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ACTIVATION OF POLYMER–METAL COMPLEXES ON ENZYME

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ABSTRACT

In this paper a new type of activation of polymer-metal complexes on an enzyme immobilized in them will be described. The polymer is partially quaternized poly(dimethylaminomethylstyrene) (PQPD). The metals (Me) include the fourth-period transition elements trivalent chromium and divalent iron, cobalt, nickel, copper, zinc, and manganese. Glucose oxidase (GO) is the enzyme. These complexed metallic ions had some activation effects on GO for most PQPD-Me-GO complexes. Specifically, the activity of the GO was increased up to 1.8-fold by chromium ion in the complex compared to immobilized GO without metal ion. The extent of activation of the polymer-metal complexes on GO was found to be related to the ionic radius and could be expressed quantitatively by a regression equation $a = (4.5 - 42.3r) \times 100$, where a represents the relative activity of the immobilized enzyme and r represents the metal ion radius in the polymer-metal-enzyme complex.

INTRODUCTION

In recent years a large number of polymer-metal complexes containing transition metals have been investigated in which the metal-containing portion is coordinated to the polymer as a pendent group. The polymer-metal complexes show some special properties such as electron semiconducting,

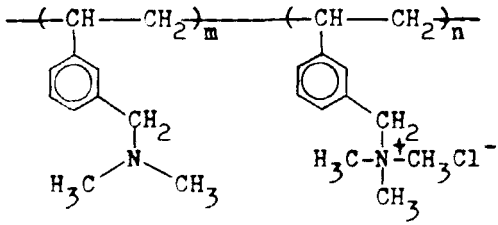
oxidation-reduction, and hydrogenation catalysis. Hence they have many potential uses as functional materials with desirable chemical and physical properties [1-3]. But certain properties related to activation of metallic ions on an enzyme immobilized on those complexes have not been reported before.

We have reported an immobilized glucose oxidase (GO) using a polycation-polyanion complex. This polyion complex is capable of preventing silver ion inhibition but causes activation of the GO [4]. Recently, we found that some polymer-metal complexes are able to activate an enzyme immobilized on them, which is a new, interesting property. This polymer is a partially quaternized poly(dimethylaminomethylstyrene) (PDPQ). The metal ions include the fourth-period transition elements trivalent chromium and divalent iron, cobalt, nickel, copper, zinc, and manganese. The glucose oxidase is the enzyme.

EXPERIMENTAL

Materials

Partially quaternized poly(dimethylaminomethylstyrene) (crosslinked with divinylstyrene), (PQPD, I); content of cationic nitrogen, 3.368 mmol/(g resin); content of neutral nitrogen, 0.0121 mmol/(g resin); Styrene Strong Anion Resin, Model-714 (Shanghai Resin Factory, Shanghai, China).



Glucose oxidase (GO), 23 000 u/g (solid), Sigma Chemical Company, U.S.A.

Preparation of PQPD-Metal Complexes

Metallic ions (salts): $CrCl_3$, $FeCl_2$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$.
Concentration of metallic ions in water: 0.1-1.0 mol/L.

Procedure. 2 mL aqueous solution of metallic ion was added to 0.5 g resin at pH 1-4. After 2 h the resin coordinated with the metallic ion was washed with deionized water until the eluate showed absence of the ion.

The amounts of metals in the resin were measured by atom absorption spectrum. The coordination of nitrogen on the resin with the metal ions was confirmed by infrared spectrum (Infrared spectrophotometer: PE580, Perkin-Elmer).

Preparation of PQPD-Me-CO Complexes

GO aqueous solution (0.5 mL, 10 mg/mL) was added to 0.5 g PQPD-Me complex. After shaking for 12 h the solid was washed with deionized water until the eluate showed absence of GO, resulting in the PQPD-Me-GO complex.

