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Preliminary communication

Superoxide-catalyzed reactions of carbohydrates*

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Recently, the importance of superoxide radicals in biological systems[1–4], in chemiluminescence, in the formation and decomposition of singlet oxygen in the upper atmosphere[5], and in many organic reactions[6] has been generally recognized. Relatively little research has, however, been devoted to reactions involving the utilization and regeneration of superoxide radicals in the oxidation of organic compounds by aqueous hydrogen peroxide.

In an investigation of the degradation of alditols by alkaline hydrogen peroxide in the presence and the absence of an iron catalyst[7], it was observed that, under certain conditions, rapid and extensive oxidation of the alditol and decomposition of hydrogen peroxide take place concurrently, with evolution of oxygen. Both reactions are accelerated by heat, and appear to depend on such chance catalysts as minute traces of salts of transition metals. These observations could not be explained by earlier theory but could be readily understood if both the degradation of the alditol and the decomposition of the hydrogen peroxide were catalyzed by superoxide radicals in much the same manner as Fenton's reaction is catalyzed by ferrous ions. We have now examined this hypothesis, and have found that it serves to explain the reactions in all important respects.

The following reactions presumably take place in the systems under discussion.

$$H_2O_2 + OOH^- \xrightarrow{\Delta} H_2O + {}^{\bullet}OH + {}^{\bullet}O_2^- \qquad (1)$$

$${}^{\bullet}O_2^- + {}^{\bullet}OH \rightarrow O_2 + OH^- \qquad (2)$$

$$H_2O_2 + Fe^{2+} \rightarrow OH^- + {}^{\bullet}OH + Fe^{3+} \qquad (3)$$

$$OOH^- + Fe^{3+} \rightarrow {}^{\bullet}OOH + Fe^{2+} \qquad (4)$$

$$ROOH + Fe^{2+} \rightarrow RO^{\bullet} + OH^- + Fe^{3+} \qquad (5)$$

$$ROH + {}^{\bullet}OH \rightarrow RO^{\bullet} + H_2O \qquad (6)$$

$${}^{\bullet}OOH \rightleftharpoons {}^{\bullet}O_2^- + H^+ \qquad (7)$$

*Reactions of Carbohydrates with Hydroperoxides. X. For Part IX, see ref. 15.

$$ROOH + {}^{\bullet}O_2^- \rightarrow RO^{\bullet} + OH^- + O_2^* \quad (8)$$

$$H_2O_2 + {}^{\bullet}O_2^- \rightarrow {}^{\bullet}OH + OH^- + O_2^* \quad (9)$$

$$O_2^* + O_2^{2-} \rightarrow 2\,{}^{\bullet}O_2^- \quad (10)$$

$$ROOH + OOH^- \xrightarrow{\Delta} H_2O + RO^{\bullet} + {}^{\bullet}O_2^- \quad (11)$$

Experimentally, we have found (see Table I) that alditols (D-arabinitol and D-glucitol) and aldonic acids (D-arabinonic and D-gluconic) are only slightly attacked by alkaline hydrogen peroxide in large excess at low temperatures (*e.g.*, 4°), but that they are largely degraded at 40°. At low temperatures, in the absence of an iron catalyst, there was relatively little, or no, evolution of oxygen, but when the temperature was raised, evolution of oxygen became rapid, and extensive oxidation occurred. The heat-induced decomposition of hydrogen peroxide and the degradation reactions were inhibited by the addition of magnesium sulfate to the reaction mixtures.

Presumably, decomposition of alkaline hydrogen peroxide by heat gives rise to hydroxyl and superoxide radicals (eq. *1*). The ${}^{\bullet}O_2^-$ radical most frequently acts as a reducing agent[8], and the ${}^{\bullet}OH$ radical is a strong oxidant[9]. Hence, they may react with each other (eq. *2*), forming O_2 and OH^-. However, some of the radicals may escape mutual reaction, and react with other substances in the environment.

We believe that the catalytic effect of ${}^{\bullet}O_2^-$ in the reactions of hydrogen peroxide is similar to the effect of ferrous ion, discovered by Fenton[10,11] in 1894. The role of ferrous salts in the reactions of hydrogen peroxide was not understood until Haber and Weiss[12] advanced the hypothesis that decomposition of hydrogen peroxide begins with the production of ${}^{\bullet}OH$ and ${}^{\bullet}OOH$ radicals by reactions *3* and *4*, and that these radicals are formed continuously from hydrogen peroxide in a chain reaction. By this means, a catalytic amount of an iron salt causes decomposition of a large amount of hydrogen peroxide.

