Study of the Haber–Weiss Reaction using a Sensitive Method for Detection of OH Radicals

By W. HUGH MELHUISH and HARRY C. SUTTON*

(*Institute of Nuclear Sciences, Department of Scientific and Industrial Research, Lower Hutt, New Zealand*)

Summary A sensitive technique has been employed to show that the rate constant of the reaction $O_2^- + H_2O_2 \rightarrow OH + OH^- + O_2$ is less than 0·3 $dm^3\ mol^{-1}\ s^{-1}$ in dilute aqueous solution, and hence that it cannot occur significantly in biological systems unless catalysed by some cellular agent.

EXTENSIVE studies on the biological role of superoxide have focussed recent attention[1] on the possible occurrence in biological systems of the Haber–Weiss reaction (1).

$$O_2^- + H_2O_2 \rightarrow OH + OH^- + O_2 \qquad (1)$$

If this reaction can compete effectively with the decomposition of superoxide in the dismutation expressed stoicheiometrically as in reaction (2), then it would greatly augment

$$2\,O_2^- + 2\,H^+ \rightarrow O_2 + H_2O_2 \qquad (2)$$

the limited chemical properties of superoxide by forming the powerful oxidant, OH. We have employed a sensitive method to seek the products of this reaction in dilute aqueous solutions of superoxide and hydrogen peroxide.

Superoxide was prepared by γ-irradiation of aerated solutions of inhibitor-free hydrogen peroxide of concentration 0·4 mmol dm^{-3}, made by prior irradiation of oxygen-saturated water, and adjusted to pH 9·6 with 1 mmol dm^{-3} of sodium pyrophosphate. Analyses for superoxide by reduction of tetranitromethane to nitroform showed that 2 μmol dm^{-3} was available 16 s after irradiation. Its decomposition over a 10-fold range could be expressed approximately as first order with a half-life of 60 $\pm$ 20 s, or second order with a rate constant of 8000 $dm^3\ mol^{-1}\ s^{-1}$ {4 times higher than reported at pH 9·6 for the decomposition of superoxide in reaction (2), with $[O_2^-]^\circ > 30$ μmol dm^{-3} in highly purified solutions containing added ethylenediaminetetra-acetic acid.[2]}

Formation of OH radicals in reaction (1) was sought by adding 0·5 mmol dm^{-3} sodium benzoate (prepared from zone-refined benzoic acid with <2 p.p.m. salicylic acid) to the solution 16 s after irradiation, and analysing for sodium salicylate or other hydroxylated products[3] by a fluorimetric method (300 nm excitation, 430 nm emission). This benzoate (adjusted to pH 9·6 with sodium hydroxide) increased the decomposition rate of superoxide to a half-life of 40 $\pm$ 15 s. The analysis was calibrated by irradiating similar peroxide–benzoate solutions, thus forming OH radicals in a yield calculated from radiation chemical data to be 0·4 μmol dm^{-3} per 1 Gy radiation dose in our solutions. The fluorescence so induced was identical spectroscopically with that caused by added salicylate, and that caused by 0·02 Gy could be detected, corresponding to the product derived from 8 nmol dm^{-3} concentration of OH. The rate of reaction (1) was inferred by comparing f, the concentration of OH consumed in forming the fluorescent product, with d, the concentration of superoxide decomposed in the same time at the rate determined experimentally. Experimentally f was less than 8 nmol dm^{-3} when d was 1·5 μmol dm^{-3}. Equating f/d to the ratio $k_1[H_2O_2]/k_3$, where k_3 refers to the 'natural' decay, reaction (3), and under our

$$O_2^- \longrightarrow \text{products} \qquad (3)$$

conditions $k_3 = (1{\cdot}7 \pm 0{\cdot}7) \times 10^{-2}\ s^{-1}$, we conclude that k_1 is less than 0·23 $\pm$ 0·09 $dm^3\ mol^{-1}\ s^{-1}$.

Rigo *et al.*[4] recently reported a related study using a less sensitive scavenger for OH but more concentrated hydrogen peroxide (0·16 mol dm^{-3}), and found k_1 to be less than 10^{-4} $dm^3\ mol^{-1}\ s^{-1}$. The difficulties associated with the use of reagent grade hydrogen peroxide solutions which may not be free of inhibitors are well known,[5] and may have led to an under-estimate of k_1. Weinstein and Bielski[6] have used reagents of extreme purity, including 0·09 mol dm^{-3} inhibitor-free hydrogen peroxide, to show by different methods that $k_1 = 0{\cdot}13 \pm 0{\cdot}07$ $mol^{-1}\ dm^3\ s^{-1}$. Our result

should be viewed as confirmation of this measurement at lower $[H_2O_2]$, but not of earlier higher measurements ranging from 1·1 to 530 $dm^3\ mol^{-1}\ s^{-1}$.[5,7] We conclude that reaction (1) cannot compete effectively with (2) under credible physiological conditions ($[H_2O_2] < 10^{-5}$ mol dm^{-3}; $[O^-_2]$ *ca.* 10^{-9} mol dm^{-3}, decomposing second order with $k_2 = 5 \times 10^5\ dm^3\ mol^{-1}\ s^{-1}$ at pH 7[4]) unless it is catalysed by some cellular species not present in our experiments.

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