Dicopper Complexes as Oxidase Catalysts Immobilized on Polystyrene and Polyacrylic Beads

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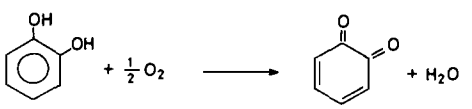
Received March 13, 1984

The simple dicopper(II) complexes FSAL(Glu)$_2$-Cu$_2$OH·2H$_2$O and FSAL(Lys)$_2$Cu$_2$·2HCl·2H$_2$O, previously used as oxidase catalysts, were anchored to polystyrene and oxirane acrylic beads. The ability of the immobilized dicopper-bead complexes to catalyze the oxidation of catechol was measured and their catalytic activities were compared with those of the simple dicopper complexes used as homogeneous catalysts. The catecholase activities of the dicopper bead complexes, although found to be reasonably high, were less than the activities of the simple dicopper complexes.

Introduction

Previous studies have shown that simple dicopper complexes are capable of catalyzing the oxidation of catechols, eqn. 1 [1, 2]. Similar type dicopper complexes were attached to the polymers, polyethyleneimine and polyvinylamine to form polymeric dicopper complexes (PEI-Cu and PVA-Cu) [3]. The polymers were expected to enhance the enzyme like properties of the dicopper complexes [4]. With cross linking of the polymer, the dicopper complexes could be immobilized in water. One of the PEI-Cu complexes performed well as a soluble enzyme like catalyst but we were unable to immobilize it in water by crosslinking the polymer. The PVA-Cu complexes were completely insoluble in water but were found to be poor oxidase catalyst [3]. Since PEI-Cu complexes as soluble catalyst do not serve our purpose and the PVA-Cu complexes which were immobilized possess little catalytic activity, an attempt was made to anchor soluble dicopper complexes to polystyrene and polyacylic beads. We report the synthesis of the dicopper-bead complexes and their ability to catalyze the oxidation of catechol.



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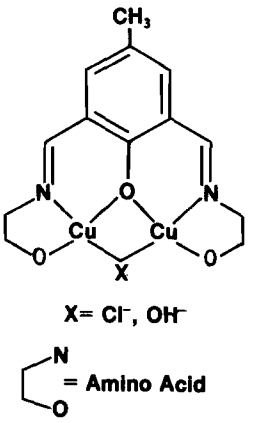

Fig. 1. Simple dicopper complexes.

Experimental

Synthesis of the Dicopper Complexes FSAL(Glu)$_2$-Cu$_2$OH·2H$_2$O and FSAL(LYS)$_2$Cu$_2$Cl·2HCl·2H$_2$O Anchored to Polystyrene Beads

The FSAL(Glu)$_2$Cu$_2$ and the FSAL(Lys)$_2$Cu$_2$ complexes were prepared according to reference [1]. Their structure is shown in Fig. 1. Chloromethylated polystyrene beads were purchased from Polyscience Corp. The beads ranged in size from 200–400 mesh and contained 2.63 meq per gram of methylchlorine. The FSAL(Glu)$_2$Cu$_2$-polystyrene beads (GluCu-Sty Bead) and FSAL(Lys)$_2$Cu$_2$-polystyrene bead (LysCu-Sty Bead) were prepared in the following manner. Reagent grade tetrahydrofuran was dried over 3 X molecular sieves for three days. One gram of the bead was placed on a watch glass and dried in a vacuum oven overnight at room temperature. The beads were then added to 200 ml of the dried tetrahydrofuran and one gram of the corresponding dicopper complex in a 500 ml round bottom flask connected to a water cooled condenser. The mixture was refluxed while stirring for 24 hours and then allowed to cool. It was filtered, washed with several aliquots of water and dried to give generally a light green appearance. The results of copper analysis are given in Table I. The coupling reactions are given as follows:

