The Kinetics of Oxidation of Cu(I) by Molecular Oxygen in Aqueous Pyridine

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The autoxidation of Cu(I) in aqueous pyridine has been studied spectrophotometrically and by following polarographically the formation of Cu(II). The rate of the reaction decreases with increasing free pyridine concentration. The reaction was first-order with respect to O_2 while the order with respect to Cu(I) was respectively 1 or 2 depending on Cu(I) concentration. The results indicate clearly the formation of a Cu(I)–O_2 adduct.

Introduction

Copper ions play an important role as catalyst in many oxidation processes both in chemical and biological systems. In particular in recent years much work has been focused on copper oxidases where copper ions appear to be stabilized mainly by nitrogen containing ligands [1, 2]. However, notwithstanding the relevance of these processes, little is known about the mechanism involved in the fast oxidation by molecular oxygen of small Cu(I) complexes, such as Cu(I)–(histidine), Cu(I)–(2,2′-bipyridine) *etc.* and of copper containing oxidases. For instance different values of the reaction order with respect to Cu(I) have been reported by different authors [3–5]. Furthermore, though the formation of the Cu(I)–O_2 adduct was postulated in view of the affinity of the soft Cu(I) towards the π acceptor O_2 [6], experimental evidence of its existence was weak.

In the present work we have investigated the kinetics of autoxidation of aqueous Cu(I)–pyridine complexes which show $E^{\circ\prime}$ values similar to those of some copper proteins [1].

Experimental

Materials and Methods

Analytical and reagent grade chemicals were used throughout. The reagent solutions were prepared in doubly distilled water; high purity mixtures of N_2 and O_2 were supplied by SIO (Milan). Catalase and superoxide dismutase were obtained from Boehringer, Mannheim Gmbh.

The Cu(I) oxidation was followed either spectrophotometrically, measuring the absorbance decrease at 218 nm, or polarographically, following the increase at 0 V versus saturated calomel electrode (SCE), of the polarographic wave corresponding to the monoelectronic reduction of Cu(II) to Cu(I). Because of the high absorbance of pyridine only the polarographic method was utilized in the presence of pyridine concentrations $>3 \times 10^{-2}$ *M*. The polarographic or spectrophotometric cells, placed in water thermostatted cell holders, were fitted with a serum cap through which nitrogen–oxygen mixtures were introduced. A Varian Techtron 635 model UV-VIS spectrophotometer and an Amel model 461 polarograph were employed.

Preparation of Aqueous Cu(I) Solutions

25 ml of 6.7×10^{-2} *M* pyridine solutions containing 5×10^{-3} *M* Cu(II) were brought to pH 5.2 with H_2SO_4 and were deaerated with high purity nitrogen. Then 2 g of Cu wire, titre 99.9%, were added and the system was vigorously stirred until equilibrium between Cu(II), Cu(I) and the metallic copper was reached. After 24 hours, according to polarographic measurements, the concentrations of Cu(I) and Cu(II) were $\sim 10^{-2}$ and $<10^{-5}$ *M* respectively.

Results and Discussion

The UV spectra of Cu(I) and Cu(II) pyridine complexes in the region 200–240 nm, where the pyridine shows an absorbance minimum, are reported in Fig. 1. In particular, absorption measurements carried out at different Cu(I) and Cu(II) concentrations in the presence of 3.2×10^{-3} *M* free pyridine (Py), gave an average value of $\epsilon_{Cu(I)} - \epsilon_{Cu(II)} = 1.95 \times 10^{+3}$ M^{-1} cm^{-1} at 218 nm. As a consequence, when Cu(I) was oxidized by molecular oxygen in pyridine solution,

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TABLE I. Cu(I) Oxidation by Molecular Oxygen: Order with Respect to Cu(I). The Experiments were performed in Aqueous Pyridine Solutions, pH 5.2, in the Presence of 5.7 × 10^{-4} M O_2.

