AN ATTEMPT TO EVALUATE THE RATE OF THE HABER-WEISS REACTION BY USING 'OH RADICAL SCAVENGERS

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1. Introduction

The reaction between superoxide and hydrogen peroxide

$$H_2O_2 + O_2^- \xrightarrow{k_1} OH^- + OH + O_2 \qquad (1)$$

was proposed in 1934 by Haber and Weiss [1] and it was recently suggested by Beauchamp and Fridovich [2] and Peters and Foote [3] to be a source of OH' in biological systems. This reaction would thus amplify the toxicity of superoxide, because of the high oxidizing power of 'OH.

The value of k_1, which would allow one to predict the amount of 'OH radical formed from O_2^-, is still uncertain. Dainton and Rowbottom [4] gave a value of 3.4 M^{-1} s^{-1}, while Bray [5], McClune and Fee [6] and Halliwell [7] were unable to show the occurrence of the reaction by various approaches. However a limiting value $k_1 \leqslant 10$ M^{-1} s^{-1} can be deduced from their data. This value, although small in comparison to other rate constants of the O_2^- reactions, is still large enough for reaction (1) to be significant under some conditions. For instance at physiological pH values, assuming $H_2O_2 \leqslant 10^{-5}$ M and $O_2^- \leqslant 10^{-8}$ M [8], reaction (1) will be competitive with the spontaneous dismutation of O_2^-:

$$O_2^- + HO_2^{\cdot} + H^+ \xrightarrow{k_2} O_2 + H_2O_2 \qquad (2)$$

It seemed therefore important to test the occurrence of reaction (1) by a direct assay of the formation of 'OH radicals. The present report is primarily concerned with setting up the experimental conditions and the theoretical basis which will permit the use of specific scavengers of 'OH for this purpose. By this approach it was possible to demonstrate that the rate constant for reaction (1) is less than 10^{-4} M^{-1} s^{-1}.

2. Materials and methods

Potassium superoxide was from K and K, USA. Other chemicals were reagent grade. *p*-Nitrosodimethylaniline (PNDA) was produced by nitrosation of dimethylaniline [9] and was recrystallized from benzene. Aqueous solutions were made with twice

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glass distilled water. Absorbance and pH were measured with a Varian (Palo Alto, USA) 635 M spectrophotometer and with an Amel (Milan, Italy) mod. 331 pH meter respectively.

Since the study of reaction (1) requires concentrated O_2^- solutions and dimethylsulfoxide or tetrahydrofurane cannot be used as solvents because they are good $^{\cdot}OH$ radical scavengers, alkaline aqueous solutions of KO_2 were used. It was found that adding 4 M solid KO_2 to 1 M NaOH results in a 2 M solution of O_2^-, as determined by gas volumetric analysis. This superoxide solution slowly decomposes by a first order process (half-time 6 h at 0°C) to O_2 and H_2O_2. The reaction of O_2^- with H_2O_2 was studied at 25°C by slow continuous addition of the O_2^- alkaline solution to a reaction vessel containing all the other reagents. A 60% H_3PO_4 solution was also added continuously into the system to keep the pH constant. Efficient stirring of the reaction assured a homogeneous composition of the reaction mixture. $^{\cdot}OH$ radicals were produced by photolysis of H_2O_2 with a black-Ray UVL-36 Lamp in the presence of variable amounts of ethanol as scavenger [10] to obtain different yields of radicals.

Gas chromatography was carried out with a Hewlett-Packard model 5700 gas chromatograph, equipped with a flame ionization detector.

3. Results

3.1. *Determination of the steady state concentration of O_2^- in the reaction with H_2O_2*

Under the experimental conditions used, after the attainment of the steady state, assuming constant volume of the reaction system and constant concentration of H_2O_2 which is present in large excess, it results as:

$$d[O_2^-]/dt = [O_2^-]_0\, u/v - k_2\,[O_2^-]\,[HO_2^{\cdot}] - k_1\,[O_2^-]\,[H_2O_2] \simeq 0 \qquad (3)$$

where $[O_2^-]_0$ is the concentration of superoxide in the continuously added alkaline solution, u is the rate (liters $\times$ s^{-1}) of superoxide addition to the system of volume v. In eq. (3) and following all the reactions of the protonated superoxide have been neglected.

Since from the data in the literature it appears that $k_2\,[O_2^-]\,[HO_2^{\cdot}] \gg k_1\,[O_2^-]\,[H_2O_2]$ the steady state O_2^- concentration is

$$[O_2^-]_{ss} \simeq \left([O_2^-]_0\, u/v \cdot K_a/k_2\,[H^+]\right)^{1/2} \qquad (4)$$

where $K_a = 1.58 \times 10^{-5}$ is the acidic dissociation constant of $HO_2^{\cdot}$.