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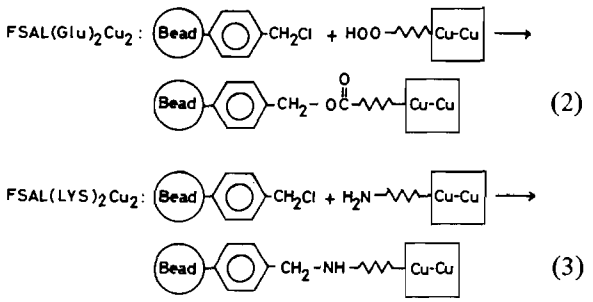
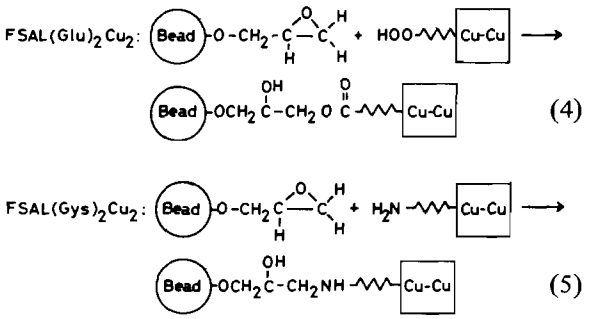


Synthesis of the Dicopper Complexes of FSAL(Glu)$_2$-Cu$_2$OH·2H$_2$O and FSAL(Lys)Cu$_2$Cl·2HCl·2H$_2$O Anchored to Oxirane Acrylic Beads

Oxirane acrylic beads were purchased from Rohm Pharmaca, Darmstadt, F.R.G. The beads range in size from 200–400 mesh and contain 1.86 meq per gram of the epoxide group. The procedure for preparing the FSAL(Glu)$_2$Cu$_2$-oxirane bead (GluCu-Ox Bead) and FSAL(Lys)$_2$Cu$_2$-oxirane bead (LysCu-Ox Bead) was the same as the procedure used to prepare the polystyrene bead except that the oxirane beads were used as received from Rohm without drying. The results of the analysis for copper are given in Table I. The coupling reactions are given as follows:



Infrared Spectra

The ir spectra were run on the complexes prepared in KBr pellets. The spectra were run on a Perkin Elmer 283 Infrared Spectrometer.

Electronic Spectra

The electronic spectra were run on a nujol sample evenly coated on filter paper. The spectra were run in the visible region between 900 nm and 350 nm on a Cary 17 Spectrophotometer.

Catecholase Activity

The catecholase activities of FSAL(Glu)$_2$Cu$_2$ and FSAL(Lys)$_2$Cu$_2$ (*o*-quinone produced and oxygen uptake during the reaction in eqn. 1) were measured using the method previously reported in reference 1. Because of the heterogeneous state of the GluCu-Bead complexes, a special apparatus (Fig. 4) was constructed to measure the rate of *o*-quinone produced. From the figure one can see that the reaction takes place outside of the spectrometer in an insulated, temperature controlled container. A portion of the reaction mixture containing the oxidation products is cycled through a flow-through cuvette in a Cary 17 spectrophotometer for measurement. The production of o-quinone is followed at λ = 390 nm, ϵ = 1719. The oxygen uptake is measured using a method described in reference 1.

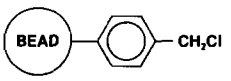


Fig. 2. Polystyrene bead chloromethylated.

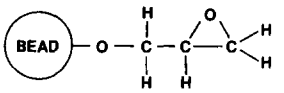


Fig. 3. Oxirane acrylic bead.

TABLE I. Percent Copper in Dicopper-Bead Complexes.