Pyridine $M \times 10^3$	Cu(I) $M \times 10^5$	order with respect to Cu(I)	overall kinetic rate constant		*k_1 M^{-1} s^{-1}	*$k_3/k_2 \times 10^{-5}$
			k (M^{-1} s^{-1})	k × 10^{-7} (M^{-2} s^{-1})		
1.2	9.5	1	322	–	161	0.86
1.2	0.6	2	–	2.76		
1.8	9.7	1	268	–	134	1.27
1.8	0.8	2	–	3.40		
2.3	9.3	1	236	–	118	1.52
2.3	0.7	2	–	3.60		
5.9	9.2	1	116	–	58	3.14
5.9	0.5	2	–	3.64		

*Calculated according to eqn. 1.

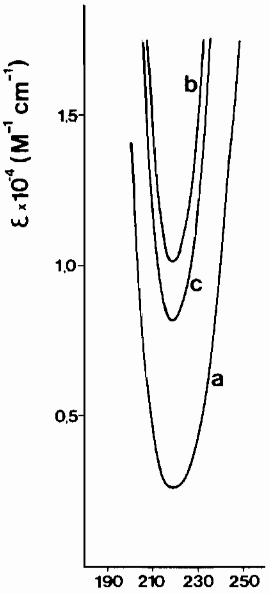

Fig. 1. UV spectra of Cu(I) and of Cu(II) in aqueous pyridine solution. a) 3.2 × 10^{-3} M pyridine, pH 5.2; b) a plus 5 × 10^{-4} M Cu(I); c) b after oxidation by molecular oxygen.

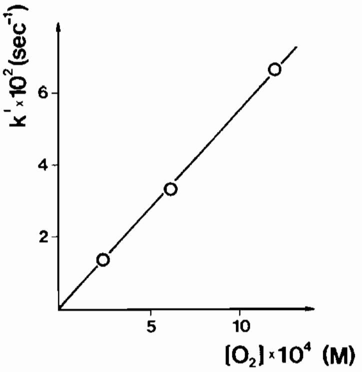

Fig. 2. Dependence of the kinetic rate constant of Cu(I) oxidation on the oxygen concentration. The measurements were carried out in 3.3 × 10^{-2} M free pyridine, pH = 5.2. The concentration of Cu(I) was 6 × 10^{-5} M.

the absorbance decreased and its time dependence was a function of O_2, Cu(I) and pyridine concentrations. In fact the oxidation rate of Cu(I) increased linearly with the O_2 concentration, see Fig. 2, showing that the reaction is first-order with respect to this reagent. The order with respect to Cu(I) was found to depend on the concentration of the Cu(I) itself. In particular the order with respect to Cu(I) increased from 1 to 2 lowering the concentration of cuprous ion from 10^{-4} to 10^{-6} M, as shown in Table I, where the reaction orders and the corresponding overall kinetic rate constants are reported for different experimental conditions. Furthermore, from this table the influence of Py concentration on the kinetic rate constants is indicated clearly. The overall second-order kinetic rate constants were then accurately measured as a function of the pyridine concentration and the results obtained are reported in Table II. This table shows that as the concentration of Py increases the kinetic rate constants decrease monotonically, while at constant concentration of free pyridine the rate of Cu(I) oxidation is independent of H^+ concentration in the pH range 5.0–6.3.

Since H_2O_2 is a reaction product of Cu(I) oxidation by molecular oxygen, the influence of H_2O_2 on the Cu(I) oxidation rate was investigated and the results are summarized in Table III. From this table it appears that the addition of 10^{-7} M catalase, which dismutes H_2O_2 very efficiently into H_2O and O_2, k = 3.2 × 10^7 M^{-1} s^{-1} [7], has little effect on the oxidation rate constants of Cu(I) by molecular oxygen. Moreover the addition of H_2O_2 to the Cu(I)–O_2 system increased the oxidation rate by less than 10%

TABLE II. Cu(I) Oxidation by Molecular Oxygen: Dependence of the Overall Second Order Kinetic Rate Constant on the Concentration of Free Pyridine. The Measurements were carried out in Aqueous Pyridine, containing 6 × 10^{-5} *M* Cu(I) and 5.7 × 10^{-4} *M* O_2.

pH	Pyridine $M \times 10^3$	k $M^{-1}\ s^{-1}$
5.2	1.2	392
5.2	1.5	316
5.2	1.8	268
5.2	2.3	236
5.2	2.8	169
5.2	3.9	152
5.2	5.9	116
5.2	7.8	90
5.2	16.2	62
5.2	33.0	46
5.2	67.0	43
5.2	335.0	40
5.0	2.3	224
6.3	2.3	244

TABLE III. Influence of H_2O_2 on the Oxidation Rate of Cu(I) by Molecular Oxygen. The Experiments were carried out with 5.7 × 10^{-4} *M* O_2 and 9 × 10^{-5} *M* Cu(I); pH 5.2.

