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Oxidation of 5-Hydroxy-6-methyluracil with Molecular Oxygen in the Presence of Copper(II) Chloride in Aqueous Solution

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Abstract—Oxidation of 5-hydroxy-6-methyluracil with molecular oxygen in the presence of copper(II) chloride involves formation of a 2 : 1 complex with copper(II). The rates of consumption of initial 5-hydroxy-6-methyluracil and oxygen were determined in the temperature range from 40 to 80°C. A probable reaction mechanism implies fixation and activation of molecular oxygen on the copper(II) complex with 5-hydroxy-6-methyluracil with formation of active oxygen species that are responsible for hydroxylation of the double C^5=C^6 bond in the uracil molecule.

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Interest in oxidation of pyrimidine bases continuously increases due to the necessity of understanding the nature of DNA degradation and repair and of synthesizing oxidative stress markers. Potassium permanganate [1], sodium peroxosulfate [2], hydrogen peroxide [3], ozone [4], and hydroxyl radicals [5] were studied as oxidants. As a rule, the oxidation requires severe conditions and gives rise to a large number of products and poor yields. On the other hand, researchers' attention is given to simulation of enzymatic systems capable of hydroxylating organic compounds under mild conditions with exceptional selectivity at high rates. Molecules of uracil derivatives possess several donor centers which can be involved in coordination to transition metal ions. Uracil derivatives can participate in model enzymatic systems as both ligands and substrates. Al-Arab and Hamilton [6] studied oxidation of some diaminouracils in the presence of copper(II) salts in aqueous solution as a model process simulating oxidation with copper-containing amine oxidases. We previously showed [7], that oxidation of 5-hydroxy-6-methyluracil with molecular oxygen in the presence of copper(II) salts yields 5,5,6-trihydroxy-6-methylhexahydropyrimidine-2,4-dione, and a probable reaction mechanism was proposed [8].

In the present work we studied the kinetics of oxidation of 5-hydroxy-6-methyluracil with molecular oxygen in the presence of copper(II) chloride in aqueous solution. The process is accompanied by consumption of the initial uracil and oxygen and formation of 5,5,6-trihydroxy-6-methylhexahydropyrimidine-2,4-dione as the only product which was characterized by X-ray diffraction and NMR data [7].

Continuous bubbling of oxygen through the reaction mixture accelerated the reaction. No oxidation of 5-hydroxy-6-methyluracil occurred under nitrogen. Copper(II) ion does not act as oxidant, for we failed to detect copper(I) ion in the reaction mixture. Addition of ethylenediaminetetraacetic acid disodium salt [which is known to form a strong complex with Cu(II)] prevents oxidation of 5-hydroxy-6-methyluracil. Taking the above data into account, we presumed that the catalytic effect of Cu(II) on the oxidation of 5-hydroxy-6-methyluracil with molecular oxygen originates from formation of a complex of 5-hydroxy-6-methyluracil with copper(II) chloride.

The composition of the Cu(II)–substrate complex (1 : 2) was determined by spectrophotometry from the dependence of optical density upon [L]/[Cu(II)] concentration ratio the molar ratio (Fig. 1). Donor centers in the ligand molecule involved in coordination to the metal ion were identified by ^{13}C NMR spectroscopy (Table 1). The largest variations in the chemical shifts were observed for the C^4 and C^5 atoms, which suggests copper(II) coordination at the oxygen

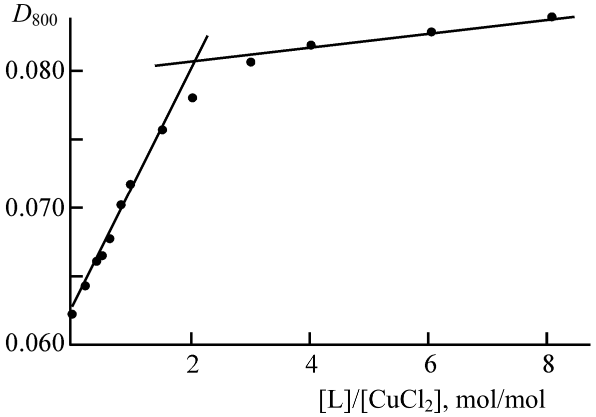


Fig. 1. Dependence of the optical density of solutions vs. concentration ratio of 5-hydroxy-6-methyluracil and copper(II) chloride.