Complex	% Copper
GluCu-Sty Bead	2.1
LysCu-Sty Bead	0.09
GluCu-Ox Bead	1.9
LysCu-Ox Bead	0.06

Results and Discussion

Synthesis

The structures of the simple dicopper complexes whch are to be coupled to the polystyrene and oxirane polymer beads (FSAL(Lys)$_2$Cu$_2$ and FSAL-(Glu)$_2$Cu$_2$) are given in Fig. 1. The Lys complex is coupled to the polymer beads through the primary amine extending out from the amino acid moiety of the complex. The Glu complex utilizes the carboxylic acid group for coupling purposes. These simple dicopper complexes are coupled through the methyl chloride group on the polystyrene bead (Fig. 2) and through the epoxide group on the oxirane acrylic bead (Fig. 3). The coupling reactions are given in eqns. (2–5). A measure of the reaction yield for the coupling reactions is given in Table I which lists the percent copper found in each dicopper-bead complex. Both the glutamic acid (Glu) complexes were found to be high in copper and are presumed to react better than the lysine (Lys) complexes with either the polystyrene chloromethylated group or

TABLE II. Visible Spectra of Dicopper-Bead Complexes in Nujol Mull.

Complex	λ, nm			
$FSAL(Glu)_2Cu_2$	830	680	620	378
GluCu-Sty Bead	820	670	615	420
GluCu-Ox Bead	800	675	610	420
$FSAL(Lys)_2Cu_2$	800	680	610	410
LysCu-Sty Bead	830	670	600	410
LysCu-Ox Bead	830	665	605	405

the oxirane epoxy group. The dicopper-bead complexes were found to be reasonably stable in water. The bead complexes were placed in water for a period of three days after which the water spectrum was run in UV region. No trace of organic decomposition product was found.

Visible Spectra

The visible spectra of the dicopper-bead complexes were made on the samples prepared in a nujol mull which was applied to filter paper. The sample was run against a bead-nujol mixture in the reference side. The results of the spectral runs are given in Table II. Although the bands are broad and not well resolved, one can in most cases make out three bands between 830 nm and 600 nm which are most likely d–d bands. They compare well with the three bands in the same region given by the simple complexes $FSAL(Glu)_2Cu_2$ and $FSAL(Lys)_2Cu_2$ whose spectra were run under the same conditions and are also presented for comparison in Table II. The band found at about 400 nm for both the simple dicopper complexes and the dicopper bead complexes is probably a charge transfer band. The simple

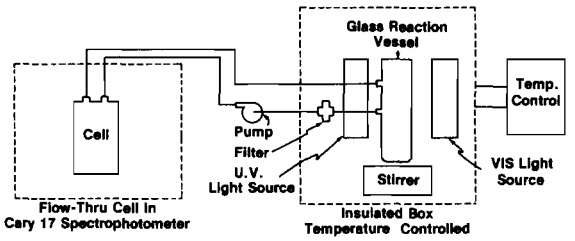


Fig. 4. Apparatus for measuring reaction rates catalyzed by immobilized catalysts.

dicopper complex bands and their analogous beaded complex bands are in good enough agreement to assume that the $FSAL(Glu)_2Cu_2$ and $FSAL(Lys)_2Cu_2$ remain in tact upon immobilization to the plastic beads.

Infrared Spectra

The infrared spectra of the dicopper-bead complexes and the polymer beads were made in KBr pellets. Because of the broadness of both spectra and because of the large excess of polymer bead in both spectral preparations, a comparison of the LysCu-Sty Bead and the Sty Bead spectra were difficult. The existence of the $FSAL(Lys)_2Cu_2$ complex in LysCu-Sty bead complex however, was confirmed by the presence of a ν(C=N) at 1655 cm^{-1}. A similar comparison of GluCu-Sty bead and Sty bead showed the presence of a broad band between 1630–1670 cm^{-1} which did not exist in the Sty bead spectrum. Part of this band results from ν(C=N). The existence of the $FSAL(Glu)_2Cu_2$ complex was also supported by the ν(Skel) phenol at 1550 cm^{-1}.

In a similar comparison, a ν(C=N) at 1715 cm^{-1} was found to be present in the spectrum of LysCu-Ox Bead which was not found in the Ox Bead spec-

TABLE III. Catecholase Activity.

