[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, COLUMBIA UNIVERSITY]

The γ-Ray Induced Oxidation of Ascorbic Acid and Ferrous Ion[1]

BY N. F. BARR AND C. G. KING

RECEIVED FEBRUARY 15, 1955

The oxidation of aqueous ascorbic acid solutions by Co^{60} γ-rays has been studied and interpreted in terms of the mechanism of oxidation of ferrous ion under similar conditions. The ratio of the rates of radiation-induced oxidation of ferrous ion in the presence and absence of molecular oxygen was determined to be 1.89 ± 0.04. The ratio of the rates of oxidation of ferrous ion to the consumption of molecular oxygen was found to be 4.15 ± 0.1. The results for both substances are interpreted in terms of radical mechanisms.

Chemical effects induced in dilute aqueous solutions by ionizing radiations apparently are indirect and result from primary energy absorption by water. The observed chemical changes then result from the interaction of chemical species produced from water as a result of this absorption of energy. Such systems can be studied to best advantage in terms of a chemical dosimeter.

In this investigation the oxidation of ascorbic acid in solution was studied in terms of the radiation-induced oxidation of ferrous ion. The great disparity among reported values for the ratio of yields in the presence and absence of molecular oxygen and the bearing of these values on the generally accepted reaction mechanism warranted investigation of this point.[2] Ascorbic acid was selected since it is a biologically important substance that is representative of a large group known to be radiation sensitive in aqueous solution.[3,4]

Experimental and Results

Irradiation Facilities.—The sources of radiation were patterned after facilities described elsewhere.[5]

The dose rate variation within the cylindrical volumes has been determined with the aid of polyvinyl chloride films[6] and in terms of the rate of oxidation of ferrous ion, and was found not to be critically dependent on the position within the cylinders. Samples positioned within the aluminum cannister were attached to the source plug and lowered into the radiation field. Irradiation vessels were irradiated with a dose of approximately one million roentgens prior to use. Dose rate values for the two Co^{60} sources were 2.9×10^{21} e.v./l. hr. and 1.2×10^{22} e.v./l. hr. for sources number 1 and 2, respectively.

Analytical Methods.—Ferric ion was determined by direct measurement of its absorption at 3040 Å.[7] The thiocyanate method[8] was found to be unsatisfactory. Solutions were held within ±0.1° during absorption measurements, since the extinction coefficient of ferric ion in 0.8 N sulfuric acid was found to increase 0.5% per degree in the region of 25°. Oxygen was determined polarographically[9,10] in a bath thermostated to 30 ± 0.1°. Ascorbic acid was determined by titration with 2,6-dichlorophenol-indophenol.[11]

Oxidation of Fe^{++}.—Solutions of triply recrystallized ferrous ammonium sulfate ranging from 1 to 100 mM were prepared by dissolving the salt in doubly distilled water which had been made 0.8 N in sulfuric acid. Samples of the solutions were irradiated in source 1 for periods of 1 to 25 minutes. The samples were then removed, thermostated to 25° and compared with unirradiated controls. Ferric ion production was linear with dose for initial concentrations of ferrous ion from 1 to 100 mM. No rate differences could be detected in solutions prepared from distilled water or triply distilled water, or in the use of redistilled sulfuric acid. Refluxing triply distilled water with alkaline permanganate followed by two more distillations, the last from acid, had no effect on the radiation yield. Sodium chloride (200 mM) was found to decrease the apparent oxidation rate about 6%.

The consumption of oxygen upon irradiation of air-saturated 0.8 N H_2SO_4, 5 mM in ferrous ion, was determined by measuring the oxygen content of solutions equilibrated with air at 30°. The rate of oxidation of ferrous ion was calculated from the optical density of a portion of the irradiated solution compared with an unirradiated control. The values calculated from six determinations carried out in source 2 were: $-(dO_2/dt) = 768 \pm 35$ μM/l. hr.; and $(dFe^{+++}/dt) = 3{,}180 \pm 60$ μM/l. hr. Identical experiments carried out in source 1 gave the following average values from six determinations: $-(dO_2/dt) = 195 \pm 3$ μM/l. hr.; and $(dFe^{+++}/dt) = 809 \pm 9$ μM/l. hr. (Table I).

