Electrolytic Univalent Reduction of Oxygen in Aqueous Solution Demonstrated with Superoxide Dismutase

Abstract. *The superoxide anion, generated electrolytically at a platinum electrode in buffered aqueous solution, was detected by its ability to cause the oxidation of epinephrine to adrenochrome. The rate of electrolytic oxidation of epinephrine varied with the applied potential in a manner reminiscent of an oxygen reduction half wave. This oxidation of epinephrine was dependent upon the presence of oxygen and was completely inhibitable by superoxide dismutase. It may be concluded that superoxide radicals, generated at the electrode, diffuse into the solution to an extent which allows reaction with small molecules such as epinephrine or with enzymes such as superoxide dismutase.*

One of the unsolved problems of the electrolytic reduction of oxygen is whether univalent reduction occurs in aqueous solutions, as it does in aprotic solvents, and whether the superoxide anion, O_2^-, if formed in aqueous media, does diffuse from the electrode surface (*1–3*). Some workers (*2*) have argued that univalently reduced oxygen, formed at the surface of the electrode, would be further reduced to H_2O_2 before it could diffuse away, whereas others (*3*) have concluded that O_2^-, generated on an electrode, would protonate and undergo dismutation spontaneously before it could escape from the vicinity of the electrode.

It has, however, been demonstrated (*4*) that sulfite oxidation, which is a chain reaction initiated by a variety of free radicals, could also be initiated at a platinum electrode and that the voltage dependency of this initiation resembles an oxygen reduction half wave. The superoxide anion, generated during the aerobic oxidation of xanthine by xanthine oxidase (*5*), has also been shown to initiate the oxidation of sulfite (*4*). Electrolytically initiated sulfite oxidation has been shown to be suppressed by impure preparations of superoxide dismutase (*6*). In view of the continued uncertainty (*1–3*) concerning the electrolytic generation of superoxide radicals in aqueous solutions, it appeared desirable to further document this point through the use of a stoichiometric, chromogenic, superoxide-detecting reagent and of pure superoxide dismutase.

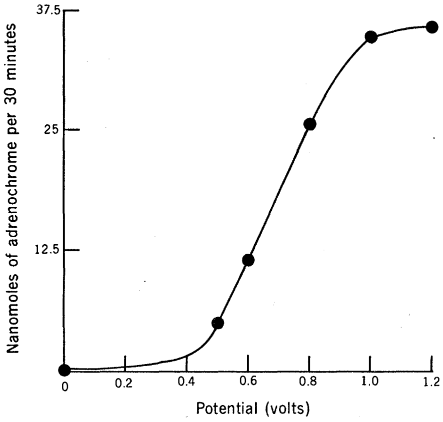

Fig. 1. Electrolytic cooxidation of epinephrine as a function of the applied potential. The rate of accumulation of adrenochrome in 0.05*M* potassium phosphate, 5 × $10^{-4}M$ epinephrine, and 1 × $10^{-4}M$ EDTA at *p*H 7.8 and 22°C is here presented as a function of the d-c potential applied to a platinum cathode immersed in the solution. These rates have been corrected for the spontaneous rate of adrenochrome production (46 nmole per 30 minutes), observed in the absence of an impressed potential.

The superoxide anion has been shown to cause the oxidation of epinephrine to adrenochrome (*7*), and the yield of adrenochrome formed per O_2^- has been shown to be close to 1, at saturating concentrations of epinephrine (*8*). Superoxide dismutase, which has been shown to catalyze the dismutation of O_2^-, in aqueous solutions, to yield oxygen and hydrogen peroxide (*7*), was prepared from bovine erythrocytes.

The anodic compartment of an H-shaped polarographic cell was filled with 0.05*M* potassium phosphate, 0.10*M* KBr, and 1 × $10^{-4}M$ ethylenediaminetetraacetic acid (EDTA) at *p*H 7.8, and the cathodic compartment was filled with 0.05*M* potassium phosphate, 5 × $10^{-4}M$ epinephrine, and 1 × $10^{-4}M$ EDTA at the same *p*H. The cathodic solution was constantly bubbled with air at 22°C, and a d-c potential was applied to platinum electrodes immersed in each arm of the cell. At intervals, we determined the concentration of adrenochrome in the cathodic compartment on the basis of its absorbancy at 480 nm (*9*), using a molar extinction coefficient of 4020 (*10*). There was some spontaneous oxidation of epinephrine under these conditions, and the rate of adrenochrome formation under the influence of the applied potential was corrected for the rate seen in the absence of an impressed voltage.

As shown in Fig. 1, the rate of electrolytic production of adrenochrome was a function of the applied potential. The curve in Fig. 1 bears a close resemblance to an oxygen reduction half wave. Thus, although the ordinate is given in terms of the adrenochrome which accumulated as a result of electrode processes, it is really a function of the amount of current utilized for the univalent reduction of oxygen. Superoxide dismutase, when added to a final concentration of 1.5 × $10^{-7}M$, caused a complete inhibition of adrenochrome formation. The imposition of anaerobic conditions, brought about by sweeping with nitrogen, also prevented the generation of adrenochrome. The shape of the curve in Fig. 1, along with the known reactivity of O_2^- with epinephrine and with the enzyme superoxide dismutase, led us to conclude that O_2 was electrolytically reduced at the cathode. Because it appears unlikely that an enzyme could act upon a small substrate while that substrate remained bound to a solid surface, it appears likely that the resultant O_2^- diffused from the cathode to an extent which permitted reaction with epinephrine and with superoxide dismutase. In addition, cathodically generated O_2^- was seen to initiate the sulfite-oxygen chain reaction (*4, 6*). Because there would be an electrostatic barrier to the close approach of sulfite to the cathode, this result suggests that O_2^- must have left the immediate vicinity of the cathode, prior to interaction with sulfite.

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References and Notes

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