	Activity, Mol/Liter × 10^6					
	o-Quinone Production			Oxygen Uptake		
Complex	10 min	20 min	30 min	10 min	20 min	30 min
GluCu-Sty Bead[a]	3.5	5.5	7.0	15.1	30.1	38.6
GluCu-Ox Bead[a]	1.7	3.5	5.2	10.8	16.6	18.1
Lys Cu-Sty Bead[a]		not measurable[b]		3.6	6.7	8.6
LysCy-Ox Bead[a]		not measurable[b]		4.1	7.0	8.9
$FSAL(Glu)_2Cu_2$[c]	10.5			36.7		

[a]The reaction condition for measuring *o*-quinone production and oxygen uptake for the bead complexes were essentially the same. The reactants consisted of 47.2 ml of 10^{-3} *M* catechol with 0.33 g of bead complex. The pH was 7.0. [b]The LysCu-Sty Bead and LysCu-Ox Bead complexes showed very little *o*-quinone production. [c]The reaction conditions for measuring *o*-quinone and oxygen uptake for $FSAL(Glu)_2Cu_2$ were 10^{-3} *M* catechol and 10^{-4} *M* in dicopper complex. The activities listed in the Table above have been adjusted to account for the differences in copper content between the simple complex reaction and the Bead reaction. (Rates for $FSAL(Glu)_2Cu_2$ are 1st order in dicopper).

trum. The GluCu-Ox Bead spectrum showed the presence of ν(C=N) at 1715 cm^{-1} and ν(skel) at 1450 cm^{-1} which also was not present in the spectrum of the Ox Bead. These comparisons clearly show the presence of the simple dicopper complexes attached to the polymer beads.

Catecholase Activity

The amounts of *o*-quinone produced and oxygen uptake measured in the catalyzed reaction given by eqn. 1 are reported in Table III. The GluCu-Sty Bead gave the highest activity both in *o*-quinone produced and in oxygen uptake for the reaction. It contained the largest amount of copper and its high activity is thus attributed to the high copper content. The other Glu complex, GluCu-Ox Bead which also was found to be high in copper exhibited high activities in *o*-quinone production and oxygen uptake. The bead complexes containing Lys, LysCu-Sty Bead and LysCu-Ox Bead, were found to be low in copper content and exhibited low oxygen uptake. The *o*-quinone production activities of the Lys Bead complexes were too low to be accurately measured by the optical method.

The stoichiometric mol ratio of oxygen to *o*-quinone according to eqn. 1 should be ½ to 1. In contrast, the experimentally found ratio was about 5 to 1. Similar results were previously reported for catalytic studies of the same reaction using the simple dicopper complexes $FSAL(Glu)_2Cu_2$ and $FSAL(Lys)_2Cu_2$ as catalysts [1]. The reported oxygen to *o*-quinone ratios were found to be about 4 to 1, in keeping with the ratio found in this work. Apparently, the oxidation reaction does not stop at the production of *o*-quinone; rather, several other oxidation products requiring additional oxygen may be shown to result.

A comparison of the activity of $FSAL(Glu)_2Cu_2$ with the activities of the dicopper bead complexes is presented in Table III. Although the concentrations of the $FSAL(Glu)_2Cu_2$ reactions were different from those of the dicopper bead complexes, the activities of the $FSAL(Glu)_2Cu_2$ were adjusted to account for dicopper differences. They show a somewhat larger value than the activity of the GluCu-Sty Bead or GluCu-Ox Bead complex at the same copper content. The greater activity is expected for the homogeneous catalyst $FSAL(Glu)_2Cu_2$. No attempt was made to compare values of $FSAL(Lys)_2Cu_2$ with its analogous Bead complex because of the difficulty in measuring the low Bead complex activities.

Acknowledgement

We thank the U.S. Environmental Protection Agency (Grant No. R809726) for support to this study, given to GSV.

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