TABLE I

SUMMARY OF DATA

Solution	Irradiation time, min.	Source	$-(dO_2/dt)_{cor}$, μM/l. hr.	dFe^{+++}/dt, μM/l. hr.	No. of determ.
50 mM Fe^{++a}	20	1	195 ± 3	809 ± 8	6
5 mM Fe^{++a}	5	2	768 ± 35	3180 ± 60	6
250 mM $AH_2{}^b$	5	2	1500 ± 50		2
50 mM $AH_2{}^b$	5	2	1450 ± 50		4
5 mM $AH_2{}^b$	5	2	1480 ± 40		5
5 mM $AH_2{}^b$	20	1	400 ± 20		9
0.5 mM $AH_2{}^b$	5	1	390 ± 10		4
0.5 mM $AH_2{}^b$	10	1	390 ± 30		3
0.5 mM $AH_2{}^b$	12	1	410 ± 25		2
0.5 mM $AH_2{}^b$	15	1	380 ± 20		3

[a] 0.8 N H_2SO_4. [b] 0.8 N H_2SO_4, 0.5 M $(COOH)_2$.

The rate of oxidation of ferrous ion in the absence of oxygen was initially estimated by the use of 50 mM solutions of ferrous ion in 0.8 N H_2SO_4 flushed with nitrogen purified by passing through four columns of Fieser's solution, one of sulfuric acid, and one of saturated lead acetate. The rate of oxidation decreased at high percentage conversion, indicating a back reaction of the ferric ion product. Hence it was decided to use high concentrations of ferrous ion containing only small amounts of ferric ion and to make optical measurements directly in the irradiated tube. When solutions of thrice recrystallized reagent grade ferrous ammonium sulfate were prepared in water free from oxygen, 100 mM solutions contained initially 20 μM ferric ion. Irradiation produced not more than 200 μM of ferric ion.

In order to permit the ferric ion determinations to be made without exposing the solution to oxygen, it was necessary to determine the effect of radiation on the ultraviolet absorption of Pyrex glass. The coloration produced by radiation may be rapidly discharged by heating the glass gently with a luminous flame. Preliminary experiments in-

---

[1] Supported in part by U. S. Atomic Energy Commission Contract #AT (30-1)-1186, and in part by The Nutrition Foundation, Inc., New York 27, N. Y.

[2] N. F. Barr and C. G. King, THIS JOURNAL, **76**, 5565 (1954).

[3] B. E. Proctor and J. P. O'Meara, *Ind. Eng. Chem.*, **43**, 718 (1951).

[4] H.-C. Kung, E. L. Gaden, Jr., and C. G. King, *Agr. Food Chem.*, **1**, 142 (1953).

[5] B. Manowitz, *Nucleonics*, **9**, 2 (1951).

[6] E. J. Henley and A. Miller, *ibid.*, **9**, 6 (1951).

[7] T. Hardwick, *Can. J. Chem.*, **30**, 17 (1952).

[8] S. L. Whitcher and N. R. Todd, U. S. Atomic Energy Commission Report, U.C.L.A.-34.

[9] E. L. Gaden, Ph.D. Dissertation, Columbia University, 1949.

[10] "International Critical Tables," Vol. 3, McGraw-Hill Book Co., New York, N. Y., p. 272.

[11] C. G. King and O. A. Bessey, *J. Biol. Chem.*, **103**, 687 (1933).

dicated that: (a) the optical density at 3040 Å. is proportional to the dose delivered up to at least 100 kiloroentgen; (b) there is no detectable fading at room temperature for periods up to 20 hours; and (c) the rate of coloration varies from tube to tube and is approximately 0.1 o.d.u./hr. for 75 mm. Pyrex tubing exposed in source 1. Optical density readings were thus corrected by determining the total optical density change of the tube due to an exposure of 1 hour and correcting for readings taken at smaller dosages on the basis of the observed linearity between dosage and optical density.

In the runs used to evaluate the ratio of the rates of oxidation in the presence and absence of oxygen, tubes were irradiated for a one-minute interval and then for two 15-minute intervals. After each irradiation the tubes were thermostated and their optical densities were determined against a control tube. The tubes were broken at the seal, emptied, rinsed, filled with air-saturated 5 m*M* ferrous ion solution, and irradiated in an identical position within the source for 15 minutes. After their optical density had been determined they were again emptied, rinsed and refilled with control 5 m*M* ferrous ion solution, and the optical density was again determined. The rate of coloration of the tubes was determined as previously described. The data from these runs are compiled in Table II. The value calculated for the ratio from these data is 1.89 ± 0.04.

