BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN, VOL. 46, 2741—2744 (1973)

Kinetic Studies of Photosensitized Oxidation through Semioxidized Eosine in an Aqueous Solution

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(Received April 5, 1973)

The rate constant for an electron transfer reaction between triplet eosine and potassium tris(oxalato)cobaltate(III) was estimated. The experimental results from flash photolysis indicate that a transient semioxidized eosine exists in a free state. When a substrate was added to the deaerated eosine and complex solution at an optimum concentration, the original dye was restored by electron transfer from a substrate to the semioxidized eosine, sensitized oxidation occurring efficiently. Choosing indole and its derivatives as a substrate, we have obtained the elementary rate constants of the eosine recovery process.

In a previous paper it was reported that semioxidized eosine (E^+) produced by electron transfer from triplet eosine to potassium tris(oxalato)cobaltate(III) ($K_3[Co(C_2O_4)_3]$) might exist as a free radical in a degassed aqueous solution and the reactivity of E^+ for leucofluorescein was discussed on the basis of a comparison with its transient complex forms such as $E^+\cdots O_2^-$ and $E^+\cdots E^-$.[1)]

This paper shows kinetically the occurrence of eosine sensitized oxidation with a high efficiency in this deaerated solution, where original eosine could be reproduced through a semioxidized form *via* an electron transfer with a substrate. Since the sensitization cycles

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1) Y. Usui, C. Iwanaga, and M. Koizumi, This Bulletin, **42**, 1231 (1969).

are not disturbed by the unreactive species of cobaltous and oxalate ions aquated from unstable potassium tris(oxalato)cobaltate(II) in a neutral solution, the mechanism is expected to be determined as the simple scheme by the retarding effect of a substrate on the eosine bleaching rate.

Including the case of a coupled formation of E^+ and E^- by D–D mechanism,[2] this sensitized oxidation pattern through the path of the semioxidized form may be significant as compared with the classical photodynamic action in which oxygen needs to take the major role of substrate oxidation.[3]

Experimental

Purification of materials except substrates and most of the experimental procedures were similar to those described previously. All substrate reagents (Tokyo Kasei G.R.); indole, 3-methylindole, and L-tryptophan *etc.* were used without further purification. All the reactions were carried out at 25.0 °C.

Results and Discussion

Reaction Mechanism of Photooxidation of Eosine by Potassium Tris(oxalato)cobaltate(III). In flash photolysis of the deaerated eosine and complex aqueous solution, three main maxima of a transient species appeared at 408 nm (semireduced form, E^-), 460 nm (semioxidized form, E^+) and 580 nm (eosine triplet, E^T). E^- was slightly accompanied by D–D mechanism.[2] The lifetime of E^+ is considerably longer (~1 ms) than that in a plain aqueous solution, where the decay is expressed as the recombination of E^+ and E^- to restore original eosine.[4] When cobalt complex (C) is added in order of magnitude greater than 10^{-4} M, E^+ could be produced by the reaction of $E^T + C \longrightarrow E^+ + C^-$, a destructive bleaching of eosine occurring with high quantum yield.

A competitive effect of the complex concentration on the decay of E^T is shown in Fig. 1. Putting $k_s = k_d^o + k_D^s[D]$, the pseudo-first order rate constant (k) is written as

$$k = k_d^o + k_D^s[D] + k_c^s[C] = k_s + k_c^s[C]$$

where k_d^o, k_D^s, and k_c^s ($=k_c^r + k_c^d$) are the rate constants on triplet eosine for a unimolecular decay, D–D interaction and a reaction with the complex, respectively. From the slope in Fig. 1, we get the k_c^s value as 9.2×10^6 M^{-1} sec^{-1}.

To clarify the genuine rate of an electron transfer from E^T to C by a steady-light illumination experiment, the quantum yield (Φ) of eosine bleaching was determined from the slope of a plot $\ln(e^E - 1)$ *vs.* time (E is optical density). A linear relationship in the reciprocal plots ($1/\Phi$ *vs.* $1/[C]_0$) holds in the dilute concentration region of the complex lower than 1×10^{-3} M (Fig. 2). A deviation at higher concentration seems to be related with the effect of ionic strength and/or the reaction in an excited singlet state of eosine with the complex. Thus, the reaction scheme can be written as follows.

