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FERRICYTOCHROME C BY O_2^{2-}

PROGRESS REPORT

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Department of Physical Chemistry
The Hebrew University of Jerusalem, Israel

Work carried out during the period:
December 1st 1977 to November 30th 1978

Prepared for the U.S. Energy Research & Development Administration
Under Contract No. EY-76-C 02-3221

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Do Copper Ions Influence the Reduction of Ferricytochrome C by O_2^- ?

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Summary

Recently, it was suggested that the measured rate of reduction of ferricytochrome c by O_2^- below pH=8, was too high in the presence of high concentrations of formate [Koppenol et al. (1976), Biochim. Biophys. Acta, 499, 157-168].

The high values were attributed to the presence of impurities of copper, which compete on O_2^- . This assumption is consistent with either a decrease in the reduction yield of CIII in the presence of copper, or with a very fast reaction of Cu(I) with CIII.

It was previously shown by us and by others that the reduction yield of CIII by O_2^- is 100%. We measured the rate of reduction of CIII by Cu(I), and found that this reaction is slow: $k=(1.5\pm0.5) \times 10^3 M^{-1} s^{-1}$.

Therefore, our results rule out the possibility that below pH=8 copper impurities affect the measured rate constant of the reduction of CIII by O_2^- .

The reduction of ferricytochrome C (CIII) by the superoxide radical (O_2^-):

was recently the subject of several investigations [1-4]. In three of these studies [1-3], a pH dependence of k_1 in the pH range 6.5-10 was observed. Koppenol et al. [4] attributed the pH dependence below pH=8 to a competition on O_2^- between cytochrome C (reaction (1)) and free or complexed Cu(II) (reaction (2)) and Cu(I) (reaction (3)).

It was suggested that copper is present as an impurity in formate and in cytochrome C [4]. The concentration of copper in a solution containing 100mM formate and 10 μ M CIII was found to be 50nM [4].

If one ignores reactions (2) and (3) at these concentrations of copper-formate complexes below pH=8, the measured value of k_1 will turn out to be too high. Above pH=8, copper-hydroxide complexes dominate. They are not reactive toward O_2^- , and therefore the presence of copper would not affect the kinetics of reaction (1) above pH=8.

Klug-Roth and Rabani [5] showed the catalytic effect of copper ions and copper complexes, including copper-formate complexes on the dismutation of O_2^- . They found that the apparent rate of reactions (2) and (3) in the presence of copper-formate complexes was proportional to the concentration of copper ions. The apparent second order rate constant was: $1.4 \times 10^9 M^{-1} s^{-1}$. Their experimental conditions were very similar to those employed in the studies of reaction (1) [1-3]. The fact that copper-formate complexes have a catalytic effect implies that k_3 cannot be much smaller than k_2 .

A significant competition of reactions (2) and (3) with reaction (1) would decrease the reduction yield of CIII simultaneously with its effect on the kinetics of reaction (1).

If reaction (4) is faster than reaction (3), the reduction yield of CIII would not be affected by the presence of the copper complexes:

The net effect will still be a catalysis of O_2^- dismutation, but the reduction yield of CIII will remain high ($\sim 100\%$). In this case, k_1 measured ignoring reactions (2) and (4) would be too high.

In order to check this possibility, we measured k_4 . Cu(I) was produced in situ using the pulse-radiolytic technique, in N_2O saturated solutions, containing $20\mu M$ CIII, $100mM$ HCOONa, $2mM$ $CuSO_4$ (pH=6.7). The details of the experimental methods were described before [1].

Under these conditions, all the primary radicals produced by the pulse are transformed into CO_2^- radicals. The CO_2^- radicals may react with either Cu(II), or CIII:

In order to calculate quantitatively the amount of CO_2^- radicals reacting with Cu(II) in competition with CIII, we determined k_5 .

We followed the decay of the absorbance of CO_2^- radicals at 260nm, in N_2O saturated solutions containing $50-200\mu M$ $CuSO_4$, $100mM$ HCOONa, and $2mM$ phosphate buffer (pH=6.8). We used low doses (~ 200 rad) in order to avoid competition with the second order decay of CO_2^- ($2k=9 \times 10^8 M^{-1} s^{-1}$ [6]). Under these conditions, the half-life of CO_2^- was proportional to the concentration of Cu(II), $k_5 = (1.5 \pm 0.3) \times 10^8 M^{-1} s^{-1}$.

In order to measure k_4 , we used solutions with $[Cu(II)] = 2mM$, and $[CIII] = 20\mu M$. Under these conditions, $k_5 \times [Cu(II)] = 3 \times 10^5 s^{-1}$, and $k_6 \times [CIII] = 2 \times 10^4 s^{-1}$ [1], thus about 94% of CO_2^- reduced Cu(II).

About 10 microsecond after the pulse, reaction (5) was completed, and then we followed reaction (4) at 550nm (the reduction of CIII). The results indicated a very slow reaction, which took several minutes to be finished.

We chose $[CIII]_0$ to be roughly equal to $[CO_2^-]_0$ being formed during the pulse. As CO_2^- almost quantitatively reduces Cu(II) to Cu(I), in our experiments, $[Cu(I)]_0 \sim [CIII]_0 = 20\mu M$.

Plots of $1/(A_t - A_\infty)$ versus time yielded straight lines, from which $k_5 = (1.5 \pm 0.5) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ was calculated. (A_t is the absorbance at time t . A_∞ is the absorbance after 500 seconds).

From these results, it is obvious that Cu(I) can not compete efficiently with CIII on O_2^- . Therefore, if Cu(II) competes with CIII on O_2^- , a decrease in reduction yield is expected, in contradiction to the experimental results.

We conclude that copper ions do not influence the kinetics of the reduction of ferricytochrome C by O_2^- . Therefore, the observed pH dependence of the rate of reduction of CIII by O_2^- below pH=8, is real and not an artifact arising from the contamination of the solutions by copper ions.

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