COPPER ION ENHANCEMENT OF THE CHEMILUMINESCENCE (CL) INTENSITY PHOTOINDUCIBLE IN AN AQUEOUS RIBOFLAVIN-HYDROGEN PEROXIDE (HOOH) SOLUTION*

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Metals and metal ions, particularly those of the transition series, are known to mediate and/or catalyze photochemical reactions. Uri (1952), and Walling (1957) discuss a few of these reactions, and Basolo and Pearson(1960) consider the charge-transfer aspects of the phenomena. While the ultraviolet decomposition of HOOH has been studied extensively for many years (Schumb et al,1955) its decomposition by visible light as sensitized by metal ions has not been widely observed or recognized. Kistiakowsky's early observation (1900) that the decomposition of HOOH, weakly catalyzed by a ferrocyanide-ferricynanide mixture, was enhanced markedly by light remains still as a rather isolated example.

In a recent paper Steele (1963 a) described a CL obtained from a buffered riboflavin-HOOH solution induced by visible light. Williams and Steele (1965) showed subsequently that this reaction was mediated in the primary photochemical phase by the senitized decomposition of HOOH to produce free radicals. In experiments comparing the kinetic aspects of the CL emission photoinduced from the riboflavin-HOOH system with the kinetics of the CL induced in a riboflavin-HOOH system by the addition of ascorbic acid and copper, one of us (JEV) discovered a marked enhancement of the CL intensity from the former system when cupric sulfate was added prior to photoinduction. The effect appears to be specific for copper, as iron, cobalt, nickel, manganese, and zinc are ineffective. Since the results are suggestive of a copper-flavin interaction,

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a rather rare occurrence (uricase, and possibly a few plant reductases, being copper-flavin enzymes), we feel that our findings may be of general interest. Of equal interest is the related problem of the mechanism and energetics of HOOH (O_2)-activation and hydroxylation. A detailed kinetic analysis of the CL photoinduced from the riboflavin-HOOH-Cu^{++} system will be presented elsewhere.

EXPERIMENTAL. Riboflavin and HOOH solutions were prepared and assayed as described previously (Steele 1963 a). Copper and iron were used as the sulfate. Histidine (Nutritional Biochemical Corp.)was used as the free base. Water was prepared by the glass distillation of water deionized previously on ion-exchange resins to an approximate level of 0-15 ppm. Phosphate buffer was freed of contaminating copper and iron with Bio-Rads Chelex 100 (Bio-Rad Laboratories: 32nd & Griffin, Richmond, Calif.). Copper and iron were determined by a chelation technique using the reagents and methods provided and described by Diehl and Smith (1958). CL was photoinduced and measured as described previously (Steele 1963 a). Chemiluminescent intensities at the transient steady state maxima are the ordinate values recorded on the figures. The kinetic features of the data were determined as described by Steele et al. (1963 b).

RESULTS. Representative traces of the photoinduced chemiluminescence intensities, plotted logarithmically, versus time for the riboflavin-HOOH system, with and without copper present, and with copper plus histidine (see below), are presented in Fig. 1. Semilog plots of the data reveal the series first order kinetic features of the data. The kinetic features of the system may be described by the following kinetic equation:

$$\text{riboflavin} + \text{HOOH} \xrightarrow[\text{hv} \pm \text{cu++}]{k_0} A \xrightarrow{k_1} B \xrightarrow{k_2} C + \text{chemiluminescence}$$

where k_0 is a zero order constant typical of photochemical reactions, and k_1 and k_2 are series first order rate constants. It is evident from the figure that all of these constants increase with increasing copper, and we have

