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Impact of nature and length of linker on the catecholase activity of a covalently immobilized copper(II) complex in continuous flow catalysis

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ABSTRACT

The catecholase activity of four immobilized mononuclear copper(II) catalysts with chains of different nature and length used as linkers is studied in continuous flow catalysis. The graphite felt support was first derivatized by cathodic reduction of 4-carboxymethyl-benzenediazonium salts. Complexes with different chains were then covalently immobilized on the functionalized porous support by esterification reaction. The successful achievement of the immobilization process is attested by the presence of the $Cu^{II/I}$ reversible system in cyclic voltammetry. Volume concentrations around 10^{-8} mol cm^{-3} of immobilized catalysts are estimated by integration of the redox peak. Comparison of the catecholase activity of the immobilized complexes allows to conclude on the effect of the chain nature and length. First, high chain length positively influences the catalytic activity. Second, the presence of oxygen atoms in the linker significantly enhances the catecholase activity of the catalyst. A possible explanation is the chain hydrophilicity, making easier the access of the catalytic center by H_2O molecules.

1. Introduction

Copper metalloproteins have been the subject of many investigations due to their catalytic activity and their ability to reversibly bind and activate dioxygen [1,2]. Among them, type 3 proteins are composed of two close copper atoms, each coordinated by three histidine residues. The most known are hemocyanin, tyrosinase and catechol oxidase. Whereas hemocyanin is used to transport and stock dioxygen, tyrosinase catalyses ortho-hydroxylation of phenol and their subsequent oxidation into *o*-quinones. Catechol oxydase does not have hydroxylation activity but catalyzes the oxidation of *o*-diphenols into *o*-quinones by dioxygen reduction. Many mono and dinuclear copper complexes have been synthesized as biomimetic models for a better mechanistic understanding of the enzyme catalytic process and to find new catalysts for oxidation reactions [3–5]. However, these molecular catalysts are often less stable and selective than the enzyme, whose active site is isolated in a protein backbone. Thus, catalysts have been immobilized on supports to more precisely mimic natural enzymes and to improve their catalytic performances [6–16]. The aim is to provide proper geometry and distance for catalytic centers and to improve the catalyst stability, reducing the possibility of side reactions to occur.

In supported catalysis, several factors influence the activity, such as the nature of the support, the catalyst density, the point of catalyst attachment and the length and nature of the linker. Many studies have shown that increasing the length and the flexibility of the linker facilitates the access of reagents to the catalytic site and enhances the catalytic activity [17,18]. Thus, long spacer chains have shown activity approaching that of the corresponding non-immobilized catalysts. However, in some cases, too long arms can change the catalyst environment and reduce the catalytic activity. Thus, it has been reported that catalyst site isolation is preferentially obtained with chain of intermediate length, whereas long spacer chains led to interactions between two immobilized catalysts [19,20] or to different coordination modes [21]. Clearly the effect of the linker on the catalytic activity is difficult to generalize.

We have previously reported the covalent immobilization of a mononuclear copper complex based on tripodal ligands on graphite felt and its catecholase activity in continuous flow catalysis [22]. Comparison with results obtained when the catalyst is in solution, underlined the interest of the flow system that gives better conversion yields with using less catalyst. Moreover, with the same catechol concentration and ratio catalyst/substrate, supported catalysis exhibited better results than unsupported one.

In this work, copper complexes with different arms containing an alcohol ending group were immobilized on graphite felt and characterized by cyclic voltammetry. We report the effect of the chain nature and length on the catecholase activity and stability of the immobilized catalyst.

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2. Experimental

2.1. Reagents and materials

Graphite felt was obtained from Le Carbone Lorraine (RVG 4000). All commercially available reagents were used as supplied. All solvents were analytical grade. Tetrahydrofuran (THF) was distilled from deep blue solutions of sodium/benzophenone ketyl prior to use. Syntheses of the tripodal ligands **L_1**–**L_3** and the corresponding copper complexes **1**–**3** have been previously described [23]. 4-Carboxymethyl-benzenediazonium salts were prepared according to literature and stored under argon in a freezer [24]. The covalent immobilization of the complexes was performed according to a previously described procedure [22].

2.2. Instrumentation

Voltammetric experiments were carried out using an EDAQ potentiostat unit, with the EChem software package. A platinum wire working electrode, a platinum wire auxiliary electrode, and a saturated calomel reference electrode were used in a standard three-electrode configuration. The graphite felt sample (1 cm^3) was mechanically fixed to the platinum wire before analysis by inserting the wire directly into the felt. Cyclic voltammetry analyses of immobilized complexes were performed in acetonitrile containing 0.1 M tetrabutylammonium hexafluorophosphate, under a dinitrogen atmosphere.

2.3. Synthesis

2-{2-[2-(2-Amino-ethoxy)-ethoxy]-ethoxy}-ethanol **6** was prepared from tetra(ethyleneglycol) **5** according to a procedure described in literature [25].