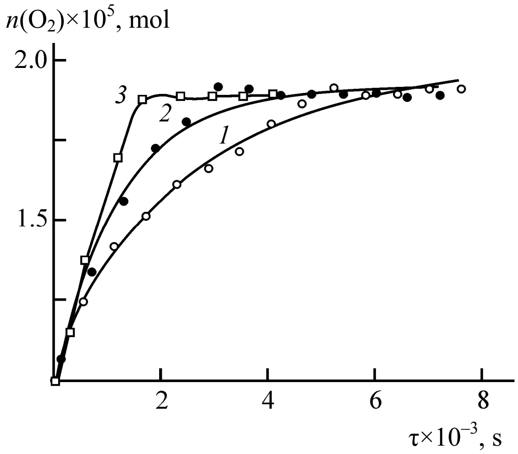


Fig. 2. Kinetic curves for oxygen absorption in the oxidation of 5-hydroxy-6-methyluracil in the presence of copper(II) chloride (60°C): (*1*) $[L]_0 = 8\times10^{-3}$ M, $[CuCl_2]_0 = 4\times10^{-3}$ M, (*2*) $[L]_0 = 6.67\times10^{-3}$ M, $[CuCl_2]_0 = 6.67\times10^{-3}$ M, (*3*) $[L]_0 = 6.67\times10^{-3}$ M, $[CuCl_2]_0 = 13.3\times10^{-3}$ M.

atoms in the carbonyl (C^4=O) and hydroxy groups. Insofar as the copper(II) ion in the complex has octahedral configuration (λ_{max} 800 nm), its coordination sphere is likely to include two water molecules in addition to two 5-hydroxy-6-methyluracil (L) molecules.

$$2L + [Cu(H_2O)_6]^{2+} \rightleftarrows [Cu(L)_2(H_2O)_2]^{2+} + 4H_2O.$$
$$\mathbf{I}$$

The kinetic curves for consumption of 5-hydroxy-6-methyluracil in the temperature range from 40 to 80° C are satisfactory linearized by first-order kinetic equation. The first-order rate constants were calculated from the semilog kinetic plots, $k_{ap}\times10^4$ (s^{-1}) = 0.28±0.03, 0.89±0.05, 3.7±0.2, 13±2, and 28±4 at 40, 50, 60, 70, and 80°C, respectively ($[L]_0 = 7\times10^{-4}$ M, $[CuCl_2]_0 = 3.5\times10^{-4}$ M). The activation parameters were calculated from the temperature dependence of the apparent rate constant.

$$\log k_{ap} = (14\pm2) - (26\pm3\ \text{kcal mol}^{-1})/\theta,$$
$$\theta = 2.3RT\ (r^2 = 0.997).$$

The oxidation of 5-hydroxy-6-methyluracil in aqueous solution in the presence of copper(II) chloride was accompanied by absorption of oxygen. Typical kinetic curves for oxygen absorption are shown in Fig. 2. The rate of consumption of 5-hydroxy-6-methyluracil is almost equal to the rate of oxygen absorption, which implies their joint participation in the process at the rate-determining stage. The moment of termination of oxygen absorption corresponds to the complete conversion of 5-hydroxy-6-methyluracil. Table 2 contains the amounts of absorbed oxygen at different temperatures. Analogous relations were observed in the oxidation of diaminouracils [6].