TABLE II

RATE OF IRRADIATION-INDUCED OXIDATION OF FERROUS ION IN THE PRESENCE AND ABSENCE OF OXYGEN

Run	Total rate in the absence of oxygen o.d.u./hr. 1st 15 min.	2nd 15 min.	Av.	Rate of browning o.d.u./hr.	Cor. rate, o.d.u./hr.	$(dFe^{+++}/dt)_{O_2}$, o.d.u./hr.	*R*
3	1.227	1.243	1.235	0.100	1.14	2.15	1.88
4	1.044	1.102	1.073	.030	1.04	1.98	1.90
5	1.208	1.204	1.206	.090	1.12	2.12	1.89
6	1.175	1.204	1.190	.090	1.10	2.14	1.94
7	1.204	1.192	1.198	.040	1.16	2.11	1.82
							1.89 ± 0.04

Ascorbic Acid Oxidation.—The loss in titer of solutions of ascorbic acid in 0.8 *N* sulfuric acid stabilized with 0.5 *M* oxalic acid was determined; the results are presented in Fig. 1. It is seen that the loss in titer is approximately linear with dose. There was no detectable difference in solutions stabilized with 0.05 *M* oxalate. The ratio of the rates of oxidation of ascorbic acid and ferrous ion were studied at initial ascorbic acid concentrations of 2.5 and 3.5 m*M*. Oxalic acid concentrations of 0.5 and 0.05 *M* were used. 5 m*M* solutions of ferrous ion were irradiated in identical positions. All solutions were 0.8 *N* in sulfuric acid. The data yield the following ratios of rates expressed in micromoles per liter per hour:

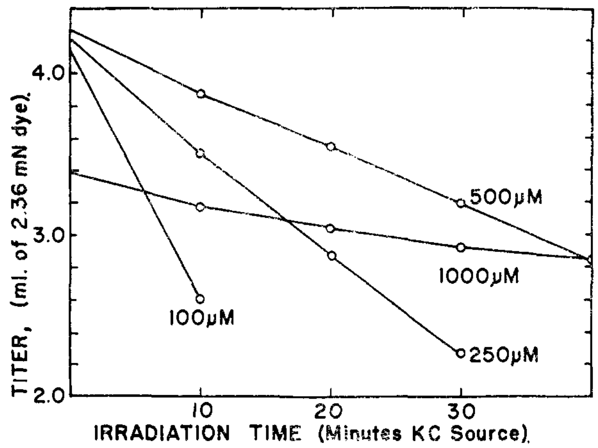

Fig. 1.—Radiation induced oxidation of ascorbic acid in 0.8 *N* H_2SO_4.

Source 1: $(dAH_2/dt/dFe^{+++}/dt) = 392 \pm 29/752 \pm 10 = 0.52 \pm 0.05$

Source 2: $(dAH_2/dt/dFe^{+++}/dt) = 1440 \pm 100/2970 \pm 30 = 0.49 \pm 0.04$

The rates of oxygen consumption were determined in both sources and the associated rates of production of ferric ion were determined in the same syringe repositioned in the source. The rates of oxygen consumption were determined in the presence of 0.5 and 0.05 *M* oxalic acid and for ascorbic acid concentrations ranging from 5 to 250 m*M*. The rate was determined for total dosages corresponding to 5, 10, 15 and 20 minutes exposures in source 1. These data are presented in Table I.

The rates of spontaneous oxygen consumption in stabilized ascorbic acid solutions were determined. Solutions 5, 50, 250 and 500 m*M* in ascorbic acid were prepared in 0.8 *N* sulfuric acid, 0.5 *M* in oxalic acid. The solutions were prepared with stabilizing solution previously thermostated to 30°. One portion of the solution was transferred to the polarograph cell and the other to a hypodermic syringe which was then capped. The syringe was then thermostated at 30°. Samples were removed at appropriate intervals and transferred to the polarograph cell. The rate of spontaneous oxygen consumption was found to increase with increasing concentration of ascorbic acid. Rates of oxygen consumption observed upon irradiation were corrected for this spontaneous oxidation.

