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SOD-LIKE ACTIVITY STUDIES OF CYTOKININ-COPPER(II) COMPLEXES

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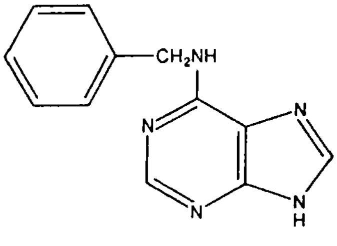
Using the pulse radiolysis technique it was shown that copper(II) complexes of kinetin and 6-benzylaminopurine (6-BAP) catalyze O_2^- dismutation very efficiently at physiological pH. The 'turnover' rate constants at pH 7 were determined to be $(1.5 \pm 0.3) \times 10^9$ and $(2.2 \pm 0.4) \times 10^9 M^{-1} s^{-1}$ for 6-BAP and kinetin, respectively. The system was studied at pH 3-10 in the case of 6-BAP, and the results show that this complex catalyzes also HO_2 dismutation efficiently.

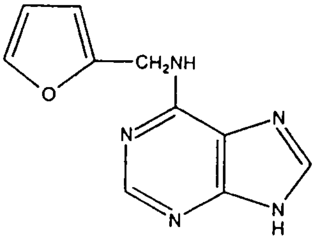
KEY WORDS: O_2^-, SOD, SOD-mimic, Cu(II)-complexes, dismutation, pulse radiolysis.

INTRODUCTION

Cytokinins (CK), a group of plant growth regulators, can efficiently retard senescence-associated parameters, where oxygen free radicals seem to play an important role.[1] CK can act as a free radical scavenger, and indeed it was shown to react directly with O_2^- in aprotic solvent by extraction of hydrogen from the α-carbon amine bond.[2] Since CK does not react fast with O_2^- in aqueous solution, it has been recently suggested that CK binds copper ions present in the chloroplast, and that this complex catalyzes O_2^- dismutation in aqueous solutions.[3] It has been shown that 6-benzylaminopurine (6-BAP) and kinetin form stable divalent complexes with copper(II) in aqueous solutions, and that both complexes remove O_2^- by superoxide dismutase (SOD) mimetic activity. The reaction rate constants with O_2^-, which were determined by polarography at pH 9.8, were $2.3 \times 10^7 M^{-1}s^{-1}$ for kinetin and $1.5 \times 10^7 M^{-1}s^{-1}$ for 6-BAP.[3]



6-Benzylaminopurine



Kinetin

The CK-Copper(II) complexes may be more reactive towards superoxide at pH 7 than at pH 9.8, and in this communication the SOD activity of 6-BAP and kinetin complexes of copper(II) was studied at pH 3–10 with the use of the pulse radiolysis technique.

MATERIALS AND METHODS

All chemicals were of analytical grade and were used as received: 6-BAP, kinetin (Sigma Chemical Co.), sodium formate, sodium phosphate, $HClO_4$, NaOH and copper sulfate (Merck). All solutions were prepared with distilled water that had passed through a Millipore ultrapurification system. The pH of the solution was adjusted with either NaOH, $HClO_4$ or phosphate buffer and it was determined with a pH meter, Model 240 (Corning). The CK:Cu(II) complexes were prepared by mixing 10 μM of the ligand with 0.1–4 μM copper sulfate. Since $\log K_1$ and $\log \beta_2$ found for kinetin are 7.8 and 15.3 and those for 6-BAP 8.3 and 15.9, respectively,[3] the only species present in the solutions is $(CK)_2Cu(II)$.

Pulse radiolysis experiments were carried out with a Varian 7715 linear accelerator with a 200 mA curent of 5 MeV electrons. Irradiations were done in a 4 cm spectrosil cell with three light passes. A 200 W Xe–Hg lamp produced the analyzing light. The detection system included a Bausch & Lomb Photomultiplier. The signal was transferred through a SONY/TEXTRONIX 390AD programmable digitizer to a micro PDP-11/24 computer, which operated the whole pulse radiolysis system. Dosimetry was carried out with KSCN dosimeter. The yield of $(SCN)_2^-$ was measured with $\varepsilon_{475} = 7600\,M^{-1}cm^{-1}$ and $G = 6.0$, where the G-value represents the number of molecules formed per 100 eV of energy absorbed by the solution. All experiments were carried out at room temperature.