2.3.1. 2-(2-{2-[2-(Bis-pyrazol-1-ylmethyl-amino)-ethoxy]-ethoxy}-ethoxy)-ethanol

L_4

A mixture of 1-hydroxymethylpyrazole (51 mg, 0.52 mmol) and 2-{2-[2-(2-Amino-ethoxy)-ethoxy]-ethoxy}-ethanol (50 mg, 0.26 mmol) was stirred without solvent for 3 h at 70 °C under vacuum (1 mbar). The product was obtained as a yellow oil in quantitative yield. ^1H NMR (400 MHz, $CDCl_3$): δ 7.55 (d, J = 3.2 Hz, 2H); 7.46 (d, J = 2.4 Hz, 2H); 6.21 (dd, J = 2.4 Hz, J = 3.2 Hz, 2H); 5.06 (s, 4H); 3.65–3.45 (m, 14H); 2.85 (t, J = 7.0 Hz, 2H) ^{13}C NMR (100 MHz, $CDCl_3$): δ 139.6; 130.1; 105.8; 72.6; 70.6; 70.5; 70.4; 70.3; 70.2; 68.4; 61.6; 49.5. HRMS (ESI): m/z calcd for $C_{16}H_{27}N_5O_4Na$ $[M+Na]^+$: 376.19607; found, 376.1956.

2.3.2. Copper complex 4

Copper(II) chloride (35 mg, 0.26 mmol) and tripodal ligand (92 mg, 0.26 mmol) were stirred in anhydrous THF for 2 h at room temperature. The solvent was evaporated. The complex was washed with a small amount of THF and dried under vacuum. Yield 0.09 g (72%) of a green compound. HRMS (ESI): m/z calcd for $C_{16}H_{27}N_5O_4ClCu$ $[M-Cl]^+$: 451.1047; found, 451.1047.

2.4. Immobilization of copper complexes on graphite felt

100 mg of 4-carboxymethyl-benzenediazonium salts, dissolved in 100 mL of degassed H_2SO_4 were reduced at −0.2 V_{SCE} for 5 min under nitrogen, using a graphite felt (48 mm diameter, 12 mm thickness) as working-electrode. Then the derivatized graphite felt was ultrasonicated 4 times in deionized water for 15 min and dried under vacuum. The functionalized graphite felt was placed in 50 mL anhydrous dichloromethane in a reactor under argon at 0 °C. 0.5 mL of diethylaminosulfur trifluoride (DAST) were added and the solution was stirred at room temperature for 5 h. The felt was ultrasonicated two times in dichloromethane. Then, the electrode was placed in a solution of anhydrous dichloromethane, 1 mL of triethylamine and 100 mg of copper complex. The solution was stirred at room temperature for 1 day. The modified electrode was ultrasonicated two times in dichloromethane and two times with acetone for 15 min.

R	Ligand	Complex
C_2H_4OH	**L_1**	$[Cu(L_1)Cl]Cl$ **1**
$C_6H_{12}OH$	**L_2**	$[Cu(L_2)Cl]Cl$ **2**
$C_2H_4OC_2H_4OH$	**L_3**	$[Cu(L_3)Cl]Cl$ **3**
$(C_2H_4O)_3C_2H_4OH$	**L_4**	$[Cu(L_4)Cl]Cl$ **4**

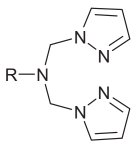

Fig. 1. Structure of the ligands **L1**–**4** with the corresponding complexes **1**–**4**.

2.5. Catalysis

Heterogeneous catalysis was performed in a flow cell [22] containing the modified graphite felt (10 mm diameter, 12 mm thickness). A fresh solution of 8.7×10^{-3} mol L^{-1} of 3,5-di-*tert*-butylcatechol in methanol maintained in air (O_2 from air was used as oxidant) at constant stirring and temperature of 25 °C percolated the porous support with a constant flow rate (3 mL min^{-1}) monitored by a Gilson minipuls 2 peristaltic pump. The number of moles of catalyst was determined by cyclic voltammetry for a graphite felt of 10 mm diameter and 12 mm thickness. To compare the 4 immobilized catalysts, the same ratio of catalyst/substrate (4.5×10^{-5}) was used for all experiments, by adjusting the volume of the solution of 3,5-di-*tert*-butylcatechol. Since 3,5-di-*tert*-butylquinone gives a strong absorption band at 400 nm in methanol, the absorbance was continually monitored at 400 nm for 1 h using a dipping probe colorimeter to follow the reaction.

3. Results and discussion

3.1. Synthesis of the complexes

Four copper complexes **1**–**4** containing tripodal ligands with pyrazole rings **L_1**–**L_4** with different nature and length chains were studied (Fig. 1). The linker was directly incorporated in the complex structure to minimize the number of steps of the immobilization process that are usually not quantitative because of the heterogeneous reaction medium and steric hindrance.

The complexes **1**–**3** were synthesized according to previously reported procedures [23]. Complex **4** was prepared by reaction of the ligand **L_4** with $CuCl_2$. **L_4** was synthesized in 5 steps from 2-{2-[2-(2-Hydroxy-ethoxy)-ethoxy]-ethoxy}-ethanol **5** (Scheme 1).

After monotosylation of the diol **5** and subsequent substitution reaction by sodium azide, reduction of the azido group by triphenylphosphine afforded the aminoalcohol **6**. A condensation reaction of 1-hydroxypyrazole on **6** performed at 70 °C for 3 h without solvent led to the ligand **L_4** with the oxoethylene arm. The corresponding copper complex was prepared in anhydrous tetrahydrofuran at room temperature for 2 h with $CuCl_2$ and conserved under argon.

3.2. Immobilization of the complexes on graphite felt

The graphite felt electrode was first derivatized by cathodic reduction of 4-carboxymethyl-benzenediazonium salts **6** at −0.2 V_{SCE} in 0.5 M H_2SO_4 for 5 min in a flow cell (Scheme 2) [23].

The passivation of the graphite fibers modified with aryl diazonium salts was investigated by cyclic voltammetry using potassium