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DEGRADATION OF REDUCING SUGARS AND RELATED COMPOUNDS BY ALKALINE HYDROGEN PEROXIDE IN THE PRESENCE AND ABSENCE OF IRON AND MAGNESIUM SALTS*†

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ABSTRACT

Traces of either ferrous or ferric salts greatly increase the rate of the stepwise degradation of reducing sugars by alkaline hydrogen peroxide, as measured by formation of formic acid; addition of larger proportions of iron salts causes relatively smaller effects. The results showed that, unless unusually strict precautions are taken to exclude traces of iron, the free-radical cleavage of the hydroperoxide adducts of reducing sugars is far more rapid than the ionic cleavage. The catalytic effect of iron salts is counteracted by addition of magnesium salts. With D-glucose, inhibition of the catalytic effect of iron by magnesium depends on both the magnesium–iron ratio and the concentration at a given ratio. Measurements with various molar proportions of the salts indicated that a magnesium–iron complex, containing six atoms of magnesium to one of iron, is formed. Presumably, removal of iron by formation of this complex inhibits the free-radical degradation of hydroperoxide adducts. In marked contrast to the results obtained with reducing sugars, the degradation of potassium glyoxylate and of glyoxal by alkaline hydrogen peroxide is extremely rapid, and not catalyzed by iron or inhibited by magnesium. The results are in accord with an ionic, rather than a free-radical, cleavage of the hydroperoxide adducts of these compounds. The rapidity of the ionic reaction may be attributed to the ready availability of an electron pair from the adjoining carbon atom.

DISCUSSION

In prior papers (refs. 1–7), the hypothesis was advanced that stepwise degradation of reducing sugars by alkaline hydrogen peroxide takes place by nucleophilic addition of the hydroperoxide anion to the carbonyl group of the sugar, followed by oxidative cleavage of the adduct. The cleavage may take place by either an ionic or a free-radical mechanism (see Schemes 1a and 1b). In measurements made during a period of years, differences have been found in the reaction rates of a sugar with

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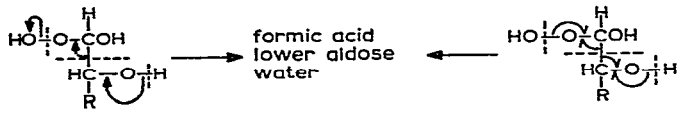


a. Ionic mechanism b. Free-radical mechanism

Scheme 1. Oxidative cleavage of the adduct of an aldose.

various samples of hydrogen peroxide. It is known that traces of iron salts greatly accelerate decomposition of hydroperoxides, by forming highly reactive hydroxyl and peroxy radicals. In view of the sensitivity of hydroperoxides to metal catalysts[8,9], it seemed probable that the observed differences in reaction rates arose from variations in minute proportions of iron salts in the reagents, and, consequently, in the relative importance of ionic and free-radical reactions. Prior workers have reported that the catalytic effect of iron salts on the decomposition of hydrogen peroxide, and on the degradation of carbohydrates by oxygen, may be inhibited by the addition of magnesium salts[10–12]. Thus, it appeared that a study of the effect of magnesium salts on the rates of oxidation of reducing sugars in the presence and absence of iron salts could be used to ascertain the relative importance of the ionic and free-radical mechanisms.

Prior investigators have suggested that hydrogen peroxide and iron salts give the following reactions:

1. $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$

2. $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}OOH + H^+$

3. $Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^-$

4. $Fe^{3+} + {}^{\bullet}OOH \rightarrow Fe^{2+} + O_2 + H^+$

5. ${}^{\bullet}OH + OOH^- \rightarrow OH^- + {}^{\bullet}OOH$

6. ${}^{\bullet}OOH \rightarrow {}^{\bullet}O_2^- + H^+$

7. ${}^{\bullet}O_2^- + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$

With an excess of hydrogen peroxide, the reactions give rise to ${}^{\bullet}OH$, ${}^{\bullet}OOH$, and ${}^{\bullet}O_2^-$ radicals in a dynamic system in which ferrous ions are oxidized by ${}^{\bullet}OH$ radicals, ferric ions are reduced by ${}^{\bullet}OOH$ radicals, and hydrogen peroxide is oxidized by ${}^{\bullet}O_2^-$ radical ions.

Experimentally, we found that very small proportions of iron salts greatly accelerate the degradation of D-glucose by alkaline hydrogen peroxide (see Table I). Surprisingly, the catalytic effect obtained by use of like quantities of ferrous sulfate or ferric sulfate were similar. Ferrous ions and hydrogen peroxide yield catalytically active ${}^{\bullet}OH$ radicals (eq. *1*), whereas ferric ions afford ${}^{\bullet}OOH$ radicals (eq. *2*). Thus, the effect of the two forms of iron should be quite different. However, with an excess of hydrogen peroxide, an equilibrium is quickly established between ferrous and ferric ions by eqs. *1* and *2*. For this reason, the two salts give similar results.