Organic hydrogen peroxides are decomposed by ferrous iron (eq. *5*) in much the same way as is hydrogen peroxide. Hydroxyl radicals are able to oxidize alcoholic groups and to abstract hydrogen atoms from a wide variety of organic compounds (eq. *6*). The ${}^{\bullet}OOH$ radical is[13] an acid (pH 4.8), and hence, under alkaline conditions, it exists in the form of the ${}^{\bullet}O_2^-$ radical (eq. *7*), which, as already mentioned, is a reducing agent[8]. The ${}^{\bullet}O_2^-$ radical is not capable of abstracting a tightly bound hydrogen atom directly, but it is particularly effective in cleaving hydroperoxides (eq. *8*). Reaction of the superoxide radical with hydrogen peroxide (eq. *9*) gives a hydroxyl radical, hydroxyl ion, and a molecule of activated singlet oxygen, O^*_2. On reaction with divalent peroxide anion (eq. *10*) the activated oxygen yields two superoxide radicals. Hence, reactions *9* and *10* provide a prolific source of superoxide radicals.

Many processes heretofore attributed to Fenton's reaction may proceed by mechanisms involving the formation and use of superoxide radicals. Fenton's reaction is particularly important in neutral or acid solutions. Under alkaline conditions, however, superoxide reactions are favored, because ${}^{\bullet}OOH$ radicals dissociate forming ${}^{\bullet}O_2^-$ radicals, whereas the concentration of the iron catalyst in Fenton's reaction is restricted by the low solubility of ferrous and ferric hydroxides.

The superoxide-catalyzed degradation of an aldose is depicted in Scheme 1.

TABLE I

OXIDATION OF ALDITOLS AND ALDONIC ACIDS BY ALKALINE HYDROGEN PEROXIDE[a]

Substrate	*Temp. (degrees)*	*Millimoles of products per millimole of substrate*											
		A			*B*			*C*			*D*		
		Formic acid	*CO_2*	*Oxalic acid*	*Formic acid*	*CO_2*	*Oxalic acid*	*Formic acid*	*CO_2*	*Oxalic acid*	*Formic acid*	*CO_2*	*Oxalic acid*
D-Arabinitol	4	0.13	nil	nil	0.13	nil	nil	5.00	nil	nil	4.91	nil	nil
	40	2.90	nil	nil	0.15	nil	nil						
D-Glucitol	4	0.08	nil	nil	0.20	nil	nil	5.80	nil	nil	5.55	nil	nil
	40	1.42	nil	nil	0.12	nil	nil						
Potassium D-arabinonate	4	0.25	nil	nil	0.10	nil	nil	3.65	0.81	0.10	3.78	0.55	0.45
	40	3.42	0.89	nil	0.12	nil	nil						
Potassium D-gluconate	4	0.20	nil	nil	0.23	nil	nil	4.56	0.76	0.04	4.68	0.60	0.28
	40	4.20	0.70	nil	0.12	nil	nil						

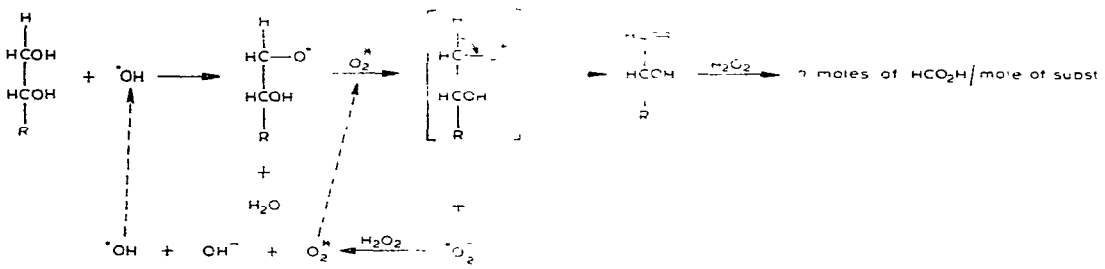
[a]Reaction mixture per millimole of substrate: 16 mL of 1.5M KOH plus 4 mL of 30% H_2O_2, plus the following additions for columns B, C, and D: B, 2 μmoles of $MgSO_4$; C, 2 μmoles of $FeSO_4$; and D, 20 μmoles of $FeSO_4$. Products determined after reaction period of 20 h.

Scheme 1 Superoxide-catalyzed degradation of an aldose (having n carbon atoms)

Scheme 2 Ferrous iron-catalyzed degradation of an aldose

Removal of the unpaired electron from the aldose radical in the transition state (shown in brackets) leaves the oxygen atom electron-deficient. The deficiency is satisfied by the cleavage reaction depicted. *The unpaired electron of the aldose radical may be accepted by any oxidizing agent in the environment.* The process is similar to the iron-catalyzed degradation (see Scheme 2), except for the replacement of ferrous and ferric ions by the superoxide radical and activated oxygen, respectively. In the iron-catalyzed system, the acceptor is usually ferric ion; in the superoxide-catalyzed system, the acceptor is activated oxygen. The process proposed differs substantially from the ionic and free-radical mechanisms previously postulated[14].