Pyridine $M \times 10^3$	H_2O_2 $M \times 10^5$	Catalase $M \times 10^7$	overall kinetic rate constant k $M^{-1}\ s^{-1}$
1.54	7.2	–	348
1.54	–	–	316
1.54	–	0.4	297
3.93	7.2	–	187
3.93	–	–	152
3.93	–	0.4	139
33.0	7.2	–	49
33.0	–	–	46
33.0	–	0.4	46

when $|H_2O_2| \lesssim |Cu(I)|$. These results led us to neglect the small contribution of the H_2O_2 generated in the autoxidation of Cu(I) to the rate of Cu(I) oxidation. We must point out that the presence of 10^{-6} *M* superoxide dismutase, which catalyses the dismutation of O_2^- into H_2O_2 and O_2, k = 2.3 × 10^9 M^{-1} s^{-1} [8], had no effect on the Cu(I) oxidation rate. This result appears to exclude an oxidation path involving the generation of superoxide ion such as:

$$Cu(I) + O_2 \longrightarrow Cu(II) + O_2^-$$

$$Cu(I) + O_2^- \xrightarrow{2H^+} Cu(II) + H_2O_2$$

Furthermore the dependence of the reaction order on Cu(I) concentration supports strongly a reaction mechanism of the type:

$$Cu(I) + O_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Cu(I){-}O_2 \xrightarrow[Cu(I),\ H^+]{k_3} 2Cu(II) + H_2O_2$$

In fact introducing the steady-state approximation for the Cu(I)–O_2 complex were obtained:

$$\frac{d|Cu(II)|}{dt} = \frac{2k_1k_3|Cu(I)|^2|O_2|}{k_2 + k_3|Cu(I)|} \qquad (1)$$

According to this equation the order with respect to Cu(I) is one for $k_3|Cu(I)| \gg k_2$ and two for $k_3|Cu(I)| \ll k_2$.

Utilising eqn. 1 we have calculated, from the experimental data of Table I, the values of k_1 and the ratio k_3/k_2. The results obtained at different pyridine concentrations are reported in the last two columns of Table I. These results indicate the formation of a complex Cu(I)–O_2 which at $|Cu(I)| < 10^{-5}$ *M* is in equilibrium with the Cu(I) and O_2, while at $|Cu(I)| > 10^{-4}$ the formation of the complex appears to be the rate determining step of the oxidation process.

The dependence of the Cu(I) oxidation rate on Py concentration, see Table II, suggests a different O_2 reactivity towards the various Cu(I)–Py complexes, whose overall formation constants are:

$$\beta_{Cu(I)-Py} = 10^{4.60}, \beta_{Cu(I)-Py_2} = 10^{7.34} \text{ and } \beta_{Cu(I)-Py_3} = 10^{8.63} \ [6].$$

The concentration of these complexes was correlated with the overall second order kinetic rate constants reported in Table II to obtain the kinetic rate constants for the autoxidation of the different Cu(I) species present in aqueous pyridine: Cu(I)–aquo = (1.7 ± 0.15) × 10^4, Cu(I)–Py = 284 ± 32, Cu(I)–Py_2 = 38 ± 2 and Cu(I)–Py_3 = 26 ± 7 M^{-1} s^{-1}. These kinetic rate constants, which are in agreement with the values reported by Zuberbühler [6], correspond to twice the k_1 values of eqn. 1.

In conclusion the kinetic data we have obtained on the autoxidation of Cu(I) in aqueous pyridine, showing the dependence of the reaction order on the Cu(I) concentration, provide strong experimental evidence of the formation of the Cu(I)–O_2 complex as a reaction intermediate. Finally the indication that at least two Cu(I) ions are involved in the fast reduction of one molecule of oxygen appears consistent with the presence of more than one copper ion in copper oxidases [1].

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