Discussion

Dosimetry.—Of great interest at the beginning of this investigation was the question of the effect of impurities on the irradiation-induced oxidation of ferrous ion—the requirement for extremely pure water being potentially a serious limitation on the utility of the dosimeter.

The data summarized in Fig. 1 indicate a constant rate of oxidation of ferrous ion with dose over an initial concentration range from 1 to 100 m*M*. The rates for the various initial concentrations are equal and do not indicate a decreased rate at high initial ferrous ion concentrations which would be predicted if organic impurities were present.

The rates are constant with dose in spite of the large variation in rate of spontaneous oxidation with initial concentration. The rates of spontaneous oxidation for initial concentrations of 500, 100, 50 and 10 m*M* are, respectively, 50, 2.5, 1 and 0.2% of the irradiation-induced rate. Due to this spontaneous oxidation, the limited concentration of oxygen in air-saturated 0.8 *N* H_2SO_4, and the oxygen consumption associated with the oxidation, the initial concentration of ferrous ion is restricted, for practical purposes, to concentrations below 100 m*M*.

Dewhurst[12] has presented evidence which suggests that sodium chloride inhibits the acceleration of the irradiation-induced oxidation of ferrous ion by organic impurities. Several oxidations were run in the presence of 200 m*M* sodium chloride in order to further test the purity of the water used. The rates of oxidation in the presence of chloride were always less reproducible than the rates in its absence, and were approximately 6% lower. Since repeated distillation of triply distilled water after long refluxing with alkaline permanganate failed to reduce the yield in the absence of chloride, it is unlikely that organic impurities affected the reaction rate. Sodium chloride increased the rate of spontaneous oxidation 4- to 5-fold, and this increased rate decreased with time, apparently due to in-

---

(12) H. A. Dewhurst, *Trans. Faraday Soc.*, **48**, 905 (1953).

TABLE III

COMPILATION OF OBSERVED AND CALCULATED STOICHIOMETRY

Ratio	Obsd. Source 1	Obsd. Source 2	Calcd.[a]	G[b]
$(dFe^{+++}/dt)_{O_2}/(dFe^{+++}/dt)_{H_2O}$	1.89 ± 0.04		1.89	$(G_{Fe^{+++}})_{H_2O}$ = 8.24
$(dFe^{+++}/dt)_{O_2}/(dO_2/dt)_{Fe}$	4.15 ± 0.1	4.14 ± 0.2	4.25	$(G_{-O_2})_{Fe^{++}}$ = 3.77
$(dAH_2/dt)(dFe^{+++}/dt)_{O_2}$	0.52 ± 0.05	0.49 ± 0.04	0.47	(G_{-AH_2}) = 7.8
$(dO_2/dt)_{AH_2}/(dO_2/dt)_{Fe}$	2.05 ± 0.1	1.93 ± 0.1	2.00	
$(dFe^{+++}/dt)_{O_2}/(dO_2/dt)_{AH_2}$	2.02 ± 0.1	2.15 ± 0.1	2.13	$(G_{-O_2})_{AH_2}$ = 7.49

[a] Using $G_{H_2}/G_{Fe^{+++}} = 0.03$. [b] Using $(G_{Fe^{+++}})_{O_2} = 15.6$ molecules/100 e.v.

creased concentration of ferric ion. When the results are corrected for this effect by extrapolation to zero time, the rates are the same, within the limits of error of the extrapolation, in the presence and absence of sodium chloride. The increased rate of spontaneous oxidation of ferrous ion in the presence of sodium chloride and its dependence on ferric ion concentration explains the apparent decrease in G for ferrous oxidation with increasing sodium chloride concentration noted by Amphlett.[13]

Mechanism of Radiation-induced Oxidation of Ferrous Ion.—The generally accepted scheme for the radiation-induced oxidation of ferrous ion in acid solution[14] leads, when it is assumed that in the absence of oxygen the reducing species disappear by $H + H \rightarrow H_2$, to a value of 4 for R, the ratio of the rates of oxidation in the presence and absence of oxygen. A value in support of this has been reported.[15]

