Redox Chemistry of 3-Iodotyrosine in Aqueous Medium

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The reactions of 3-iodotyrosine (ITyOH) with the primary $^{\bullet}OH/^{\bullet}O^{-}$, $H^{\bullet}$, and e_{aq}^{-} and selected secondary radicals in irradiated aqueous solutions have been studied. The pK_a values of ITyOH are 2.1 ± 0.1 (−COOH), 8.3 ± 0.05 (Ph-OH), and 10.0 ± 0.1 ($-NH_3^+$), and its reactivity is pH-dependent. The phenoxyl radical ($ITyO^{\bullet}$) formed during oxidation exhibits λ_{max} at 275 nm ($\epsilon = 8200 \pm 400$ M^{-1} cm^{-1}) and 405 nm ($\epsilon = 3300 \pm 270$). The rate of oxidation increases with the pH; e.g., for the Br_2^- radical, $k = 8.1 \times 10^7$ M^{-1} s^{-1} at pH 6.7 and 5.0×10^8 M^{-1} s^{-1} at pH 11.7. The $^{\bullet}OH$ radical reaction leads to the formation of the adduct radical $ITy(OH)_2^{\bullet}$ in 30% of the cases while 70% lead to rapid formation of $ITyO^{\bullet}$. Oxygen shows negligible reactivity toward $ITyO^{\bullet}$, but it reacts with $ITy(OH)_2^{\bullet}$ with $k = 1 \times 10^7$ M^{-1} s^{-1}. The respective one-electron reduction potentials (E) for the $ITyO^{\bullet}$, H^+/ITyOH, and $ITyO^{\bullet}$/$ITyO^-$ couples are 0.82 ± 0.03 V at pH 7.4 and 0.73 ± 0.04 V vs NHE at pH 11.5. Reduction by e_{aq}^- ($k = 1.2 \times 10^{10}$ M^{-1} s^{-1} at pH 6.5, 7.5×10^9 M^{-1} s^{-1} at pH 10, and 5.0×10^9 M^{-1} s^{-1} at pH 12) follows different paths at different pH. Deamination and deiodination occur at pH < 8, at nearly equal proportions. However, at pH 8−11, the yield of ammonia decreases whereas the yield of I^- increases. The radical produced by deiodination, $^{\bullet}TyOH$, exhibits λ_{max} at 280 nm with $\epsilon = 6400 \pm 400$ M^{-1} cm^{-1} and reacts with oxygen with $k \approx 10^7-10^8$ M^{-1} s^{-1}. In the absence of oxygen, $^{\bullet}TyOH$ decays by radical dimerization reaction with $2k = 5.3 \times 10^9$ M^{-1} s^{-1}. In alkaline pH, however, the $^{\bullet}TyO^-$ radical rearranges into $TyO^{\bullet}$. The latter species oxidizes $ITyO^-$ to $ITyO^{\bullet}$ with $k = 10^8$ M^{-1} s^{-1}. Formation of the semioxidized transient during reduction in alkaline pH is unique to 3-iodotyrosine and has not been reported for either tyrosine or 3,5-diiodotyrosine. The $H^{\bullet}$ atoms reaction takes place with $k = 8 \times 10^9$ and 5×10^9 M^{-1} s^{-1} at pH 1.5 and 5, respectively, and leads to the formation of the adduct $ITyOH_2^{\bullet}$ with λ_{max}= 360 nm and $\epsilon = 1370 \pm 100$ M^{-1} cm^{-1}.

Introduction

3-Iodotyrosine (ITyOH) is an intermediate in the synthesis of the growth hormones triiodothyronine (T3) and thyroxine (T4) in the thyroid.[2−4] Iodination of tyrosine residues in the protein thyroglobulin leads to the formation of ITyOH. Physiological disorders resulting in oxidative or reductive stress in the thyroid may affect the hormone synthesis by opening up new channels for its chemistry.

To study the redox chemistry of ITyOH in the laboratory, radiation chemical techniques have been used. The redox environments in aqueous media were generated employing dilute solutions of ITyOH in the range of $10^{-6}-10^{-3}$ M. To study these reactions, conditions generating appropriate radical initiators were selected following well-accepted methodology. Some of these radicals are also involved in in vivo chemistry. The pulse radiolysis technique was used to study transient behavior, and γ-radiolysis experiments complemented these results to confirm the reaction mechanism. Although some studies have been reported on the actions of α- and X-radiations on ITyOH at low pH[5] and also on its γ-radiolysis in aqueous−alcohol solutions,[6] no details are available on the transient behavior, reaction mechanisms, and nature of degradation products in aqueous solutions at a pH of biological relevance. In this paper, some of these reaction mechanisms have been elucidated, separately under oxidizing and reducing conditions. Keeping in view the ubiquitous nature and efficient radical scavenging property of oxygen in biology, its effects on the progress of these reactions have been checked.