TABLE I

DEGRADATION OF D-GLUCOSE BY ALKALINE HYDROGEN PEROXIDE IN THE PRESENCE AND ABSENCE OF IRON AND MAGNESIUM SULFATES[a]

Expt. No.	Nanomoles of salts per ml of solution			Mg:Fe ratio	HCO_2H formed	
	$MgSO_4$	$FeSO_4$	$Fe_2(SO_4)_3$		Millimoles per millimole of D-glucose	Theoretical[b] (%)
1		—			0.47	8.0
2		8.33			3.07	51
3		16.7			3.16	52
4		33.4			3.26	54
5		83.3			3.86	64
6		167			4.10	68
7			8.33		2.41	40
8			16.7		2.17	36
9			33.4		3.10	52
10			83.3		3.16	53
11			167		3.67	61
12	1670	—		—	0.09	1.5
13	1670	8.33		200	0.09	1.5
14	1670	16.7		100	0.08	1.3
15	1670	41.5		40	0.12	2.0
16	1670	83.3		20	0.21	3.5
17	1670		8.33	200	0.06	1.0
18	1670		16.7	100	0.06	1.0
19	1670		33.4	50	0.08	1.3
20	1670		83.3	20	0.09	1.1
21	1670		167	10	0.33	5.5
22	1670		167	20	0.26	4.3
23	167	8.33		20	2.03	37
24	167	16.7		10	2.95	47
25	167	33.4		5	3.09	52
26	167	83.3		2	3.29	55
27	167	167		1	4.63	57
28	167		8.33	20	1.65	27
29	167		16.7	10	1.60	27
30	167		83.3	2	2.86	48
31	167		167	1	3.66	61

[a]Reaction conditions are given in the Experimental section. [b]Based on the carbon content of the substrate.

Table I shows that the catalytic effect of both iron salts is relatively greater for low than for higher concentrations. This finding is in accord with the hypothesis that the concentration of the hydroxyl radical is limited by the reactions of eqs. *3* and *5*. Decomposition of $^{\bullet}OH$ radicals, as in eq. *3*, terminates the chain reaction responsible for the degradation of the sugar.

The effect of magnesium salts on the degradation of cellulose by hydrogen peroxide and by oxygen has been studied in considerable detail, but there is still

uncertainty concerning reaction mechanisms involved[12]. Our results show that inhibition of the catalytic effect of iron salts by magnesium salts, in the oxidation of sugars, depends on both the magnesium–iron ratio and the concentration at a given ratio. Thus, in a solution that was 1.67mM with respect to magnesium, and 83.3μM with respect to iron (a 20:1 Mg:Fe ratio), the catalytic effect of iron was largely inhibited (see expts. 20 and 10); but, in a solution one-tenth as concentrated with respect to both ions, the catalytic effect of iron was only partially inhibited (see expts. 28 and 7).

TABLE II

CORRELATION OF THE MOLAR PROPORTION OF MAGNESIUM AND IRON SALTS WITH THE DEGRADATION OF D-GLUCOSE BY ALKALINE HYDROGEN PEROXIDE[a]

Micromoles of salts per ml of solution		*Mg:Fe ratio*	*HCO_2H formed*	
$MgSO_4$	*$FeSO_4$*		*Millimoles per millimole of D-glucose*	*Theoretical[b] (%)*
1.67	—	—	0.06	1.0
1.49	0.18	8.3	0.53	8.8
1.41	0.26	5.4	2.34	39.0
1.33	0.34	3.9	4.32	72.0
1.25	0.42	3.0	5.32	88.7
1.17	0.50	2.3	5.65	94.2

[a]Reaction conditions are given in the Experimental section. [b]Based on the carbon content of the substrate.

