

Preliminary Note

Primary processes in the methylene blue photosensitized oxidation of L-ascorbic acid

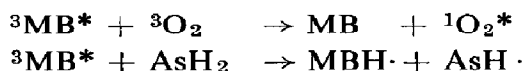
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(Received November 20, 1972)

Recently, we reported that a mixture of Methylene Blue (MB) and L-ascorbic acid (AsH₂) is a very efficient photosensitizing system for the oxidative depolymerization of hyaluronic acid¹. We are interested in the mechanism of this important biological process in connection with problems of damage to the vitreous humour of the eye caused by ultra-violet and, in the case of pigment sensitized processes, by visible light².

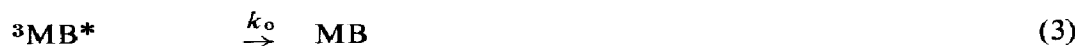
As a first step, a detailed study of the Methylene Blue sensitized photo-oxidation of L-ascorbic acid was carried out. This note reports a mechanism for the reaction and also draws attention to our conclusion that the reaction is initiated by two distinct primary processes:



This conclusion, in our view, is also of general interest, in view of the current controversy concerning the relative importance of hydrogen atom abstraction processes compared with that of direct excited singlet oxygen attack in the mechanism of photodynamic action³⁻⁶.

Evidence was obtained from a detailed study of the kinetics of oxygen absorption using a sensitive irradiation cell⁷. The light source was a Hanovia medium pressure mercury lamp filtered by a Pyrex plate. Typical rate data are shown in Fig. 1 (a). Consumption of reactants and generation of products were also followed during the course of the reaction where appropriate.

The following reaction mechanism is consistent with our results:



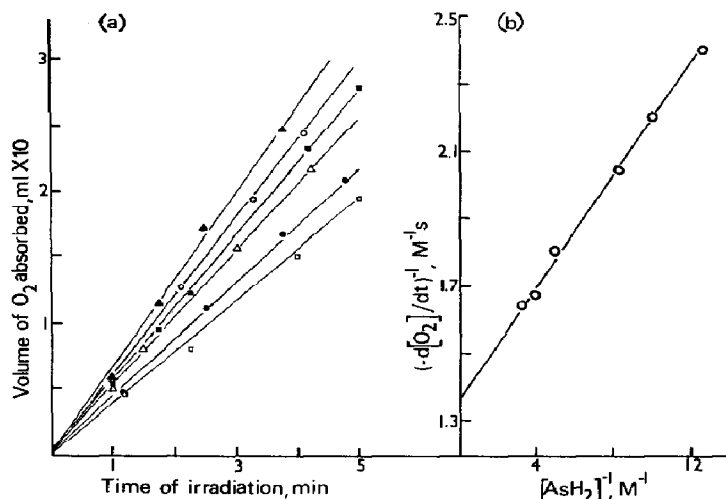
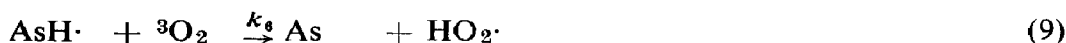
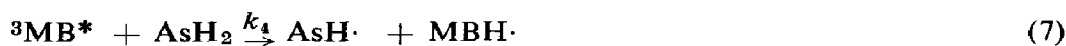


Fig. 1. (a) Typical plots of oxygen absorbed *versus* time of irradiation for aqueous solutions of $5 \times 10^{-5} M$ MB and various concentrations of AsH_2 ; $\blacktriangle = 3.0 \times 10^{-3} M$, $\circ = 2.5 \times 10^{-3} M$; $\blacksquare = 2.0 \times 10^{-3} M$; $\triangle = 1.2 \times 10^{-3} M$; $\bullet = 1.0 \times 10^{-3} M$; $\square = 8.0 \times 10^{-4} M$; (b) kinetic plot from rate data.

Formation of excited singlet oxygen by reaction (4) is well established⁸. Hydrogen peroxide was detected as a product of the overall reaction, using the specific quantitative method described by Belcher and Nutten⁹. Formation of hydrogen peroxide is accounted for by reaction (10). We obtained a value of ~ 0.5 for the ratio $R = \text{H}_2\text{O}_2 \text{ formed} / \text{O}_2 \text{ absorbed}$, at 25°C which can be readily understood if reactions (5) and (7) compete equally for ascorbic acid at this temperature. The ratio R increases linearly with temperature over the range $12\text{--}50^\circ\text{C}$, a fact which can be explained if it is assumed that the activation energy of reaction (7) exceeds that of reaction (5).

p-Methoxyphenol reduced the rate of oxygen absorption by $\sim 60\%$, probably by scavenging the radicals produced in reaction (7). The reaction scheme also explains why 1,4-diazabicyclo [2,2,2] octane, an efficient quencher of singlet

oxygen^{10,11}, reduced the rate of oxygen absorption while, at the same time, increasing R to its limiting value of 1.0.

A steady-state treatment of eqns. (1) to (10) gives the following expression:

$$-\frac{1}{d[\text{O}_2]/dt} = \frac{1}{I} \left\{ \frac{k_0}{k_1[\text{O}_2]} + 1 + \frac{k_4 k_3}{k_2 k_1 [\text{O}_2]} + \frac{k_4 [\text{AsH}_2]}{k_1 [\text{O}_2]} \right\} + \frac{k_3}{k_2 I [\text{AsH}_2]} \left\{ 1 + \frac{k_0}{k_1 [\text{O}_2]} \right\}$$

To simplify this expression, if k_4 is assumed to be of a similar magnitude to the rate constant for the reaction of triplet thionine with allyl thiourea¹² ($k = 1.1 \times 10^7$ mol l⁻¹ s⁻¹), then using this value together with¹³ $k_1 = 3 \times 10^9$ mol l⁻¹ s⁻¹, $[\text{O}_2] = 4.34 \times 10^4$ mol l⁻¹ and $[\text{AsH}_2] = 1 \times 10^{-3}$ to 5×10^{-3} mol l⁻¹, the following limits for the term $k_4 [\text{AsH}_2]/k_1 [\text{O}_2]$ are obtained: $0.008 \leq k_4 [\text{AsH}_2]/k_1 [\text{O}_2] \leq 0.04$. The contribution from this term is thus small and, for the range of ascorbic acid concentrations studied, can be neglected. At a fixed concentration the expression simplifies to:

$$-\frac{1}{d[\text{O}_2]/dt} = \frac{1}{I} \left\{ k' + \frac{k''}{[\text{AsH}_2]} \right\}$$

Figure 1 (b) shows that a plot of $(-d[\text{O}_2]/dt)^{-1}$ against $[\text{AsH}_2]^{-1}$ is linear and has a positive intercept on the ordinate. The rate of oxygen absorption was also found to be directly proportional to the absorbed light intensity, as required by the final expression.

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