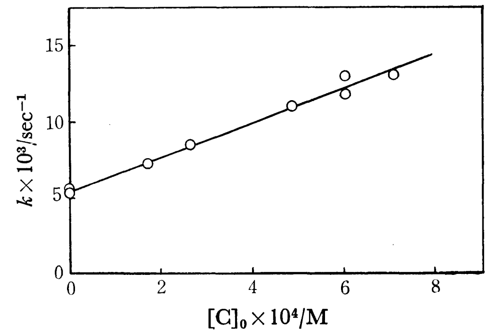

Fig. 1. Dependence of triplet eosine decay constant on $K_3[Co(C_2O_4)_3]$ concentration. ($[E] = 1.0 \times 10^{-5}$M)

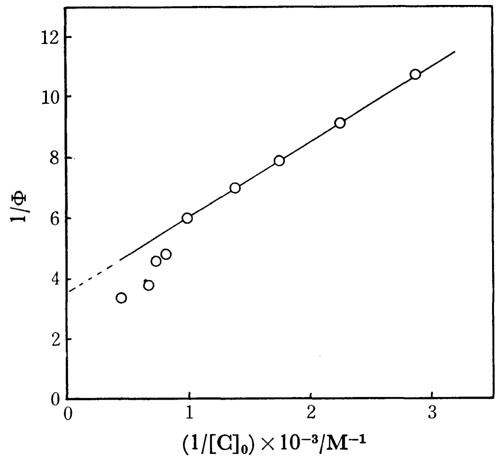

Fig. 2. Plots of $1/\Phi$ against $[K_3(Co(C_2O_4)_3)]_0^{-1}$. pH=7.0, $[E] = 1.0 \times 10^{-5}$M.

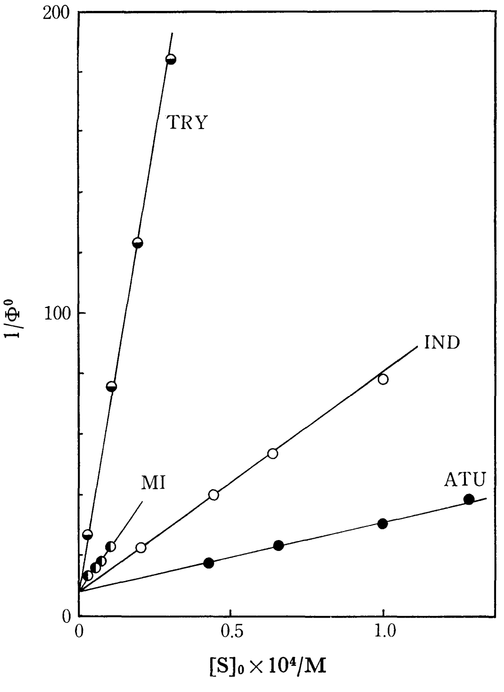

Fig. 3. Plots of $1/\Phi^0$ against substrate concentration.
—●—: Allylthiourea (ATU), —○—: Indole (IND),
—◐—: 3-Methylindole (MI), —◒—: L-Tryptophan (TRY)

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2) Y. Usui and M. Koizumi, *Mol. Photochem.*, **4**, 57 (1972).

3) L. I. Grossweiner, *Photochem. and Photobiol.*, **10**, 183 (1969).

$$\begin{array}{lll}
(1) & \mathrm{E} \xrightarrow{h\nu} \mathrm{E}^* \longrightarrow \mathrm{E}^{\mathrm{T}} & \phi_{\mathrm{st}} I_{\mathrm{ab}} \\
(2) & \mathrm{E}^{\mathrm{T}} \longrightarrow \mathrm{E} & k_{\mathrm{d}}^{\mathrm{o}} \\
(3) & \mathrm{E}^{\mathrm{T}} + \mathrm{E} \longrightarrow 2\mathrm{E}\ (\text{and } \mathrm{E}^+ + \mathrm{E}^-) & k_{\mathrm{D}}^{\mathrm{s}} \\
(4) & \mathrm{E}^{\mathrm{T}} + \mathrm{C} \longrightarrow \mathrm{E}^+ + \mathrm{C}^- & \left.\begin{array}{l} k_{\mathrm{c}}^{\mathrm{r}} \\ k_{\mathrm{c}}^{\mathrm{d}} \end{array}\right\} k_{\mathrm{c}}^{\mathrm{s}} \\
 & \qquad\quad\ \longrightarrow \mathrm{E} + \mathrm{C} & \\
(5) & \mathrm{E}^+ \longrightarrow \text{Decomposition} & k_+
\end{array}$$