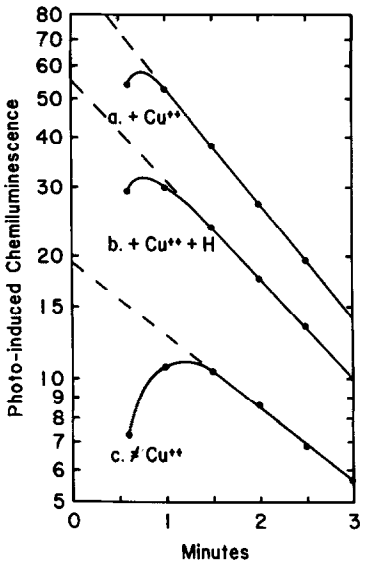

Fig.1. Photoinduced chemiluminescence vs time for a riboflavin-HOOH system. All systems: riboflavin, 0.45 μmoles; HOOH 5 mmoles; PO_4-buffer 1.5 mmoles, pH 6.2. a) 0.09 μmoles Cu++; b) 0.09 μmoles Cu++ + 3.75 μmoles histidine; c) ≠ Cu++ & ≠ histidine. Volume, 15 ml. Temp.24°C. Illumination time 30 seconds.

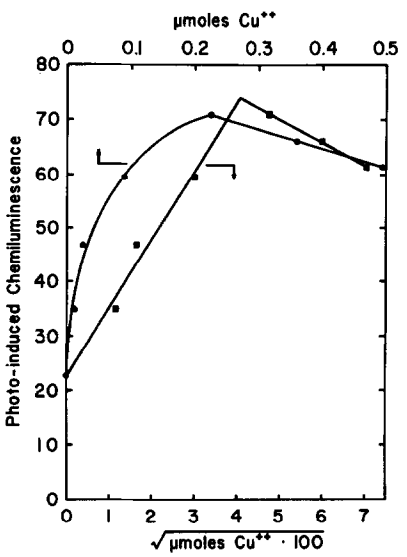

Fig.2. Photoinduced chemiluminescence (CL) vs copper concentration for a riboflavin-HOOH system. Upper curve: CL plotted vs linear concentration of Cu++; lower curve, CL plotted vs square root of Cu++. Riboflavin, 0.45 μmoles; HOOH, 5mmoles; PO_4-buffer, 1.5 mmoles, pH,6.1 Volume, 15 ml. Temp.24°C. Illumination time, 30 seconds.

found k_1 and k_2 to increase linearly.

In Fig. 2 we have plotted the maximum steady state chemiluminescent intensity versus the copper concentration expressed linearly and as the square root. The maximum intensity occurs at a copper concentration of 0.225 μm (upper curve) which is equal to one-half the riboflavin concentration present (0.45 μm), and suggests that a functional stoichiometry is important in the mechanism. The photoinducibility of the CL in the absence of copper should be noted for it emphasizes the fact that the copper effect is one of enhancement and not an obliquitory requirement. This fact is demonstrated kinetically more decisively in Figs. 3 and 4 where it can be seen that the photoinducible CL versus riboflavin concentration varies as a linear function of concentration when copper is present (Fig. 3), but as the square root of the concentration when copper is absent (Fig. 4).

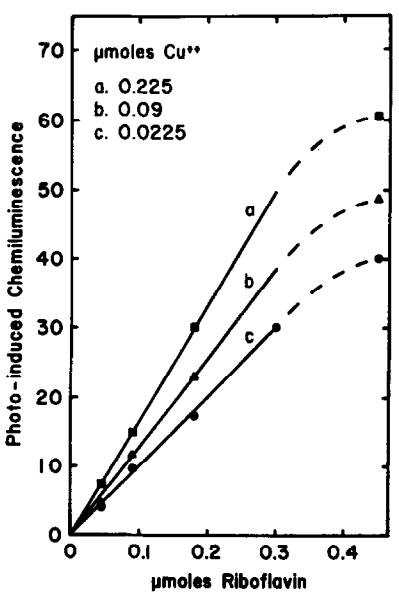

Fig. 3. Photoinduced chemiluminescence vs riboflavin concentration for different constant concentrations of copper (see insert) HOOH, 5 mmoles; PO_4-buffer, 1.5 mmoles, pH, 6.2. Volume 15 ml. Temp. 24°C., Illumination time, 30 seconds.

