Detection of Oxidation Radicals of 2,3-Enediol Form of 2,3-Dioxoguluno-δ-lactone by Pulse Radiolysis

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The OH adduct radicals of the 2,3-enediol form of 2,3-dioxoguluno-δ-lactone (2,3-End.DKGL) have been observed in aqueous solution by pulse radiolysis (the adduct radicals were converted into the one-electron oxidation radical in alkaline solutions by dehydration); the oxidation radical has also been produced by oxidation of 2,3-End.DKGL by the azide radical ($N_3\cdot$).

L-Ascorbic acid (AsA) is easily oxidized to dehydro-L-ascorbic acid (DHA), and then hydrolysed to 2,3-dioxo-L-gulonic acid (DKG) in neutral and alkaline solutions. DKG can be converted into 2,3-End.DKGL and the 3,4-enediol form of 2,3-dioxoguluno-δ-lactone (3,4-End.DKGL) by enolization and subsequent cyclization of DKG in neutral solution,[1,2] but 3,4-End.DKGL is rather unstable in aqueous solution. Both 2,3-End.DKGL and AsA have the 2,3-enediol group in their structures. The study of the oxidation mechanism of 2,3-End.DKGL is important in relation to the metabolism of AsA

in living organisms. Therefore, we are interested in studying the oxidation intermediates of 2,3-End.DKGL in order to clarify the oxidation mechanism of 'reductone' including AsA,[3] adrenaline, dopamine and triose reductone.[4,5]

This paper deals with the oxidation of 2,3-End.DKGL in aqueous solution by the OH radical ($\cdot$OH) and $N_3\cdot$ using the pulse radiolysis technique. 2,3-End.DKGL was obtained by addition of sodium hydroxide to an ethanolic DHA solution and purified by diethylaminoethyl cellurofine column chromatography.[6] The pK_a was determined to be 3.68 from the
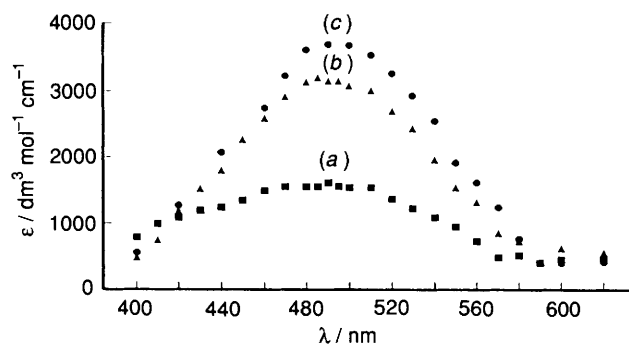
Fig. 1 Spectra of radicals obtained from 2,3-End.DKGL by pulse radiolysis; (a): pH 6.25, immediately after the pulse; (b): pH 8.75, 0.1 ms after the pulse; 2×10^{-4} mol dm^{-3} 2,3-End.DKGL, N$_2$O saturated and (c): 0.02 mol dm^{-3} NaN$_3$, pH 7.86, immediately after the pulse, 2×10^{-4} mol dm^{-3} 2,3-End.DKGL, N$_2$O saturated.

**Scheme 1.** Proposed mechanism for the formation of radicals from 2,3-End.DKGL and ·H or N$_3$·
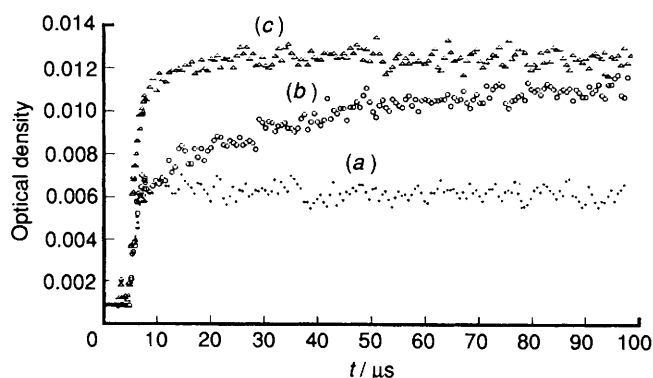
Fig. 2 Time course of the radical formation observed in pulse-irradiated solutions of 2,3-End.DKGL at 490 nm; (a): pH 6.25, (b): pH 8.75, (c): pH 10.61; 2×10^{-4} mol dm^{-3} 2,3-End.DKGL, N$_2$O saturated; 4.1 Gy per pulse.

dependence of the absorbance at 300 and 345 nm on the pH of 2×10^{-4} mol dm^{-3} 2,3-End.DKGL solutions.