The assay of the enzyme activity for this complex was carried out by measuring the rate of oxygen consumption [4]. The activity of enzyme in the PQPD-Me-GO complex was calculated by

$$u = (10^6/32)qvpd \ [\mu\text{mol/min}], \tag{1}$$

where q = solubility of oxygen in water at 30°C, 273.32×10^{-9} g(oxygen)/mL(water)
v = volume of the reaction system, 6.6 mL
p = percentage consumption of oxygen of the original enzymatic reaction per minute
d = specific gravity of water at 30°C, ~1 g/mL

The relative activity, a, of the enzyme in the polymer-Me-GO complexes in percent is calculated by

$$a = \frac{\text{activity of polymer-Me-GO complex}}{\text{activity of polymer-GO complex}} \times 100.$$

RESULTS AND DISCUSSION

Structure of the Polymer-Metal-Enzyme Complex

The infrared spectrum showed a splitting of the characteristic peak of the aliphatic dimethylamino group of the PQPD-Me complex at 1130 cm^{-1}, and a shift toward low wavenumbers (see Fig. 1). This shows that the C–N bond in the complex coordinates to the metal ion through the nitrogen atom, leading to a decrease of the C–N bond energy. This proved the coordination of the nitrogen atoms of the neutral dimethylamino groups with metallic ions.

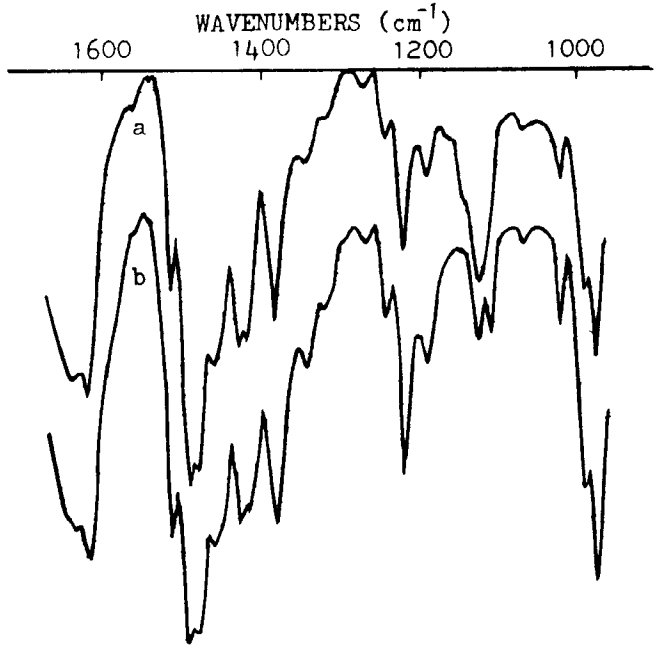


FIG. 1. Infrared spectra of PQPD and its metal complexes. a: PQPD; b: PQPD-metal complex.

In addition, the quaternary nitrogen atom on the PQPD combined with the carboxyl groups on the GO, forming salt linkages [5] and immobilized GO. The segment of the polymer-metal-enzyme complex is shown schematically in Fig. 2.

Stability of GO Immobilized in the Complex

The experiments showed that the activity of the PQPD-Me-GO complex did not decline after 35 days at room temperature (10-20°C). In addition, the immobilized GO still showed about 100% of its original activity after being used 20 times and 80% after 100 times. This stability can be attributed to "multipoint combination." That is, there are very many carboxyl groups on the enzyme and quaternary nitrogen atoms on the polymer-metal complex to form salt linkages. There are thus so many of the latter that multipoint combinations between the enzyme and the complex are formed. Hence,