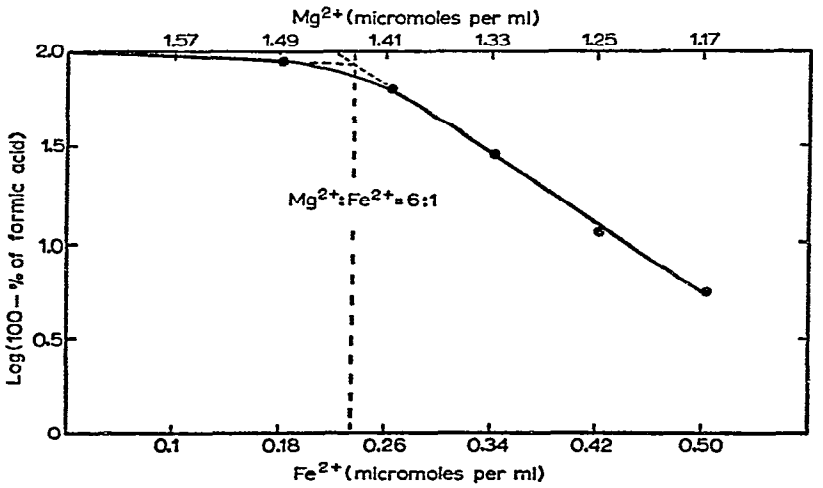


Fig. 1. Degradation of D-glucose by alkaline hydrogen peroxide in aqueous solutions containing magnesium sulfate and ferrous sulfate in various proportions.

The effect of variation in the molar proportions of magnesium and iron was studied by the method of continuous variation (see Table II and Fig. 1). One-millimole quantities of D-glucose were treated for 3 h with alkaline hydrogen peroxide in the presence of various quantities of magnesium sulfate and ferrous sulfate. As shown in Fig. 1, a plot of log (100 − % of formic acid) is diphasic, with an inflection corresponding to a magnesium–iron ratio of 5.9:1. The inflection suggests that the catalytic effect of iron salts is inhibited by a magnesium–iron complex containing six atoms of magnesium to one of iron. Presumably, the complex has a structure analogous to that of a ferrocyanide or a ferricyanide ion. The anion of a ferro complex would have a charge of 4 electrons, whereas that of a ferri complex would have a charge of 3.

Measurements similar to those for D-glucose showed that, in general, the degradation of aldoses and ketoses with alkaline hydrogen peroxide is accelerated by iron salts, and that the catalytic effect of iron is inhibited by magnesium salts. The results for lactose are of particular interest, because traces of iron salts cause not only more-rapid degradation of the carbon chain, but also rapid rupture of the disaccharide linkage (see Table III). With a solution that was 8.33μM with respect to iron, 52.5% of the lactose was degraded to formic acid in 3 h, and 70.9% in 6 h. With a solution that was also 1.67mM with respect to magnesium, only 0.3% of the lactose was degraded in 3 h, and 0.7% in 6 h. Other disaccharides gave similar results.

TABLE III

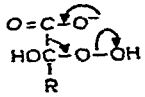
DEGRADATION OF LACTOSE BY ALKALINE HYDROGEN PEROXIDE IN THE PRESENCE AND ABSENCE OF IRON AND MAGNESIUM SULFATES[a]

Nanomoles of salts per ml of solution		*Mg:Fe ratio*	*HCO_2H formed; % of theoretical*[b]	
$MgSO_4$	*$FeSO_4$*		*3 h*	*6 h*
	—		4.7	17.1
	8.33		52.5	70.9
	83.3		65.8	69.1
	167		76.5	81.9
	334		84.5	85.4
1670	—	—	0.1	0.5
1670	8.33	200	0.3	0.7
1670	83.3	20	0.3	1.2
1670	167	10	3.7	4.2
1670	334	5	10.7	23.2

[a]Reaction conditions are given in the Experimental section. [b]Based on the carbon content of the substrate.

The data in Table I indicate that, in the presence of traces of iron, cleavage of the hydroperoxide adducts of reducing sugars takes place, in large measure, by the free-radical mechanism of Scheme 1b. However, with certain structures, and under other conditions, cleavage of the hydroperoxide adduct may take place by the ionic mechanism of Scheme 1a. The O–O bond is inherently weak, and, upon addition of a

proton, becomes so strongly polarized that O–O fission occurs. Ionic rupture of the O–O bond is also favored by the presence of a readily available electron-pair from the adjoining carbon atom, as, for instance, in the hydroperoxide adduct of an *alpha* keto acid.



Prior workers have shown that alkaline hydrogen peroxide oxidizes an *alpha* keto acid to the next lower acid plus carbon dioxide[15,16]. Experimentally, we found that, on treatment with hydrogen peroxide and alkali, either in the presence or absence of iron or magnesium salts, potassium glyoxylate yields formic acid and carbon dioxide almost quantitatively. The reaction is rapid, and not accelerated by iron salts or inhibited by magnesium salts.

