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Oxidation Processes. XXII. Some Biological Implications in Autoxidation Mechanisms

BY JAMES E. LUVALLE

Autoxidation, *i. e.*, oxidation by molecular oxygen, supplies much of the energy needed for synthetic processes by biological systems. Frequently the idea is expressed that enzymes react by chain mechanisms, and in the case of reactions with oxygen, *via* organic peroxide formation. Both suggestions are usually based upon the data for the autoxidation of organic systems involving unsaturated hydrocarbons. The postulates of chain reactions and organic peroxide formation are not necessary, however, to explain enzymatic action, and an alternate mechanism involving semiquinone formation is set forth here which gives a better explanation of the observed facts.

Two mechanisms have been shown to occur in the oxidation of organic systems by molecular oxygen. Hydrocarbon (saturated and unsaturated) systems have been shown to autoxidize by a chain mechanism involving free radicals and the formation of organic peroxides (hydroperoxides) as the bivalently oxidized product.[1] The intermediate free radical, R·, is assumed to be stabilized to some extent by resonance. All the hydrocarbons which have been shown to undergo this chain autoxidation are characterized by the extreme instability of the bivalently oxidized molecule, R″ (the primes denote that two electrons have been removed); the addition of an electron donor stabilizes it; hence, the radical O_2^- combines with R″ to form RO_2^-.

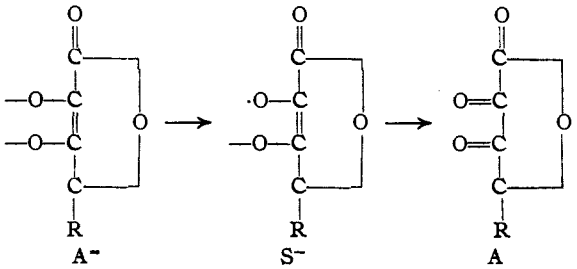
$$R'' + :O\dot{\cdot}\dot{\cdot}O: \longrightarrow R{-}\ddot{O}{-}\ddot{O}:$$

which then reacts

$$R{-}\ddot{O}{-}\ddot{O}: + RH \longrightarrow R{-}\ddot{O}{-}\ddot{O}{-}H + R\cdot$$

to form the hydroperoxide and another radical, R·. Aldehydes also autoxidize by a chain mechanism involving peroxide formation.

A second mechanism, named the semiquinone mechanism, has been established for the autoxidation of enediols, eneamines and their vinylogs like hydroquinone and *p*-phenylenediamine.[2] The semiquinones (free radicals) are much more stable than the free radicals formed in the chain mechanism. The bivalently oxidized product is stabilized by a rearrangement of bonds to form a quinonoidal structure. The compound undergoing autoxidation is frequently characterized by the stability of the neutral molecule and the extreme reactivity of one or more of its ions.[3] Thus, in *l*-ascorbic acid, the divalent ion is much more reactive than the monovalent ion or the neutral molecule.



This molecule shows the typical bond rearrangement which takes place in the semiquinone mechanism.

In metallic ion catalysis of either mechanism and in inhibition of the semiquinone mechanism, the catalyst or inhibitor apparently forms a complex with the substrate and this complex reacts with oxygen.[1a,1e,2a,3c]

In the peroxide mechanism, the free radical is stabilized to some extent by resonance, and the bivalently oxidized product is stabilized only by addition of an electron donor to form the peroxide radical. In the semiquinone mechanism, the free radical is stabilized quite strongly by resonance, the bivalently oxidized product is stabilized by a rearrangement of bonds, and the ion (or ions) is (are) frequently more reactive than the parent substance.

Many enzymatic mechanisms involve oxidation by oxygen. Waters[4] has recently discussed autoxidation in biological systems. He suggests chain mechanisms to explain the data and he also points out the objections to chain mechanisms. Hinshelwood[5] has proposed a group of enzymes spatially arranged, with free radical groupings at given spots (active centers) on their surfaces. These radicals are assumed to have a temporal existence. Michaelis[6] has suggested that both electron donor and acceptor are bound in adjacent sites on the enzyme surface and that they remain there until reaction takes place.

Virtually every coenzyme discussed by Waters[4]

(1) Only a few of the pertinent authors and references are cited: (a) Farmer, *Trans. Faraday Soc.*, **42**, 228 (1946); (b) Bolland and Gee, *ibid.*, **236**, 244 (1946); (c) George, *ibid.*, 210 (1946); (d) Robertson and Waters, *ibid.*, 201 (1946); (e) George, Rideal and Robertson, *Proc. Roy. Soc. (London)*, **A185**, 288 (1946); (f) Zuidema, *Chem. Rev.*, **38**, 197 (1946); (g) Bolland and Ten Have, *Trans. Faraday Soc.*, **43**, 201 (1947).