Pulses of 10 MeV electrons of ~0.5 μs duration (~10 Gy) were delivered from a linear accelerator to sample solutions. In the ·OH system, the solutions were saturated with N$_2$O which converted the hydrated electron [e(aq)] into ·OH. In the N$_3$· system, NaN$_3$ was added to N$_2$O saturated solutions in which ·OH reacted with the azide anion (N$_3^-$) to produce oxidizing N$_3$·. Fig. 1(a) shows optical absorption spectrum of the transient intermediates immediately after the pulse produced by the reactions of 2,3-End.DKGL with ·OH at pH 6.25. At pH 8.75 under the same conditions, a similar spectrum to that at pH 6.25 was also observed immediately after the pulse. Fig. 1(b) shows the spectrum 0.1 ms after the pulse; the spectra in Fig. 1(a) and (b) are similar in shape but the absorbance is increased at pH 8.75. Fig. 1(c) shows the spectrum of the transient intermediates produced by the reaction of 2,3-End.DKGL with N$_3$·. Again the spectrum is similar in shape to Fig. 1(a) and (b) but the absorbance is ca. 15% greater in Fig. 1(c) than in Fig. 1(b). This indicates that some types of the OH adduct radicals could not yield the oxidation radical by dehydration. Fig. 2 shows the time profiles of absorbance at 490 nm in pulse-irradiated solutions at pH 6.25, 8.75 and 10.61. At pH 8.75 another transient intermediate is generated successively after the formation of the first-stage intermediate immediately after the pulse. At pH 10.61 the generation rate of the second-stage intermediate is too rapid to distinguish the two processes. These facts suggest that the OH radical reacts with 2,3-End.DKGL to form the OH adduct radicals which give the oxidation radical by dehydration in alkaline solutions. Similar phenomena have been reported for the case of AsA.[3] The AsA radical and the triose reductone radical have much smaller values of pK$_a$ (pK$_a$ = −0.457 and 1.45) than those (pK$_a$ = 4.3 and 5.0) of the
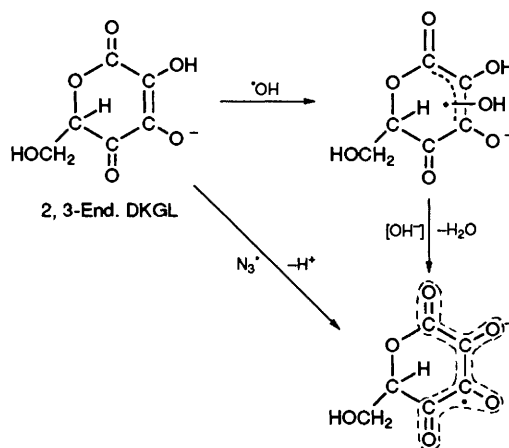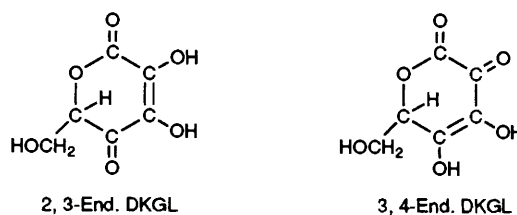
original acids. This fact enables us to presume that the pK$_a$ value of the 2,3-End.DKGL radical is smaller than that of the acid by ~4. Hence, the 2,3-End.DKGL radical must be in a dissociated form at neutral pH.

The rate constant for the dehydration of the OH adduct radicals by OH$^-$ was found to be $k = (1.6 \pm 0.2) \times 10^4 + (6.8 \pm 0.4) \times 10^8$ [H$^+$] s^{-1} from the relation of k vs. concentration of OH$^-$ (5.6 × 10^{-6} to 4 × 10^{-4} mol dm^{-3}) in N$_2$O saturated solutions of 2 × 10^{-4} mol dm^{-3} 2,3-End.DKGL. On the other hand, 2,3-End.DKGL is directly oxidized by N$_3$· to yield the oxidation radical. The rate constants for reactions of 2,3-End.DKGL with ·OH and N$_3$· were found to be $(8.8 \pm 0.8) \times 10^9$ dm^3 mol^{-1} s^{-1} at pH 7.65 and $(3.5 \pm 0.4) \times 10^9$ dm^3 mol^{-1} s^{-1} at pH 7.76, by kinetic analyses of the formation of the transient intermediate at 490 nm in the range of 2 to 4 × 10^{-6} dm^{-3} mol 2,3-End.DKGL, respectively. The OH adduct radical and the oxidation radical of 2,3-End.DKGL decay by second-order kinetics with rate constants of $(1.4 \pm 0.1) \times 10^8$ dm^3 mol^{-1} s^{-1} at pH 6.3 and $(6.8 \pm 0.4) \times 10^7$ dm^3 mol^{-1} s^{-1} at pH 7.9, above 5 Gy per pulse.

The loss of 2,3-End.DKGL for OH radical oxidation at pH 6.25 was observed to be $(3.4 \pm 0.1) \times 10^{-7}$ mol J^{-1} by HPLC analyses after ^{60}Co γ-ray irradiation. This result indicates that the transient intermediate decays by disproportionation, since the radiation chemical yield of ·OH is 6.3 × 10^{-7} mol J^{-1}.[7] A proposed mechanism for the oxidation of 2,3-End.DKGL is shown in Scheme 1.

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