It is also known that hydrogen peroxide cleaves *alpha* dicarbonyl compounds, with formation of two carboxyl groups[15,16]. We have found that slow addition of alkali to a solution of glyoxal containing hydrogen peroxide results in an almost quantitative yield of formic acid. As with potassium glyoxylate, the reaction is rapid, and not affected by iron or magnesium salts. The lack of acceleration of the reaction of both compounds by iron salts supports an ionic, rather than a free-radical, mechanism for the decomposition of the hydroperoxide adducts. The rapidity of the ionic reaction may be attributed to the ready availability of an electron pair from the adjoining carbon atom.

EXPERIMENTAL

General. — The sugars and reagents were of the highest commercial grade. The 30% hydrogen peroxide was represented as containing <0.005% of Fe, and the potassium hydroxide (A.C.S. grade), <0.001% of Fe.

Measurements in Table I. — In each experiment, the following ice-cold reagents were added successively to Pyrex test tubes cooled in ice–water: (*a*) 1 ml of a solution containing 0.4 millimole of D-glucose in water, or in a solution having sufficient magnesium sulfate to afford the final concentrations cited in the Table; (*b*) 1 ml of water containing 0 to 1 micromole of ferrous or ferric sulfate; (*c*) 3 ml of 2M potassium hydroxide; and (*d*) 1 ml of 30% hydrogen peroxide. The resulting solutions were kept in an ice-bath for 3 h, and each was then diluted to 100 ml. Aliquots (50 ml) were used for determining formic acid by the mercuric chloride method, as previously described[4,17]. The percentage of degradation was calculated on the premise that one mole of D-glucose affords 6 moles of formic acid.

Measurements in Table II. — The reaction conditions for the measurements were like those described for Table I, but with such variations of the proportions of magnesium sulfate and ferrous sulfate as to maintain a total concentration of the salts of 1.67 μmoles per ml of solution. The data are plotted in Fig. 1.

Measurements in Table III. — The reaction conditions were like those described for Table I, except for the substitution of 0.2 millimole of lactose for 0.4 millimole of D-glucose, and inclusion of data for a reaction period of 6 h. The percent of degradation was calculated on the premise that one mole of lactose affords 12 moles of formic acid.

Reaction of potassium glyoxylate with alkaline hydrogen peroxide in the presence and absence of magnesium and ferrous sulfates. — A solution (0.1M) of potassium glyoxylate was prepared by neutralizing glyoxylic acid hemihydrate (m.p. 72°) with 0.2M potassium hydroxide, and adding sufficient water to afford a 0.1M solution. Portions of this solution (10 ml each) were diluted with 1 ml of (*a*) water, or (*b*) 0.02M magnesium sulfate, or (*c*) 100μM ferrous sulfate. The mixtures were cooled in an ice-bath, and, after the addition of 2 ml of 30% hydrogen peroxide, were titrated with 0.1M potassium hydroxide (to a lasting pink color with phenolphthalein). Sufficient time was allowed, after addition of each drop of alkali, for decolorization of the indicator. Titration of each solution required 10.0 $\pm$0.1 ml of alkali, and was accomplished in $\sim$6 min.

To ascertain possible differences in the rates of reaction, measurement was made of the time required for oxidation of 75% of the substrate, in solutions prepared by methods (*a*), (*b*), and (*c*). Potassium hydroxide (7.5 ml of 0.1M) was added to each solution, held in an ice-bath. Hydrogen peroxide (2 ml of 30%) was added rapidly, and the time required for decolorization (phenolphthalein) was measured from the moment of addition. Solutions prepared by methods (*a*), (*b*), and (*c*) required, respectively, 28, 29, and 33 sec.

Reaction of glyoxal with alkaline hydrogen peroxide in the presence and absence of magnesium and ferrous sulfates. — A solution (0.1M) of glyoxal was prepared by diluting 1.3 ml of a commercial, 40%, aqueous solution of glyoxal to 100 ml. Portions (5 ml) of this solution were treated by the three methods previously described for the 10-ml portions of a solution of potassium glyoxylate. The titrations required $\sim$8 min and used 2 millimoles of potassium hydroxide per millimole of glyoxal.

To determine the time required for oxidation of 75% of the substrate, solutions were prepared by the three methods, and held in an ice-bath. After addition of 1 ml of 30% hydrogen peroxide, 7.5 ml of ice-cold, 0.1M potassium hydroxide was added, dropwise, during 75 sec. The time required for decolorization of the indicator (phenolphthalein) was measured from the addition of the first drop of alkali. Solutions prepared by methods (*a*), (*b*), and (*c*) required, respectively, 108, 110, and 110 sec.

Surprisingly, when the potassium hydroxide was added to the glyoxal solution *prior* to the hydrogen peroxide, erratic results were obtained, and less than two equivalents of acid were formed per mole of glyoxal. The subject was not pursued further.

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