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Pulse Radiolysis Study of Reaction of OH Radicals with Triose Reductone

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The reaction between the hydroxyl radical (OH) and triose reductone (2,3-dihydroxy-2-propenal) in an aqueous solution has been studied by spectrophotometric pulse radiolysis. The OH radical adds to triose reductone (TRH$_2$) to form the transient OH adduct (TRH(OH)$^{-\cdot}$) with an absorption maximum at 398 nm, ε= 3150 M^{-1} cm^{-1} (1 M=1 mol dm^{-3}) in a neutral solution. The OH adduct decays by second-order kinetics in the pH range 2—12. The rate constants for the reactions of the OH radical with dissociated and undissociated triose reductone are 1.4×10^{10} M^{-1} s^{-1} and 9.9×10^{9} M^{-1} s^{-1}, respectively. The dependence of the transient absorption on the pH shows that the OH adduct has a pK_a value of 4.7.

The structure of triose reductone (2,3-dihydroxy-2-propenal) was determined to be CH(OH)=C(OH)-CHO by H. von Euler and C. Martius.[1] A very important characteristic of "reductone" is a strong reducing ability due to its enediol group, -C(OH)=C(OH)-.[2] Well-known reductones, L-ascorbic acid,[3–5] adrenaline,[6] and dopamine[7] which play significant roles in biological systems, have been studied by pulse radiolysis. These reductones react with OH radicals to form OH adducts or one-electron oxidized radicals. The OH adducts can be converted to one-electron oxidized radicals by dehydration. Thus, the oxidation of the reductones has been shown to proceed through the intermediate radicals. Triose reductone is yielded in biological systems[1,8–10] and in the process of the browning reaction of milk.[11] In addition, it reacts with amino compounds to yield anil compounds[2] or causes a DNA-breaking action in the presence of Cu^{2+}.[12] The enzymatic oxidation of triose reductone has also been reported to produce a free radical in an ESR study.[13,14] It is useful to study the oxidation mechanism of triose reductone, as it is an important compound in biological systems[2] and is one of the simplest reductones. We have kinetically studied the oxidations of triose reductone using oxygen and hydrogen peroxide,[15] a peroxodisulfate ion,[16] and a hexacyanoferrate(III) ion,[17]. In our previous paper[18,19] it was found that triose reductone is oxidized by $X_2^{-\cdot}$ (X=SCN, Cl, Br, I) and $N_3\cdot$ to yield a one-electron oxidized radical. In the present research we studied the reaction of the OH radical with triose reductone kinetically and found a different transient species from the one-electron oxidized radical (TR$^{-\cdot}$).

Experimental

Pulse irradiation was accomplished using the 18 MeV linac (High Voltage Engineering Co.) of the Radiation Center of Osaka Prefecture at room temperature (about 20 °C). Details of the detection systems, dosimetry, and solution handling techniques were previously described.[19] All solutions were prepared from triply distilled water. Dinitrogen monoxide (Showa Denko Co., High Purity, upper limit of O$_2$ content: 10 ppm) was used after the deaeration of solutions by argon (Osaka Sanso Co., For Instrument Grade). The solutions were bubbled before irradiation for ≈60 min with Ar and N$_2$O. This procedure converts hydrated electrons into OH radicals. Triose reductone was prepared and purified by the method of Euler and Martius.[1] All other chemicals used were of analytical reagent grade. The pH of solutions was generally adjusted by an addition of the appropriate amounts of HClO$_4$ or NaOH.

Results and Discussion

Absorption Spectra of the Transient Intermediates Yielded by OH Radicals. A N$_2$O-saturated solution containing 300 μM of triose reductone at pH 7.8 was pulse-irradiated at a dose of about 500 rad per pulse. The spectrum of the transient intermediate immediately after a pulse at pH 7.8 is shown in Fig. 1. The transient absorption almost disappears upon the addition of 0.2 M *t*-BuOH, which scavenges OH