Increase in the concentration of copper(II) chloride leads to expected acceleration of oxygen consumption,

Table 1. Carbon chemical shifts (δ_C, ppm) in the ^{13}C NMR spectra of 5-hydroxy-6-methyluracil and its complex with $CuCl_2$ (DMSO-d_6)

Atom no.	Ligand	2L·CuCl$_2$	$\lvert\Delta_C\rvert$
2	149.79	148.49	1.30
4	161.18	159.65	1.53
5	128.18	126.65	1.53
6	131.95	130.59	1.36
7	12.80	11.40	1.40

Table 2. Temperature dependence of the amount of consumed oxygen in the oxidation of 5-hydroxy-6-methyluracil in the presence of $CuCl_2$ ($[L]_0 = 8\times10^{-3}$ M, $[CuCl_2]_0 = 4\times10^{-3}$ M)

T, K	$n(O_2)\times10^5$, mol	$n(L)\times10^5$, mol	$n(O_2)/n(L)$
323	1.8±0.3	4	0.45±0.07
333	1.9±0.2	4	0.47±0.06
343	1.9±0.3	4	0.48±0.08
353	2.0±0.3	4	0.49±0.06

Table 3. Dependences of the amount and initial rate of oxygen absorption in the oxidation of 5-hydroxy-6-methyluracil upon concentration of $CuCl_2$ (60°C)

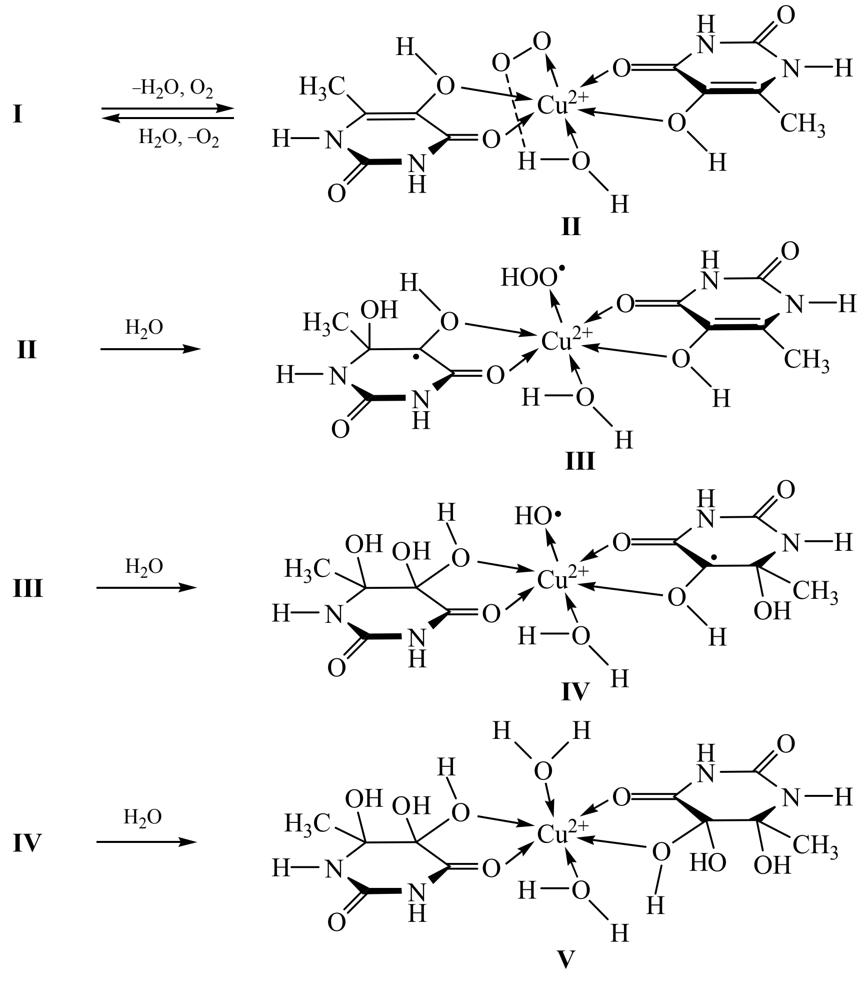
$[CuCl_2]_0 \times 10^3$, M	$[L]_0 \times 10^3$, M	$[L]_0/[CuCl_2]$	$n(O_2) \times 10^5$, mol	$n(O_2)/n(L)$	$W_0 \times 10^6$, mol l^{-1} s^{-1}
3.34	6.67	2	1.9±0.2	0.47±0.06	1.6±0.4
6.67	6.67	1	1.7±0.2	0.43±0.05	2.4±0.5
13.3	6.67	0.5	1.8±0.4	0.45±0.09	2.5±0.5