The steady state concentration of O_2^-, calculated according to eq. (4) was checked by quenching the reaction system with 2×10^{-5} M tetranitromethane (TNM), after addition of catalase, (TNM reacts rapidly with H_2O_2). Under these conditions O_2^- reacts quantitatively with a $k = 1.9 \times 10^9$ M^{-1} s^{-1} and gives the nitro form with $A_{350} = 1.46 \times 10^{-4}$ M^{-1} cm^{-1} [11].

Table 1 reports the results obtained, which show that the calculated values are in the same order of magnitude as the experimental ones.

3.2. *Revaluation of the rate constant of the Haber-Weiss reaction through the reaction of PNDA and benzene with $^{\cdot}OH$ radicals*

At sufficiently high concentrations of scavenger all $^{\cdot}OH$ radicals react with it and, as a consequence, the amount of scavenger reacted is equal to the amount of O_2^- reacted with H_2O_2. The ratio between O_2^- reacted in reactions (1) and (2) is thus:

$$\frac{\text{moles } O_2^- \text{ dismuted}}{\text{moles of scavenger reacted with } ^{\cdot}OH} = \frac{k_2\,[O_2^-]_{ss}\,[HO_2^{\cdot}]}{k_1\,[O_2^-]_{ss}\,[H_2O_2]} = \frac{\frac{k_2}{K_a}\,[H^+]\,[O_2^-]_{ss}}{k_1\,[H_2O_2]} \qquad (5)$$

Since k_2 is known [12] the value of k_1 can be calculated from the amount of scavenger reacted with $^{\cdot}OH$. For this purpose PNDA and benzene were used as scavengers. PNDA is a good $^{\cdot}OH$ trapping agent because of its high rate constant (1.07×10^{10} M^{-1} s^{-1}) and high extinction coefficient of its absorption band ($A_{440} = 3.4 \times 10^4$ M^{-1} cm^{-1}) which disappears upon reaction with $^{\cdot}OH$ [13].

We checked also its specificity by reacting it with H_2O_2 and O_2^-. The reactivity with O_2^- was tested

Table 1
Steady state concentration of O_2^- in the reaction with H_2O_2

pH	O_2^- concentration at steady state (M × 10^{-4})	
	Calculated according to eq. (4)	Found by reaction with TNM
5.4	0.04	not measured
7.5	0.45	0.26
8.4	1.28	0.5
9.4	4.0	2.1

2 ml of a 2 M aqueous O_2^- solution and 0.5 ml 60% H_3PO_4 were continuously and separately added to 20 ml 0.1 M phosphate buffer containing 0.16 M H_2O_2 and 1.4×10^{-4} M EDTA, under vigorous stirring at 25°C.

by addition of concentrated O_2^- – crown complex in dimethylsulfoxide [14]. Neither H_2O_2 nor O_2^- affected the PNDA absorption. This indicated that also singlet oxygen which arises from the spontaneous dismutation of O_2^- [15] does not react with PNDA. The reaction with ·OH was tested by measuring the decrease of PNDA absorption at 440 nm upon reaction with ·OH radicals produced through H_2O_2 photolysis. Under our conditions, the lower limit of detection of ·OH by this procedure is approximately 2×10^{-4} M. When the Haber-Weiss reaction was tested under the conditions of table 1 and in the presence of 7×10^{-3} M PNDA, the results were negative even at the highest steady state concentration of O_2^- (pH 9.4). The limit value of k_1 was thus calculated from eq. (5) to be $\leqslant 5 \times 10^{-3}$ M^{-1} s^{-1}.

·OH adds quantitatively to the benzene ring with a rate of 3×10^9 M^{-1} s^{-1} [10] to form hydroxycyclohexadienyl radical, which in the presence of O_2 gives phenol and small amount of biphenyl [16,17]. Under our conditions the lower limit of detecting phenol by gas chromatography is 1×10^{-5} M. We were not able to detect phenol in experiments carried out with benzene under conditions analogous to those used for PNDA. From these experiments the limit value of k_1 was found to be lower than 10^{-4} M^{-1} s^{-1}.

4. Discussion

Our results permit us to conclude that the Haber-Weiss reaction can not occur under most biological conditions. In fact with a k_1 value of the order of 10^{-4} M^{-1} s^{-1}, even assuming H_2O_2 as high as 10^{-3} M and O_2^- as low as 10^{-10} M, reaction (1) can not compete with reaction (2). Since considerable evidence has been accumulated during recent years that O_2^- and H_2O_2 gives ·OH radicals in some biological systems (see [7] and the references therein), a catalytic mechanism by which reaction (1) occurs should be operating. Work is in progress to detect such a mechanism.

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