On pulsing air-saturated solutions containing 50 mM formate, the following reactions take place:

$$H_2O \rightarrow e_{aq}^-, OH^\cdot, H^\cdot, H_2O_2, H_2, H_3O^+$$

$$e_{aq}^- + O_2 \rightarrow O_2^-$$

$$OH^\cdot + HCO_2^- \rightarrow H_2O + CO_2^-$$

$$CO_2^- + O_2 \rightarrow CO_2 + O_2^-$$

$$H^\cdot + O_2 \rightarrow HO_2$$

$$HO_2 \rightleftharpoons H^+ + O_2^- \qquad pK_a = 4.8^4$$

Because of the high rate constants of these reactions, all the primary radicals are converted into O_2^- within the end of the pulse. The initial concentrations of O_2^- thus generated was about 15 μM under the experimental conditions, and the decay of its absorbance was followed at 240–265 nm.

RESULTS

The O_2^- decay was first order under all experimental conditions due to catalytic impurities present in the solutions. When 1 μM EDTA was added, the decay of the radical obeyed a second order rate law, and the half life of O_2^- increased. We were unable to use EDTA in our experiments since it will sequester the copper out of the

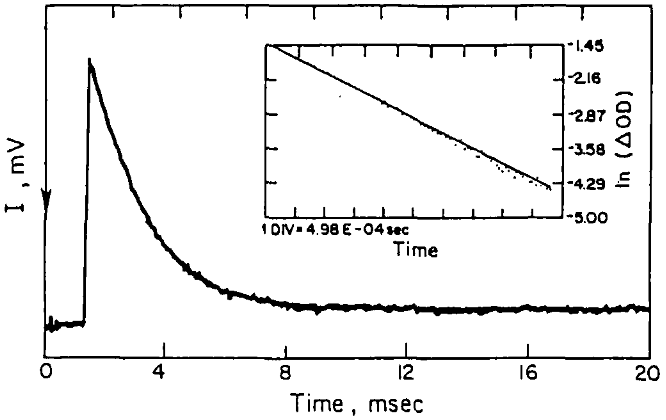


FIGURE 1 Typical kinetic plot of a pulse radiolysed air-saturated solution containing 0.4 μM $(6\text{-BAP})_2$-Cu(II) and 50 mM formate at pH 7. The optical pathlength was 12.1 cm, λ = 240 nm, I_0 = −434 mV, ΔI = 192 mV.

CK-Cu(II) complexes. No acceleration of O_2^- decay was observed in the presence of 10 μM 6-BAP or kinetin alone. When catalytic concentrations of copper(II) were added ($[Cu(II)]_0 < [O_2^-]_0$, [CK]), the decay of O_2^- followed a pseudo-first-order reaction. A typical kinetic trace is given in Figure 1. The observed rate constant depended linearly on the initial concentrations of copper(II) (Figure 2), and from the slope of such lines the second order rate constant, k_{cat}, was determined at the various pH studied (Table I).