$$\Phi = \frac{\phi_{\mathrm{st}} k_{\mathrm{c}}^{\mathrm{r}}[\mathrm{C}]}{k_{\mathrm{s}} + k_{\mathrm{c}}^{\mathrm{s}}[\mathrm{C}]} \tag{I}$$

ϕ_{st} and k_{s} are known to be 0.64 and $4.5\times10^3\ \mathrm{sec}^{-1}$ ($[\mathrm{E}]_0=1.0\times10^{-5}$ M), respectively.[4,5] From an intercept and slope of the line in Fig. 2, the values of $\phi_{\mathrm{st}}k_{\mathrm{c}}^{\mathrm{r}}/k_{\mathrm{c}}^{\mathrm{s}}$ and $k_{\mathrm{s}}/k_{\mathrm{c}}^{\mathrm{s}}$ were evaluated respectively as 0.2 and 0.71×10^{-3} M, in which the latter value coincides with the value 0.60×10^{-3} M obtained independently from the slope and interception in Fig. 1. Substituting ϕ_{st} and $k_{\mathrm{c}}^{\mathrm{s}}$ values into $\phi_{\mathrm{st}}k_{\mathrm{c}}^{\mathrm{r}}/k_{\mathrm{c}}^{\mathrm{s}}$, we get the genuine rate constant $k_{\mathrm{c}}^{\mathrm{r}}$ to be $3.2\times10^9\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$. Thus the efficiency of an electron transfer between eosine triplet and the complex is roughly estimated to be one third.

Photosensitized Oxidation of Added Substrates. It was confirmed by flash photolysis that the rate constant for the reaction $\mathrm{E}^+ + \mathrm{FH}_2 \longrightarrow \mathrm{E} + \mathrm{FH} + \mathrm{H}^+$ was of the order of magnitude of $10^9\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$ (FH_2, leucofluorescein)[1], and the reformation behavior of eosine was observed on the transient spectral change when phenanthroline and indole *etc.* were added.

Keeping the concentration of substrates and the complex as 50 μM and 1.0 mM, respectively (the competitive reaction of substrates with triplet eosine is neglected), we carried out semiquantitative survey for the retarding effect on quantum yield of eosine bleaching. Quite a large effect was obtained in the case of hydroquinone and indole-type substrates (Table 1).

It is noticeable that in the flash experiment of direct photoionization of a tryptophan alkaline solution,[6] the formation quantum yield (0.12) for a tryptophane radical cation was roughly of the same magnitude as its value listed in Table 1. By comparing the light energy (102 kcal) of maximum absorption wavelength which yields the same quantity of a tryptophan radical cation, its gain in energy is about two times higher in the eosine sensitization (55 kcal) than in the case of direct photoionization. From the viewpoint of a photoinactivation of aqueous lysozyme in which electron ejection from the essential tryptophan residues could inactivate the enzyme,[6] indole and its derivatives are taken as substrates for a kinetic treatment in the sensitized oxidation.

Kinetic Treatment of Photosensitized Oxidation of Several Substrates (S). From the above results, we see that the phenomenon could be interpreted by introducing following eosine recovery process in addition to the scheme (1) to (5);

Table 1. Relative recovery effect to bleaching eosine on the quantum yield (Φ°)[a)]

Substrate	Φ°	$(\Phi_{\mathrm{n}}^{\circ}-\Phi^{\circ})/\Phi_{\mathrm{n}}^{\circ}$
Leucofluorescein	0	1.00
Hydroquinone	0	1.00
L-Tryptophan	0.003	0.99
3-Methylindole	0.010	0.93
Indole	0.025	0.85
Phenylalanine	0.044	0.64
Trimethylamine	0.050	0.62
Allylthiourea	0.067	0.49
EDTA	0.070	0.47
Histidine	0.031	0.82
Imidazole	~0.17	~0
Adenine	~0.14	~0
none	0.13 ($\Phi_{\mathrm{n}}^{\circ}$)	—

a) [Eosine]=10 μM, [Substrate]=50 μM, $[\mathrm{Co(OX)_3}^{3-}]=1.0$ mM 25.0 °C, pH=7.0