(2) References to earlier papers are contained in the papers cited: (a) LuValle and Weissberger, THIS JOURNAL, **69**, 1567, 1576, 1821 (1947); (b) LuValle and Weissberger, *ibid.*, **70**, 2223 (1948).

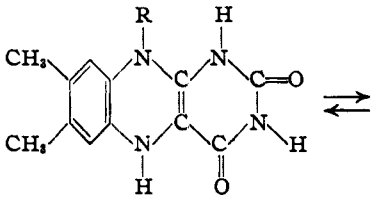
(3) (a) James and Weissberger, *ibid.*, **60**, 98, 2084 (1938); (b) Weissberger, LuValle and Thomas, *ibid.*, **65**, 1934 (1943); (c) Weissberger and LuValle, *ibid.*, **66**, 700 (1944).

(4) Waters, "The Chemistry of Free Radicals," Oxford Press, 1946, Chap. XII.

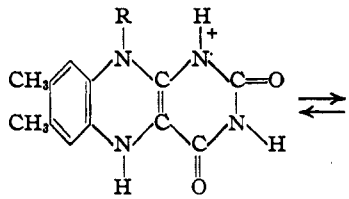
(5) Hinshelwood, "The Chemical Kinetics of the Bacterial Cell," Oxford Press, 1946, Chap. I.

(6) Michaelis, *Ann. N. Y. Acad. Sciences*, **11**, 37 (1940).

that reacts with oxygen can stabilize itself in the oxidized state by a bond rearrangement, *i. e.*, there is no necessity of proposing the formation of organic peroxides. For example, riboflavin nucleotides are the prosthetic groups of the flavo (yellow) enzymes. Let R represent the ribose group, the phosphate linkage, and the adenine group.

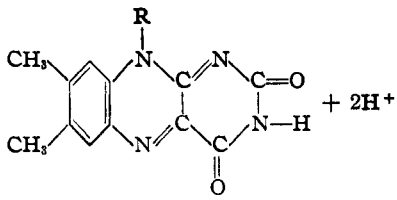


Reduced



S

Semiquinone



T

Oxidized

On oxidation, the bonds rearrange similarly to the rearrangement for the semiquinone mechanism. It is probable that even the oxidases that reduce oxygen to water do not form organic peroxides. Formation of enzyme peroxide probably leads to inactivation of the enzyme. Ionization preceding or following the oxidation adequately explains the hydrogen balance without introducing the idea of atomic hydrogen exchange (Wieland's dehydrogenation theory). Certainly in aqueous solution at the *p*H of normal cell activity there is an adequate supply of hydrogen ions to give instantaneous exchange.

LuValle and Goddard[7] have suggested that electron donor and electron acceptor are bound in a complex and that reaction takes place by the semiquinone mechanism. Either the donor or acceptor may combine with the enzyme, followed by a univalent oxidation–reduction, and this enzyme–semiquinone complex will then react with the other reactant to form the final products. By this mechanism the semiquinones are never free in the solution; they only exist bound to the enzyme. It is shown that the semiquinone mechanism can explain the observed kinetics for many respiratory enzymes. This mechanism does not involve bound peroxide formation or exchange of hydrogen atoms. Chain mechanisms are not necessary to explain the observed data.

Summary

The chain peroxide mechanism of autoxidation is briefly compared with the semiquinone mechanism. It is pointed out that the coenzymes involved in biological oxidation–reductions are much more likely to undergo reaction with oxygen *via* the semiquinone mechanism than by the chain, bound peroxide mechanism.

(7) LuValle and Goddard, in publication, "Quarterly Review of Biology."

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CONTRIBUTION FROM THE RADIATION LABORATORY AND DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA[1]

Preparation of 1-C^{14}-Propene-1 and the Mechanism of Permanganate Oxidation of Propene[1]

BY B. A. FRIES[2] AND M. CALVIN

The preparation of 1-C^{14}-propene-1 was undertaken in order to have available propene labelled in a terminal position and, incidentally, to study the stability of the double bond to migration when preparing propene under a variety of conditions. During the course of this investigation, a reliable procedure for the degradative analysis of the propene had to be developed. This analytical problem led to a study of the oxidative degradation of propene with permanganate.

A number of methods were available for the preparation of propene. Several of these methods were tried with C^{14} labelled materials, while others were discarded when preliminary tests with nonradioactive materials indicated either very poor yields or impure products. The first three of the following methods were actually employed for radioactive propene synthesis: (1) dehydration of *n*-propanol with metaphosphoric acid, (2) dehydration of *n*-propanol over heated alumina, (3) pyrolysis of *n*-propyltrimethylammonium hy-

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(2) While on leave California Research Corporation, Richmond, California.