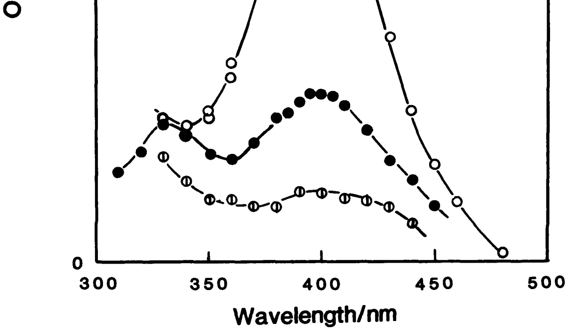
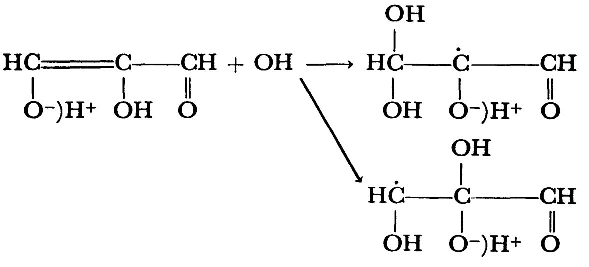


Fig. 1. Transient absorption spectra produced by pulse irradiation of N$_2$O saturated 3×10^{-4} M triose reductone solution. Normalized dose, 500 rad; pH 7.8 (○), pH 4.63 (●), pH 3.03 (⦶).

radicals. The result indicates that a transient absorption is produced by a reaction of triose reductone with the OH radical. The absorption spectrum shows an almost symmetrical (Gaussian-type) band with a maximum at 398 nm and a width at half-maximum of 71 nm. The shape of this spectrum is similar to that[19] of the triose reductone radical ($TR^{-\cdot}$, λ_{max}=398 nm) yielded through the oxidation of triose reductone by the inorganic radicals ($N_3\cdot$, $(SCN)_2^{-}\cdot$, $Br_2^{-}\cdot$, etc). The absorption coefficient around a neutral pH was obtained to be 3150 $M^{-1}\,cm^{-1}$ at 398 nm from the linear slope of absorbance vs. pulse dose. This value is about half of that of the triose reductone radical (5500 $M^{-1}\,cm^{-1}$).[19] Analogous facts have been found through a pulse radiolysis of ascorbic acid.[3] The absorption coefficients of the spectra become smaller with a decrease in the pH. The spectra of the transients at pH 4.63 and 3.03 are also shown in Fig. 1. The absorption at 398 nm almost disappears at pH 3. The triose reductone radical ($TR^{-\cdot}$) has a pK_a of 1.4.[19] If this intermediate is the triose reductone radical, the absorbance around 400 nm should remain unchanged at a pH >3. From these facts, it is seen that this transient species is not the triose reductone radical. It should be the OH adduct of triose reductone, since the OH radical is easy to add to an unsaturated bond. Triose reductone has two dissociation constants[15] (pK_1=5.0, pK_2=13.0). Therefore, the reaction scheme can be described by the following equations.

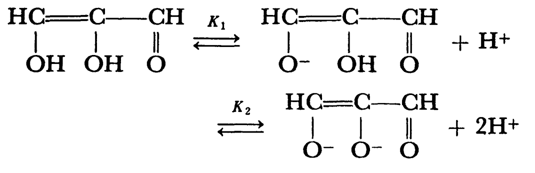
$$H_2O \rightsquigarrow e_{aq}^-,\ H,\ OH,\ H_2O_2,\ H_2 \tag{1}$$

$$N_2O + e_{aq}^- \longrightarrow OH + OH^- + N_2 \tag{2}$$



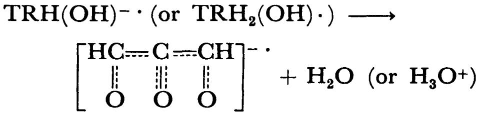
$$\mathrm{HC(O^-)H^+{=}C(OH){-}CH{=}O + OH \longrightarrow HC(OH){-}\dot{C}(OH)(O^-)H^+{-}CH{=}O} \tag{3a}$$

$$\mathrm{HC(O^-)H^+{=}C(OH){-}CH{=}O + OH \longrightarrow H\dot{C}(OH){-}C(OH)(O^-)H^+{-}CH{=}O} \tag{3b}$$



$$\mathrm{HC(OH){=}C(OH){-}CH{=}O \overset{K_1}{\rightleftharpoons} HC(O^-){=}C(OH){-}CH{=}O + H^+ \overset{K_2}{\rightleftharpoons} HC(O^-){=}C(O^-){-}CH{=}O + 2H^+} \tag{4}$$