Table 2. Rate constants for reaction (6)

Substrate	$k_+^{\mathrm{s}}\ \mathrm{M}^{-1}\ \mathrm{sec}^{-1}$
L-Tryptophan	6.4×10^{8} [a)]
3-Methylindole	1.9×10^{8}
Indole	7.3×10^{7}
Allylthiourea	2.0×10^{7}

a) pH=6.27

$$\begin{array}{lll}
(6) & \mathrm{E}^+ + \mathrm{S} \longrightarrow \mathrm{E} + \mathrm{S}^+ & k_+^{\mathrm{s}} \\
(7) & \mathrm{S}^+ \longrightarrow \text{Decomposition} &
\end{array}$$

The eosine bleaching reaction was found to occur with a good linear relationship between $\ln(e^E-1)$ and time at the initial stage of a constant substrate concentration. Thus under conditions when reaction (4) predominates greatly over the reaction between E^{T} and S, the concentration dependence of the quantum yield of eosine bleaching in an initial stage (Φ^0) on a substrate is expressed as follows.

$$\Phi^{\circ} = \phi_{\mathrm{st}} \frac{k_{\mathrm{c}}^{\mathrm{r}}[\mathrm{C}]_{\mathrm{o}}}{k_{\mathrm{s}}+k_{\mathrm{c}}^{\mathrm{s}}[\mathrm{C}]_{\mathrm{o}}} \frac{k_+}{k_+ + k_+^{\mathrm{s}}[\mathrm{S}]_{\mathrm{o}}} \tag{II}$$

Figure 3 shows a linear relationship between the reciprocals of Φ° and initial substrate concentration $[\mathrm{S}]_0$ as is expected from Eq. (II). Putting $[\mathrm{C}]_0=1.0$ mM, $k_{\mathrm{s}}=4.5\times10^3\ \mathrm{sec}^{-1}$ and $\phi_{\mathrm{st}}=0.64$, we obtain

$$\frac{1}{\Phi^{\circ}} = 7.5\left(1+\frac{k_+^{\mathrm{s}}}{k_+}[\mathrm{S}]_{\mathrm{o}}\right) \tag{III}$$

From a flash experiment, we get k_+ as $1\times10^3\ \mathrm{sec}^{-1}$ by analysing the decay of free semioxidized eosine as a first order.[1] Thus the rate constant k_+^{s} of reaction (6) for several substrates could be obtained from Eq. (III) and Fig. 3. The results are listed in Table 2. The k_+^{s} value for L-tryptophan is in good agreement with the value obtained in the flash photolysis experiment by Kepka and Grossweiner.[7] It seems that E^+ (and E^-) produced *via* D–D mechanism may exist freely during millisecond after flash irradiation.

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4) T. Ohno, S. Kato, and M. Koizumi, This Bulletin, **39**, 232 (1966). V. Kashe and L. Lindqvist, *Photochem. and Photobiol.*, **4**, 923 (1965); E. F. Zwicker and L. I. Grossweiner, *J. Phys. Chem.*, **67**, 549 (1963).

5) M. Nemoto, H. Kokubun, and M. Koizumi, This Bulletin, **42**, 1223 (1969).

6) L. I. Grossweiner and Y. Usui, *Photochem. and Photobiol.*, **11**, 53 (1970), **13**, 195 (1971).

7) A. G. Kepka and L. I. Grossweiner, *ibid.*, **14**, 621 (1971).

Although it could be noted that all the compounds sensitized by eosine have lower ionization potential than that of eosine, investigations on k_+^s value should be extended to other combinations of sensitizer and substrate.

The authors wish to express their hearty thanks to Professor Masao Koizumi, Tohoku University, for his helpful discussion and encouragement, and to Miss Michiko Ohsawa who carried out a part of the experimental work. This work was supported in part by a grant (Y.U) from the Ministry of Education.