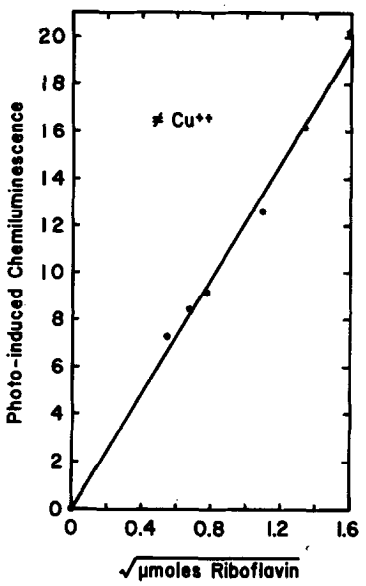

Fig. 4. Photoinduced chemiluminescence vs square root of the riboflavin concentration. NO copper added. HOOH, 5 mmoles; PO_4-buffer, 1.5 mmoles, pH 6.2. Volume 15 ml, Temp. 24°C., Illumination time 30 seconds.

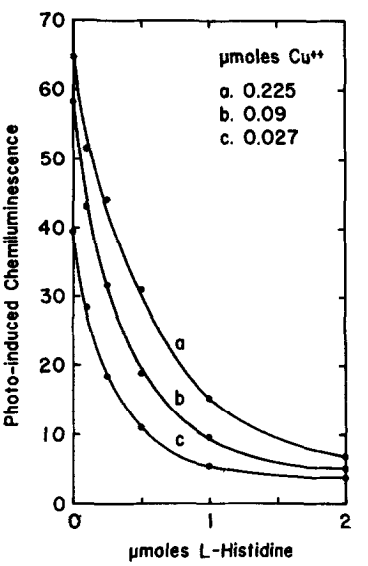

Fig.5. Photoinduced chemiluminescence vs histidine concentration for different constant concentrations of copper (see insert). Riboflavin, 0.45 µmoles; HOOH 5 mmoles; PO_4-buffer, 1.5 mmoles, pH, 6.2. Volume 15 ml. Temp 24°C. Illumination time 30 seconds.

In Fig. 5 we present data which show the suppression of the copper-enhanced photoinduced CL by the addition to the riboflavin-HOOH system of histidine which is a well known metal chelator with a high affinity for copper (Doran, et al 1964). The fact that the CL intensity, at elevated histidine concentrations is suppressed below the intensity found for the system containing no copper (See Fig.2) suggests that the suppression is due to a mechanism (s) in addition to copper chelation. In view of the strong hydroxylating potential of the system (Williams and Steele, 1965) this may be due to the hydroxylation of histidine by the free hydroxyl radicals generated.

DISCUSSION. There are several ways by which copper can enhance the intensity of the photoinduced CL in the riboflavin-HOOH system: (a) by increasing the redox dismutation of HOOH, and increasing thereby, the free radical concentration. This would result in a steady state CL upon which the photoinducible CL would be then superimposed (See vorhaben, 1965); (b) by increasing the lifetime of the excited triplet energy term (Shiga and Piette 1964) and increasing thereby, the probability for effective sensitized collisions with HOOH: (c) by stabilizing the possibly reactive riboflavin-semiquinone, which Hemmerich et al(1959, 1963) and Hemmerich (1964) have shown forms a rather unique metal chelate with cupric copper, and which can also be formed by the reaction of cuprous copper with oxidized riboflavin; (d) by the interaction of copper with a HOOH derivative to form a sterically unique complex,as suggested by Vercauteren and Massart (1962) which may be a reactant in the light reaction; or (e) conceivably by stabilizing reactive hydroperoxide derivatives generated in the photochemical reaction; or (f) by forming a transient charge-transfer complex between the reactants which may result in HOOH cleavage or activation. An assignment of mechanism must await the results of experiments in progress.

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