The electron density is greater on C2 than C3 from the results of ab initio MO calculations of triose reductone.[20] It is also presumed by electronic effects. Hence, an electrophilic attack of the OH radical is more likely to occur through reaction 3b than 3a. Ascorbic acid reacts with the OH radical to yield the OH adduct and the adduct loses H_2O (or OH^-) to form the ascorbic acid radical.[3] However, the conversion of the OH adduct of triose reductone into the $TR^{-\cdot}$ radical was not observed. This OH adduct should be rather stable and can not be converted into the $TR^{-\cdot}$ radical by dehydration as in the following reaction (5).



$$\mathrm{TRH(OH)^{-}\cdot\ (or\ TRH_2(OH)\cdot) \longrightarrow \left[HC{\cdots}C{\cdots}CH\ (O)(O)(O)\right]^{-}\cdot + H_2O\ (or\ H_3O^+)} \tag{5}$$

Rate Constants for the Reactions of Dissociated and Undissociated Triose Reductone with OH Radicals. The rate constant for the reaction of dissociated triose reductone with the OH radical was measured by the competition between TRH^- and t-BuOH with OH (Eqs. 3 and 6).

$$t\text{-BuOH} + OH \longrightarrow t\text{-BuOH radical} + H_2O \tag{6}$$

From this competition it follows that

$$\frac{D_0}{D_s} = 1 + \frac{k_6[t\text{-BuOH}]}{k_3[\mathrm{TRH^-}]}, \tag{I}$$

where D_0 and D_s are the optical densities at 400 nm in the absence and presence of t-BuOH, respectively. t-Butyl alcohol is a well-known scavenger of OH redicals. A plot of D_0/D_s versus $[t\text{-BuOH}]/[\mathrm{TRH^-}]$ gives a straight line (Fig. 2a). From the slope, k_3 was determined to be $1.4\times10^{10}\,M^{-1}\,s^{-1}$, taking $k_6=5.2\times10^8\,M^{-1}\,s^{-1}$.[21] Below a pH of 3.5, the transient absorption is very small above a wavelength of 310 nm. Therefore, the rate constant for a reaction of undissociated triose reductone with OH radicals was measured by the competition between TRH_2 and SCN^- with OH radicals. (Eqs. 3 and 7)

$$SCN^- + OH \longrightarrow \cdot SCN + OH^- \tag{7}$$

The $\cdot$SCN radical reacts with SCN^- to form $(SCN)_2^{-}\cdot$ which has an absorption maximum at 472 nm, according to the following reaction.

$$SCN^- + \cdot SCN \longrightarrow (SCN)_2^{-}\cdot \tag{8}$$

From this competition it follows that

$$\frac{D_0}{D_s} = 1 + \frac{k_3[\mathrm{TRH_2}]}{k_7[\mathrm{SCN^-}]}, \tag{II}$$

where D_0 and D_s are the optical densities at 472 nm, in the absence and presence of TRH_2, respectively. A plot of D_0/D_s versus $[\mathrm{TRH_2}]/[\mathrm{SCN^-}]$ gives a straight line (Fig. 2b). From the slope, k_3 was determined to be $9.9\times10^9\,M^{-1}\,s^{-1}$, taking $k_7=1.1\times10^{10}\,M^{-1}\,s^{-1}$,[22] which is most reliable in the competition method of thiocyanate. The rate constants of both the dissociated and the undissociated forms are summarized in Table 1. For the sake of a comparison, the data for L-ascorbic acid[3] (where $6\times10^9\,M^{-1}\,s^{-1}$ is used for k_7 and the recalculated data using $k_7=1.1\times10^{10}$