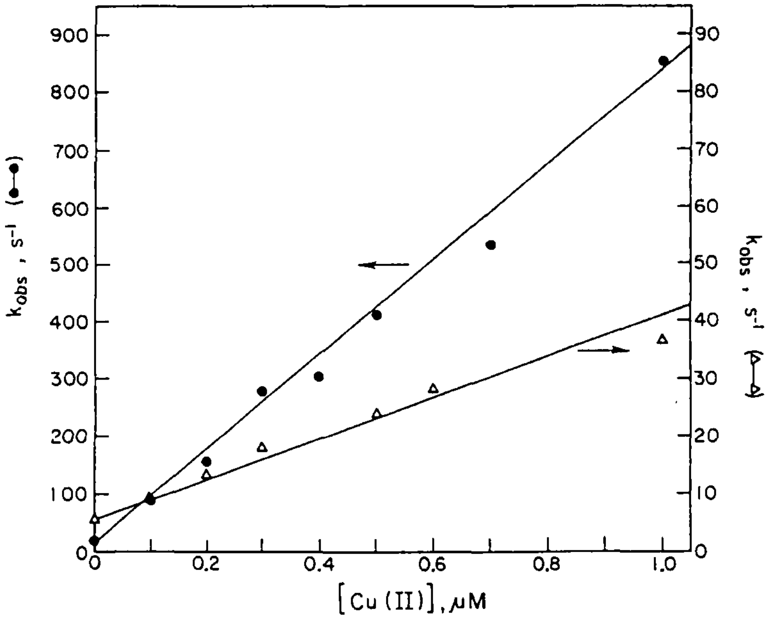


FIGURE 2 The osbserved rate constant of the decay of O_2^- at 240 nm as a function of the initial concentration of cupric ions at a constant concentration of 10 μM 6-BAP. The solutions were air-saturated and contained 50 mM formate at ● – pH 7.8; Δ – pH 10.

TABLE I
k_{cat} of $(CK)_2Cu(II)$ complexes at various conditions

CK	pH	[Cu(II)] μM	$k_{cat} \times 10^{-9}$ $M^{-1}s^{-1}$
6-BAP	3.1	0.4–4.0	0.37
6-BAP	4.1	0.4–2.0	1.22
6-BAP	5.2	0.4–1.2	1.50
6-BAP	6.1	0.2–4.0	1.30
6-BAP	7.0	0.2–2.4	1.60
6-BAP	7.8	0.2–2.0	0.85
6-BAP	10.0	0.1–0.6	0.03
Kinetin	7.0	0.4–1.6	2.20

Solutions were air saturated and contained 50 mM formate. The initial concentration of O_2^- was about 15 μM.

DISCUSSION

We have found that both $(6\text{-BAP})_2Cu(II)$ and $(\text{kinetin})_2Cu(II)$ catalyze O_2^- dismutation at pH 7 with similar efficiency to that of bovine SOD.[5] We assume that both complexes catalyze O_2^- dismutation via the same mechanism excepted for Cu,Zn-SOD as well as for many other copper complexes which is the so-called the 'ping-pong' mechanism:[5-8]

$$Cu(II) + O_2^- \rightarrow Cu(I) + O_2 \quad (1)$$

$$Cu(I) + O_2^- + 2H^+ \rightarrow Cu(II) + H_2O_2 \quad (2)$$

When $[Cu(II)]_0 \ll [O_2^-]_0$, equation (3) is obtained

$$-d[O_2^-]/dt = k_{cat}[Cu(II)]_0[O_2^-] \quad (3)$$

where

$$k_{cat} = 2k_1k_2/(k_1 + k_2) \quad (4)$$

We were unable to determine k_1 directly when $[Cu(II)]_0 \gg [O_2^-]_0$ since the CK:Cu(II) complexes have high absorbance in the U.V. region where O_2^- absorbs.

The catalytic activity of $(6\text{-BAP})_2Cu(II)$ decreased at pH < 4 and at pH > 7 (Table I). At pH 10 it reached almost the same value obtained with the polarographic method.[3] The decrease at pH < 4 cannot be attributed to the inability of the complex to catalyze HO_2 dismutation since pK_a of the radical is 4.8.[5] The decrease in k_{cat} may be due to the formation of another inactive form of the ligand that has low SOD activity as the dissociation constants of the ligand were determined to be 3.9 and 9.84.[3] This behavior is similar to that obtained for the native enzyme,[9] except that for the native enzyme reversible transitions involving Cu(II) occur at pH 3–5, while at pH < 3 further transitions cause inactivation of the enzyme.[10]

As O_2^- is more stable and reactive at a lipophilic site than in the cytosol, where the native SOD is less effective, the cytokinins complexes of copper(II), which are lipophilic, may play an important role. Thus, since the chloroplast is abundant in copper, if these complexes are formed, and hence have SOD activity, they may participate in senescence retardation by catalyzing O_2^- dismutation.

Acknowledgements

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