whereas the amount of absorbed oxygen remains almost unchanged (Table 3). The oxidation of 1 mol of the initial uracil requires approximately 0.5 mol of oxygen. Presumably, the complex of Cu(II) with uracil fixes and activates oxygen, and activated oxygen species oxidize 5-hydroxy-6-methyluracil. The proposed scheme rationalizes the data on the amount of absorbed oxygen and participation of water in the hydroxylation of the substrate.

Thus we have determined the composition of the complex formed by copper(II) chloride with 5-hydroxy-6-methyluracil and donor centers in the latter involved in coordination and proposed a mechanism for the formatin of active oxygen species.

EXPERIMENTAL

The electronic absorption spectra were recorded in the λ range from 200 to 800 nm on a Specord M40 spectrophotometer using quartz cells with cell path lengths of 0.2 and 5 cm. Doubly distilled water was used as solvent.



Complex formation of 5-hydroxy-6-methyluracil with copper(II) chloride. The composition of the copper(II) complex with 5-hydroxy-6-methyluracil was determined by spectrophotometry. For this purpose, a series of solutions containing 5-hydroxy-6-methyluracil and $CuCl_2$ at different ratios were prepared. To avoid oxidation of 5-hydroxy-6-methyluracil, the solutions were purged with argon over a period of 15 min and transferred into a hermetically capped cell (l = 5 cm) preliminarily purged with argon. The optical density was measured at λ 800 nm corresponding to the absorption maximum of $Cu(H_2O)_6^{2+}$.

The donor centers in the ligand molecule responsible for complex formation were determined by ^{13}C NMR. Solutions of $CuCl_2 \cdot 2H_2O$ (0.0310 g in 0.4 ml of DMSO-d_6) and 5-hydroxy-6-methyluracil (0.0515 g in 0.8 ml of DMSO-d_6) were purged with argon over a period of 15 min and transferred into NMR ampules. The ampules were sealed with a paraffin film, and ^{13}C NMR spectra were recorded.

Oxidation of 5-hydroxy-6-methyluracil with atmospheric oxygen in the presence of $CuCl_2$. A solution of copper(II) chloride was added to a solution of 5-hydroxy-6-methyluracil ($[L]_0$ 7×10^{-4}–8×10^{-3} M) to attain an L–$CuCl_2$ molar ratio of 2 : 1. The oxidation was carried out in a 100-ml two-necked reactor equipped with a reflux condenser and a magnetic stirrer in the temperature range from 40 to 80°C. The required temperature was maintained constant with the aid of an MLW 4 thermostat. Samples of the reaction mixture were withdrawn at definite time intervals, and the concentration of 5-hydroxy-6-methyluracil was determined by spectrophotometry at λ 278 nm (absorption maximum, $\varepsilon = 7800$ l mol^{-1} cm^{-1}).

Determination of the amount of absorbed oxygen. The amount of oxygen absorbed in the oxidation of 5-hydroxy-6-methyluracil in the presence of copper(II) chloride was determined using a UMD universal differential pressure gauge setup [9]. The reaction was performed in a two-necked reactor maintained at a constant temperature. A solution of $CuCl_2$ with a concentration of 2×10^{-2} to 4×10^{-2} M, 1–2 ml, was added using a syringe to a solution of 5-hydroxy-6-methyluracil in water ($[L]_0$ = 1×10^{-2} M), 4 ml, adjusted to a required temperature.

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