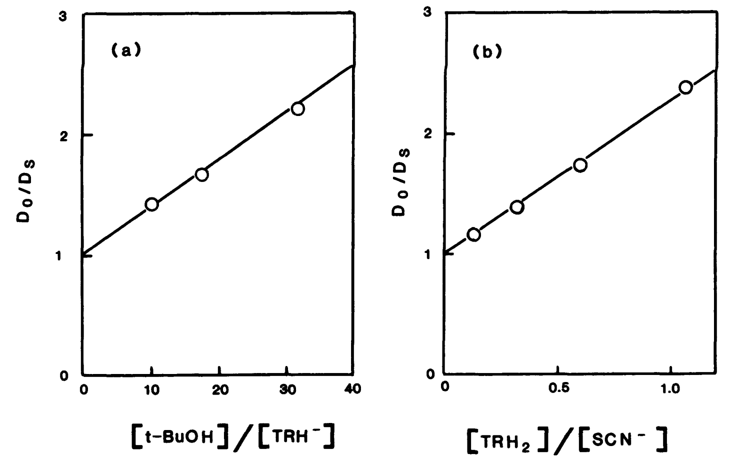


Fig. 2. Competition for OH by triose reductone and *t*-BuOH (or SCN^-). (a): N_2O saturated solution of 1×10^{-4} M triose reductone, pH 9.7, 1—3.2×10^{-3} M *t*-BuOH. (b): N_2O saturated solution of 4×10^{-4} M KSCN, pH 3.01, 0.5—4.3×10^{-4} M triose reductone.

Table 1. Rate Constants in $M^{-1}\,s^{-1}$ for the Reactions of Triose Reductone and Ascorbic Acid with OH Radicals

$k(TRH_2)$	$k(TRH^-)$	$k(AH_2)$	$k(AH^-)$
9.9×10^9	1.4×10^{10}	8.2×10^{9} [a)] 4.5×10^{9} [b)]	1.3×10^{10} [a)] 7.0×10^{9} [b)]

TRH_2: triose reductone, TRH^-: monoanion of triose reductone, AH_2: ascorbic acid, AH^-: monoanion of ascorbic acid.

a: recalculated data from Ref. 3 using $k_6=1.1\times10^{10}\ M^{-1}\,s^{-1}$.

b: Ref. 3.

$M^{-1}\,s^{-1}$) are also listed in Table 1. The rate constants for an addition of OH radicals with both reductones are almost diffusion controlled, but a little larger in dissociated forms than undissociated ones, respectively.

pK_a of the OH Adduct of Triose Reductone. The molar extinction coefficients (ε) of the OH adduct of triose reductone (determined at 400 nm as a function of pH) are shown in Fig. 3. The numerical values of ε were corrected for the transient species ($\varepsilon_{400\,\mathrm{nm}}=300$ $M^{-1}\,cm^{-1}$)[19] produced by a reaction of triose reductone with the H radical. The smooth line in Fig. 3 was computed by assuming that the pH dependence of the extinction coefficient results from an acid-dissociation process, using $\varepsilon_{400\,\mathrm{nm}}^{TRH_2(OH)\cdot}=250\ M^{-1}\,cm^{-1}$, $\varepsilon_{400\,\mathrm{nm}}^{TRH(OH)^{-}\cdot}=3150\ M^{-1}\,cm^{-1}$. The acid-dissociation constant giving the best fit to the data was pK_a=4.7. The acid-base equilibrium is given as follows.

$$TRH_2(OH)\cdot \xrightleftharpoons{pK_a=4.7} TRH(OH)^{-}\cdot + H^+ \qquad (9)$$

The pK_a value of the OH adduct transient species is a little smaller than that of triose reductone (pK_1=5.0). This fact shows that the OH adduct radical is a little more acidic than triose reductone.

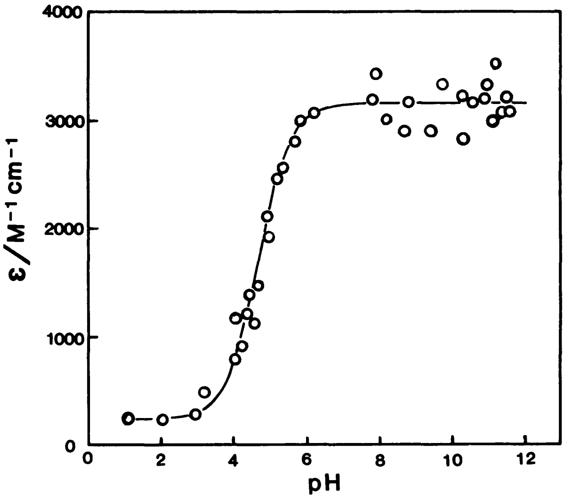


Fig. 3. Dependence of molar extinction coefficient(ε) at 400 nm, of the OH adduct of triose reductone on pH. N_2O saturated solutions of 1—5×10^{-4} M triose reductone.

