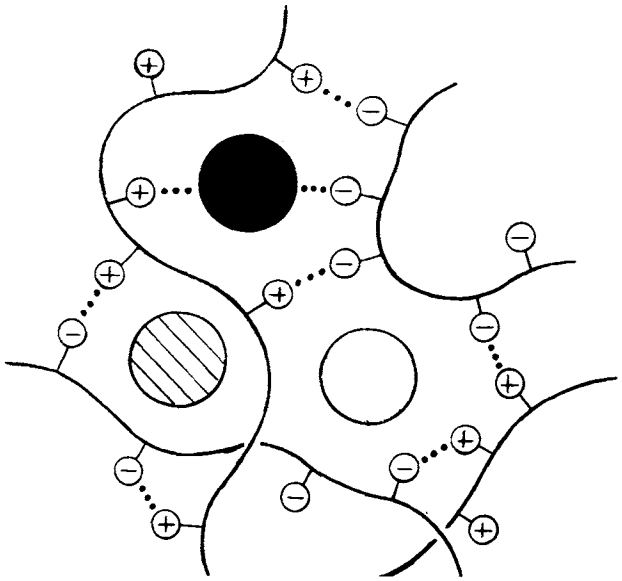


Fig. 4. The structure fragment of the complex: ● enzyme molecules binding with polyions by salt linkages; ○ enzyme molecules entrapped by network of polyion complex; ⊕ $-N^+(CH_3)_3$ groups on the polycation; ⊖ $-SO_3^-$ groups on the polyanion.

Specific Activity

As shown in Fig. 4, a great number of the active groups in the polyions and closer network are able to combine enzyme to a large extent. Thus, the immobilized glucose oxidase has a specific activity of 71 U/g, which greatly exceeds that of glucose oxidase immobilized by PEI glass beads (particle range, 13–14 μM); 589.4 pmol O_2 per mg support, (36 U/g).[10]

Stability

The glucose oxidase molecules combine with the polyions in a "multipoint" based on salt linkage. Therefore, the immobilized enzyme displays an enhancement in stability. It is not easy for the enzyme molecule to change conformation (denaturation) during storage because of multipoint

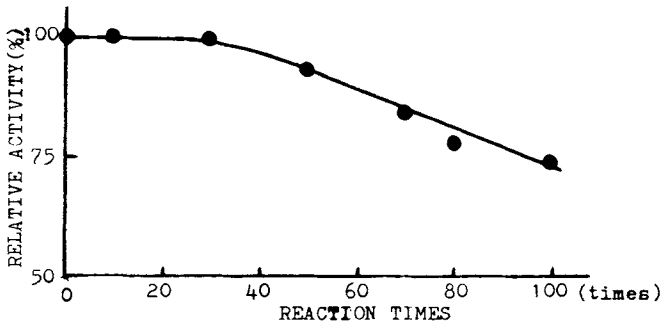


Fig. 5. Usage stability of polyion-enzyme complex.

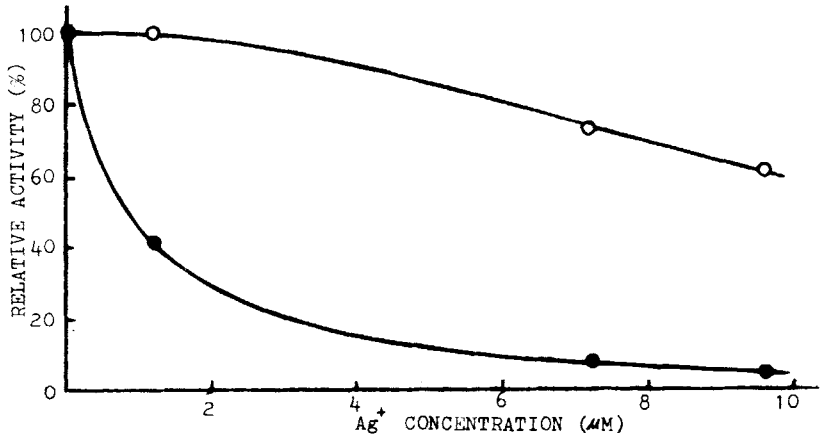


Fig. 6. Relationships between concentration of inhibitor Ag^+ and activity of free and immobilized glucose oxidase; ○ immobilized enzyme, 9.5 mg (30.8 U/g); ● free enzyme solution, 25 μL (24 U/mL); phosphate buffer 0.2 *M*, pH 6.0; *T* 30±0.01°C; reaction system volume, 6.6 mL.

combination. The immobilized glucose oxidase still showed 100% of activity after standing for 30 days, whereas a soluble glucose oxidase showed only 8% activity after the same amount of time. At the same time, the enzyme molecules combined by multipoint salt linkages could not be destroyed during the enzymatic reaction. Finally, the enzyme molecules did not easily leak out, being closely entrapped. Thus, the immobilized enzyme still showed 100% of activity after having been used 40 times and 74% after 100 times (see Fig. 5).

Anti-inhibitor Behavior

An interesting feature of the polyion-enzyme complex is that the complex can provide a protective effect against enzyme inhibitors. It is well known that Ag^+ is an inhibitor of glucose oxidase. It is clear from Fig. 6 that the QPVDMAB-KPABS complex can protect glucose oxidase from Ag^+ under conditions in which the free enzyme rapidly loses activity. Complete denaturation occurred at $[Ag^+] = 10$ μM for the free enzyme, but the complexed enzyme kept 61% of activity under the same conditions. This can be explained by the polyions containing a large number of positive and negative side groups. The former are able to exclude Ag^+, and the latter can attract Ag^+. Both effects cause a decrease of the diffusion of Ag^+ ion to enzyme.

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