Kinetics of the Decomposition of the Cupric Ion Sulfhydryl Bond in Beef Plasma Albumin

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KINETICS OF THE DECOMPOSITION OF THE CUPRIC ION - SULPHHYDRYL BOND IN BEEF PLASMA ALBUMIN

Presented to
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Ouachita Baptist University

In Partial Fulfillment
of the Requirements of
Honors Special Studies H491

by
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PURPOSE: The purpose of this work was to study the kinetics of the binding of cupric ion to beef plasma albumin, in particular the decomposition of the Cu$^{++}$-BPA complex which absorbs light at 375 millimicrons.

THEORY: The work of Klotz, Urquhart, Klotz, and Ayers has shown that the characteristic absorption band at 375 mu produced by the binding of cupric ion to beef plasma albumin is due to a copper-sulfhydryl bond. They have further demonstrated that the disappearance of this absorption band with time is accompanied by irreversible changes in the structure of the protein. The intensity of the initial absorption was shown to increase with increasing pH up to about pH 8, where it appeared to decrease again, possibly because of the increased rate of decomposition of the complex at high pH.

PROCEDURE: The solutions to be used were all prepared in .20M acetate buffer. The BPA solutions were made up as $6 \times 10^{-4}$ M, and the copper solutions as $6 \times 10^{-3}$ M. Some experiments were carried out with more dilute solutions, but were generally unsatisfactory. The prepared reagents were mixed 1:1 by pumping them individually through a polystaltic pump. Mixing was carried out in a tee joint, and the mixture run into a flow cell in position to be

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read. All absorbance readings were made with a Beckman model DU
spectrophotometer. Readings were taken for up to $4\frac{1}{2}$ hours after
mixing. The data will be found in the notebook accompanying
this report.

**CALCULATIONS:** A reaction is shown to be first order if a plot of $\log(B)$
against time is linear, where $B$ is any quantity proportional to
the concentration of the reactant in question. The reaction is
shown to be second order if a plot of $1/B$ against time is linear.
If it is assumed that the absorbance of a protein-copper solution
at 375 m$\lambda$ is proportional to the concentration of the copper-
sulphhydryl bonded complex, it should be possible to follow the
disappearance of that complex with time by taking the absorbance
of the solution periodically. The kinetics of the initial
formation of the complex should also be susceptible to this
technique, but the competing reaction, the very small changes in
absorption, and the rapidity of the reaction cause great difficulty
with the procedure followed here. Several plots of $1/A$ and $\log A$
are shown on plates I - III. All plots show some initial
nonlinearity, probably because some of the absorbing specie was
still being formed. The break on the plots of $R - 15$ was
caused by precipitation of the complex; the solution was exposed
to the atmosphere on this particular run, and a cloudy precipitate
was formed in the top of the cell and subsequently settled to the
bottom, as opposed to the precipitations encountered in some of
the earlier runs, which were more or less homogenous precipitations.
In plate IV, it was assumed that the product formed by the decomposition of the complex also absorbed at 375 μm, and that its absorbivity was approximately 1/3 that of the reactant, or $A_r = A - \frac{1}{3}A_0$, where $A_r$ is the absorbance of the reactant. The plots obtained are generally similar to the plots obtained by using the total absorbance $A$ of the solution. It can be seen that all plots could conceivably be called linear, with the possible exception of those on plate III, which are too scattered to be really informative.

**CONCLUSION:** No attempt will be made here to assign the order of the reaction by the graphs obtained. It would seem that the reaction did not proceed nearly to completion in any of the runs carried out. Thus, the seemingly contradictory linearity of all types of plots would result from the linear appearance of a small portion of a curved line. Further investigation of the reaction over longer periods of time or at higher reaction rates appear to be necessary before the order of the reaction can be determined.
R - 15 and R - 16: plots of
1/A vs. time

R - 15 and R - 16: plots of
(1 + logA) vs. time

Time, minutes
PLATE II

R - 17: plot of $1/A$ vs. time

R - 17: plot of $(1 + \log A)$ vs. time

10 Millimeters to the Centimeter
R = 18: plot of $1/A$ vs. time

R = 18: plot of $(1 + \log A)$ vs. time
R - 17: plot of $1/(A - \frac{1}{3}A_0)$ vs. time

$1 + \log(A - \frac{1}{3}A_0)$ vs. time