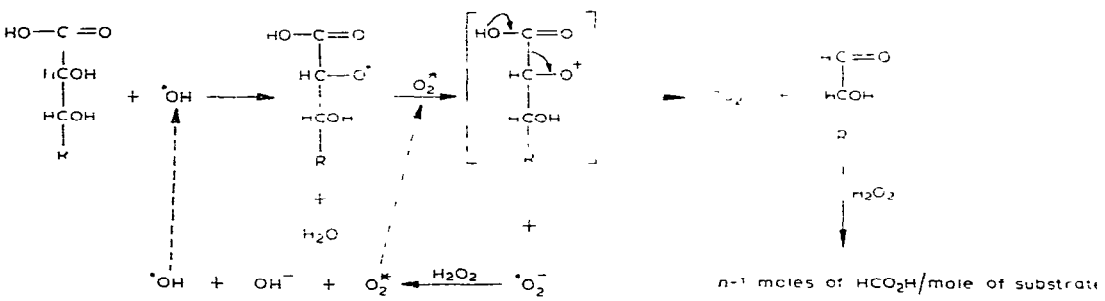
The superoxide-catalyzed degradation of an alditol (see Scheme 3) begins



Scheme 3 Superoxide catalyzed degradation of an alditol

with formation of a hydroxyl radical by eq. *9*. Reaction of this with the primary group of the alditol results in an alditol radical, which may react with activated oxygen, generating a superoxide radical, and forming a transition state that rearranges to the corresponding aldose. By reaction with hydrogen peroxide, the superoxide radical regenerates the hydroxyl radical and activated oxygen, completing a chain reaction.

The superoxide-catalyzed degradation of an aldonic acid having n carbon atoms (see Scheme 4) presumably begins with formation of a hydroxyl radical by eq. *9*. Reaction of the •OH radical with the α-hydroxyl group of the acid gives an aldonic acid radical. This may react with activated oxygen, generating a superoxide radical and forming carbon dioxide and the next lower aldose through the transition state shown in brackets. Complete degradation of the lower aldose yields $n-1$ moles of formic acid.



Scheme 4 Superoxide-catalyzed degradation of an aldonic acid

The reaction differs from that found in the presence of a ferrous salt, in that little or no oxalic acid is formed. In a prior publication[15], Isbell and co-workers noted the formation of oxalic acid in the degradation of aldonic acids by hydrogen peroxide in the presence of a ferrous salt, and suggested that this arose from oxidation of the primary alcoholic group of the acid, and subsequent degradation of the intermediate uronic acid[16]. This reaction does not occur in the absence of a ferrous salt. The effect of the ferrous salt on the course of the reaction may be ascribed to formation of a ferrous iron chelate, which blocks, to some extent, reaction at C-2, and favors reaction at the terminal carbon atom, with production of an intermediate uronic acid.

Production of superoxide radicals from hydroperoxide intermediates may account for the fact that addition of D-glucose, or other carbonyl-containing compounds, accelerates the decomposition of hydrogen peroxide and the degradation of cellulose and other polysaccharides by oxygen under alkaline conditions[17–19]. We have noted that addition of D-glucose to a slightly warm, alkaline solution of hydrogen peroxide results in rapid evolution of oxygen gas. Apparently, the hydroperoxide adduct, formed by nucleophilic addition of hydrogen peroxide to D-glucose, decomposes on heating with hydrogen peroxide anion (eq. *11*) more rapidly than does hydrogen peroxide (eq. *1*). The superoxide and D-glucose radicals formed by eq. *11* account for enhancement in the oxidative properties of alkaline hydrogen peroxide by addition of carbonyl compounds.

Dismutation of superoxide radicals, on the other hand, may account for the protective effect of magnesium compounds on the decomposition of alkaline hydrogen peroxide, and on the degradation of cellulose by oxygen. The protective effect of magnesium compounds on the degradation of cellulose has been widely investigated[20–23], and the following hypotheses have been advanced to explain the phenomenon: (*1*) that magnesium compounds form stable, not-readily-oxidizable complexes with transition-metal salts, and thus inhibit their catalytic effect[21,23]; (*2*) that magnesium hydroxide forms a complex with the primary oxidized derivative of cellulose, and thus inhibits further degradation[22]; and (*3*) that magnesium compounds deactivate active forms of peroxide molecules, or derivatives thereof, by forming stable complexes[17,18].

None of these ideas seem adequate to account for the very effective, anti-catalytic action of magnesium ions. The action appears comparable to that of superoxide dismutase in biological systems[1,24]. Hence, it seems possible that magnesium ions promote dismutation of superoxide radicals by the following reaction.

$$2\,{}^{\bullet}O_2^- + Mg^{2+} \rightarrow \underset{\text{complex}}{\text{magnesium superoxide}} \rightarrow MgO_2 + O_2$$

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