If the direct disproportionation of water into hydrogen and hydrogen peroxide which has been demonstrated by Allen[16] is considered, a lower value for R is predicted. The ratio approaches its maximum value of 4 only when the particles producing the ionization (both primary and secondary) have low ionization densities. For γ-rays from cobalt[60] the ratio of hydrogen production to ferrous ion oxidized[17] predicts a value of R which is consistent with the data of several workers.[18]

Rigg, Stein and Weiss have recently obtained a value of 2.0 for the limiting value of R at low pH, and suggest a scheme to account for this value.[14] The present values of 1.89 ± 0.04 for R and 4.15 ± 0.10 for the relative rates of ferric ion production and oxygen consumption are in excellent agreement with the values predicted on the basis of the above scheme when it is modified to include radical cross termination (Table III). The new values are in sharp disagreement with many values reported earlier. A widely accepted value has been 2.8.[19]

The most likely explanation of earlier values is associated with the decrease in rate of oxidation at high percentage conversion in the absence of oxygen which has been observed in this study. High concentrations of ferric ion, resulting either from high percentage conversions or large amounts of ferric ion present initially, would favor a back reaction with hydrogen atoms, $H + Fe^{+++} \rightarrow H^+ + Fe^{++}$. This reaction would reduce the apparent rate of oxidation and hence would increase the ratio. Strong support for this is offered by the marked pH dependence of the ratio observed by Rigg, Stein and Weiss.[14] The fact that no dependence of rate on dose was noted for dosages several times those used for the evaluation of R indicates that this reaction is not appreciable for low ferric ion concentrations and low pH.

Ascorbic Acid Oxidation.—The data summarized in Table III indicate the stoichiometry of irradiation-induced oxidation of ascorbic acid to be: $AH_2 + O_2 \rightarrow A + H_2O_2$. The relative rates of ferrous ion and ascorbic acid consumption indicate the reaction per ion pair to be: $2AH_2 + 2O_2 \rightarrow 2A + 2H_2O_2$.

These facts are consistent with a reaction scheme in which the oxidation is effected by hydrogen and hydroxyl radicals, as in the case of the ferrous ion oxidation

$$\begin{array}{c} H_2O \rightsquigarrow H + OH \\ H\cdot + O_2 \longrightarrow HO_2\cdot \\ AH_2 + OH \longrightarrow H_2O + AH\cdot \\ AH_2 + HO_2 \longrightarrow H_2O_2 + AH\cdot \\ AH\cdot + O_2 \longrightarrow AHO_2\cdot \\ AHO_2\cdot + AH\cdot \longrightarrow 2A + H_2O_2 \end{array}$$

If the cross termination reactions are considered to occur to the same extent as in the ferrous ion oxidation, the scheme predicts a stoichiometry which is summarized in Table III. Included in this table are the experimental values for both sources. Good agreement between the experimental values and the values calculated on the basis of the above scheme is indicated.

The data show no indication of a reaction of the type $AHO_2\cdot + AH_2 \rightarrow AH\cdot + A + H_2O_2$, which would lead to a chain utilization of oxygen. While the stoichiometry would be satisfied if this type of chain propagation were to occur with a chain length of approximately two, it is unlikely that this chain length would be maintained over a fifty-fold change in the concentration of ascorbic acid (Table I). The absence of a chain utilization of oxygen by ascorbic acid is consistent with the known protective effect of ascorbic acid and other easily oxidizable substances, such as thiourea, tocopherol and hydroquinone, on biological systems. The members of this class of protective compounds are characterized by their ease of oxidation and by the fact that they form stable one electron oxidation products. These substances apparently exert their protective action by limiting extended chain utilization of oxygen.

NEW YORK, N. Y.

---

(13) C. B. Amphlett, *Disc. Faraday Soc.*, **12**, 153 (1952).

(14) T. Rigg, G. Stein and J. Weiss, *Proc. Roy. Soc.* (*London*), **211A**, 375 (1952).

(15) F. H. Krenz and H. A. Dewhurst, *J. Chem. Phys.*, **17**, 1337 (1949).

(16) A. O. Allen, *Disc. Faraday Soc.*, **12**, 79 (1952).

(17) E. R. Johnson and A. O. Allen, THIS JOURNAL, **74**, 4147 (1952).

(18) E. J. Hart, *ibid.*, **73**, 1891 (1951).

(19) R. H. Betts, *et al.*, *Ann. Repts. Prog. Chem.*, **44**, 75 (1952).