Radical Decay Rate. The transient species yielded by a reaction of triose reductone with OH radicals decay by second-order kinetics over a wide pH range (pH 2—12). The reaction rate for two ions is dependent on the ionic strength (μ), as approximately given[23] by

$$\log k/k_0 = Z_A Z_B(1.02\mu^{1/2})/(1+\mu^{1/2}) = Z_A Z_B m, \qquad (III)$$

where k_0 is the value of k at zero ionic strength, Z_A and Z_B are the charges of the two ions, and μ is the total ionic strength. The effect of the ionic strength on the decay rate of the OH adduct of triose reductone was studied at pH 9.4, where the radical was presumed to be dissociated from the value of pK_a (4.7), using sodium sulfate to vary μ. A plot of log $k_{obsd}/\varepsilon_{400}$ vs. m gave a straight line with a slope of

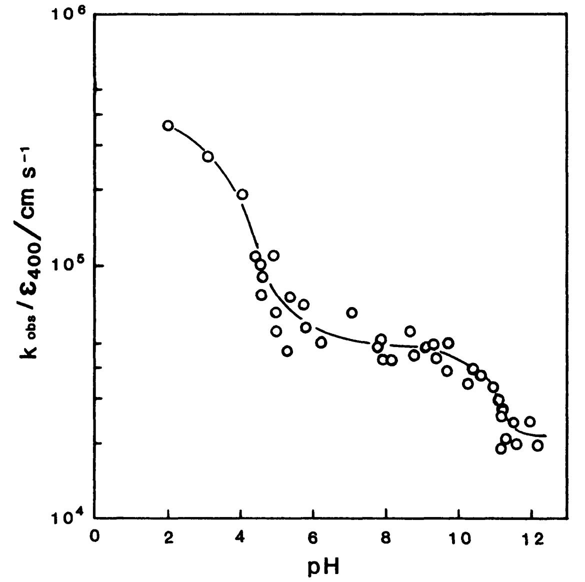


Fig. 4. Dependence of decay rate ($k_{obsd}/\varepsilon_{400}$) of the OH adduct of triose reductone radical corrected for ionic strength on pH.
N_2O saturated solutions of 1—5×10^{-4} M triose reductone.

0.77. Hence, this intermediate probably has a unit negative charge and can be assigned to the one-dissociated OH adduct radical ($TRH(OH)^{-\cdot}$) in the pH range 5—10. Figure 4 shows the pH dependence of $k_{obsd}/\varepsilon_{400}$, where k_{obsd} is the observed second-order rate constants of the transients at 400 nm in triose reductone solutions saturated with N_2O. The rate constants were corrected for ionic strength. The values of k_{obsd} are almost constant in the pH range 5.5—9.5 ($k_{obsd}=1.5\times10^8\ M^{-1}\ s^{-1}$). However, in the pH range 3—5.5, complex reactions as a result of the acid-base equilibrium of the intermediate occur according to the following equations.

$$TRH(OH)^{-\cdot} + TRH(OH)^{-\cdot} \longrightarrow \text{Products} \qquad (10)$$

$$TRH_2(OH)\cdot + TRH(OH)^{-\cdot} \longrightarrow \text{Products} \qquad (11)$$

$$TRH_2(OH)\cdot + TRH_2(OH)\cdot \longrightarrow \text{Products} \qquad (12)$$

The effect of the H adduct of triose reductone seems to become greater around pH 2. The rate constant of k_{obsd} at pH 11.5 is $7\times10^7\ M^{-1}\ s^{-1}$. The change of the rate constant around pH 11 is not yet well understood. This result might suggest the effect of a second dissociation of the OH adduct. However, since the data vary to a considerable extent, it is difficult to give a clean explanation.

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