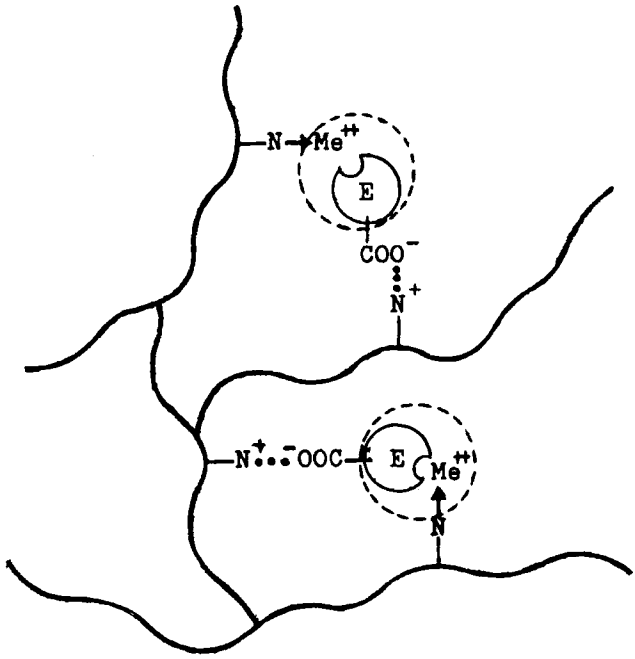


FIG. 2. Segment of the polymer-metal-enzyme complex. Me^{++}: Metal ion; →: coordinate bond; ...: salt linkage; E: enzyme; ( ): effect of metal ion on enzyme.

it is not easy for the enzyme molecule to change conformation of the active sites (denaturation) during storage or the enzymatic reaction.

Activation of Metallic Ions on GO

For the enzymatic reaction system of *soluble* GO, we found that copper ion was an inhibitor for the enzyme, giving relative activities less than 100% (see Table 1). Table 1 shows a maximum relative activity (93%) at 0.60 mmol/L copper ion.

When the GO was immobilized in some polymer-metal complexes, we found that most of the metallic ions coordinated with the complexes had some activation effects on the GO immobilized on the complexes. Moreover, the activation effects on the enzyme were related to the content of the metallic ions in the complexes (see Table 2). Specifically, the activity of the immobilized GO

TABLE 1. Effect of Copper Ion Concentration on the Relative Activity of *Soluble* Glucose Oxidase

Concentration of Cu^{2+}, mmol/L	0	0.35	0.60	2.50
Relative activity, %	100	74	93	52

was raised up to 1.8-fold by chromium ion. These phenomena can be explained in several ways.

It is well known that an enzyme molecule has a specific ionic atmosphere, and the specific electrostatic field stabilizes the basic conformation of its active sites. Changes of the surface charge on the enzyme molecule will perturb this stabilization and lower the enzyme's activity.

Thus, if there is an interaction between the residues of the enzyme's surface and a chemical, the surface charge will be altered, and this will perturb the conformation of the active site of the enzyme. This is exactly the pattern observed when GO is separated from a living system and it loses some of its original activity. In this paper, when GO is immobilized on the PQPD-Me complex, the metallic ions in the polymer-metal complexes act on its surface, altering the electrostatic field back and changing the active site close to its original conformation. In other words, the action of these metallic ions in the polymer-metal complexes on the GO puts the enzyme into an optimum conformation for its active site, more or less like that in living systems.

Thus, the uncoordinated copper ion only inhibits the GO, but when it is coordinated in the polymer, it not only inhibits the GO but also activates it. This is because the electronic state of Cu^{2+} coordinated in the polymer is different from that of free copper ions.

In addition, if a certain metal content gives an optimum conformation of the active site, optimum activity can result. That is why every ion shows an increase of the activity at a proper content in the complex, sometimes showing a decrease at a higher or a lower content (see Table 2).

Effects of Electronic Configuration and Radii of Metal Ions on the Activity of Enzyme in the Complexes

An important and unforeseen finding was that the activation of the polymer-metal complexes on the GO depended on the electronic configuration of the outer orbits and the radii of the metal ions (see Table 3). This can be explained as follows. According to Pauling's principle and Hund's rule, if

TABLE 2. Concentration of Ion in the Polymer-Metal-Enzyme Complex and Relative Activity[a]

Cr^{3+}		Ni^{2+}		Co^{2+}		Fe^{2+}	
Concentration, mmol/kg	Activity, %	Concentration, mmol/kg	Activity, %	Concentration, mmol/kg	Activity, %	Concentration, mmol/kg	Activity, %
1.0	88	0.5	96	2.0	120	0.1	100
1.1	107	0.6	120	2.1	132[b]	3.0	113[b]
1.5	100[b]	0.7	130[b]	2.2	127	3.3	80
2.3	37	1.3	59	2.6	70	4.4	13

Cu^{2+}		Zn^{2+}		Mn^{2+}	
Concentration, mmol/kg	Activity, %	Concentration, mmol/kg	Activity, %	Concentration, mmol/kg	Activity, %
5.1	63	0.0	100	0.24	50
5.5	75	4.0	91[b]	0.31	93
6.0	115[b]	15.0	59	2.50	120[b]
6.1	93	21.0	44	4.20	40

[a]Ion concentration in mmol/kg resin. Activity: relative enzyme activity a in %.
[b]Maximum activity.

TABLE 3. Effect of Electronic Configuration and Ionic Radius on Relative Activity

Metallic ion	Cr^{3+}	Ni^{2+}	Co^{2+}	Fe^{2+}	Cu^{2+}	Zn^{2+}	Mn^{2+}
Electronic configuration	$3d^3$	$3d^8$	$3d^7$	$3d^6$	$3d^9$	$3d^{10}$	$3d^5$
Maximum relative activity, %	180	130	132	113	115	91	120
Ionic radius, nm	0.064	0.074	0.078	0.080	0.080	0.083	0.091

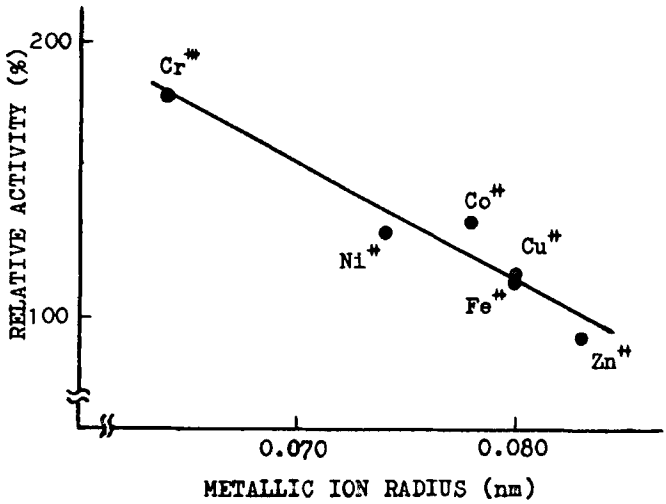


FIG. 3. Relationship between activity of the polymer-metal-enzyme complexes and ionic radius.

the outer electron orbits of a metal ion are half or completely filled, they are in a stable state, but if they are not filled, they are in an unstable state. The outer orbits of the manganese ion are in a stable state, but they are only half filled, so that the enzyme is activated. On the other hand, the outer orbits of the zinc ion are not only in stable state but also completely filled, so that the enzyme is inhibited.

It is important that the outer orbits of the trivalent chromium and divalent nickel, cobalt, iron, and copper ions are not only in an unstable state, but also unfilled. When the electronic atmosphere of an enzyme's surface interacts with the unstable and unfilled electron cloud of those ions, it will tend to change and recover the conformation of the enzyme's active site and, therefore, its activity. Thus, one of the conditions to activate an enzyme is an unfilled outer orbit of the metal ion.

Furthermore, the smaller the metallic ion radius, the stronger the action on the enzyme. Hence the effect of the chromium ion on the enzyme is the strongest and that of the iron and copper ions is very weak.

Figure 3 shows the relation between the maximum relative activity (Table 2) and the ionic radius (Table 3) of the ions with unfilled outer orbits. The

straight line through the points is represented by the regression equation

$$a = 450 - 4230r,$$

where a represents the relative activity of the immobilized GO and r represents the ionic radius.

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