Experimental Section

Materials. 3-Iodo-L-tyrosine (ITyOH) and L-tyrosine (TyOH) (Fluka, purity>99%) were used without further purification. All other chemicals used were of AR or similar grades available from Sigma and JT Baker. Solutions were prepared in Barnstead Nano Pure water (resistivity >17 Mohm cm^{-1}). Stock solutions of ITyOH were prepared and deoxygenated. (At 25 °C a saturated 5×10^{-3} M solution was obtained.) The gases N_2, O_2, and N_2O used for saturating the samples were obtained locally from Indian Oxygen Limited (Iolar grade, 99.95% purity). Unless stated otherwise, solutions within the pH range 3−12 were prepared in 5×10^{-3} M phosphate buffer (mixture of Na_2HPO_4 and KH_2PO_4), and the pH of more acidic or alkaline solutions was adjusted to the required value either with $HClO_4$ or KOH, respectively. All organic solvents used in these experiments were distilled before use.

General Experimental Procedures. Some details of the nanosecond pulse radiolysis setup (LINAC) employed in this study have appeared earlier in the literature.[7] Its current features are as follows: pulse widths of 25, 50, and 500 ns and 2 μs duration are available from the unit. The absorbed dose in each is variable from a lower 20% of the maximum value to an upper limit of ≈10 Gy for 25 ns to ≈130 Gy for 2 μs with proportional values for the intermediate pulse widths. Variation between successive pulses was less than 3%. While low-dose conditions

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using nanosecond pulses were preferred for kinetic estimations, higher doses with 500 ns or 2 μs were used in spectral studies. Dosimetry was performed at 500 nm using 0.01 M aerated solution of KCNS using the $G\epsilon(CNS)_2^-$ value of 2.23×10^{-4} m^2 J^{-1} from the literature.[8] The optical detection setup consists of a kinetic spectrophotometric unit in the range 250−800 nm, and data analysis is possible within a time scale of 500 ns−10 ms after pulse. The wavelength resolution for spectral measurements is better than 2 nm in the near-UV−vis range and marginally higher at 4 nm below 300 nm. The contribution of scattered light was less than 10% at 250 nm, decreasing to a negligible value above 300 nm. A user-controlled pumped (peristaltic) flow system was used for changing samples. The data analysis was performed on an IBM-PC using dedicated software.

For γ-radiolysis experiments, a Co-60 irradiation facility was used. The dose rate, determined by Fricke dosimeter, was 8 Gy min^{-1}.[9a] Optical absorption spectra were measured on a Hitachi 330 UV−vis spectrophotometer. Sample fluorescence was measured on a Hitachi F-4010 fluorescence spectrophotometer. Frequently, an aliquot of the sample used in the γ-radiolysis experiment was also exposed to 2 μs electron pulses from the accelerator, and the results for the same absorbed dose (at very different dose rates) were compared. The total absorbed dose in these experiments was maintained at a level such that the ratio [ITyOH]/[all radicals produced] $\geq$10 in each case. This was necessary to minimize secondary reactions between the radicals and the reaction intermediates or products. Depletion of ITyOH was estimated from the difference in its absorption spectra in the unirradiated and irradiated samples. In these measurements, the extent of positive interference due to absorption by reaction products was estimated from the changes in the absorbance values at the peak and troughs on either sides of the ITyOH absorption peak. (The estimated error was $\leq$15%.) Stable end products I^-, NH_3, and tyrosine from irradiated samples were analyzed to estimate the extent of reactions following different paths. These are discussed later in the text.

Generation of Oxidizing and Reducing Radicals. High-energy electrons produce a mixture of primary oxidizing and reducing radicals (eq 1). The quantities in parentheses indicate their radiation chemical yields (at pH 7) in μmol J^{-1}.[9b,10]

$$H_2O \rightsquigarrow H_3O^+ (0.28), {}^\bullet OH (0.28), H^\bullet (0.06), e_{aq}^- (0.28), H_2O_2 (0.071), H_2 (0.056) \quad (1)$$

The reactions of the $^\bullet$OH radical are studied by prior saturation of the matrix with N_2O. At pH > 4 it leads to an efficient scavenging of the hydrated electron, eqs 2 and 3, that causes the yield of $^\bullet$OH to double.

$$N_2O + e_{aq}^- \rightarrow N_2 + {}^\bullet O^- \quad (2)$$

$${}^\bullet O^- + H_2O \rightleftharpoons {}^\bullet OH + OH^- \quad (3)$$

$$H^\bullet + OH^- \rightarrow e_{aq}^- \quad (4)$$

The effect of H$^\bullet$, produced in comparatively lower yield, has been neglected in this study unless specifically discussed in the text. At pH < 3.5, scavenging e_{aq}^- by H_3O^+ increases the yield of H$^\bullet$ (ca. $G(H^\bullet) = 0.34$ μM J^{-1} at pH = 2, N_2 saturated). To study the reactions of the $^\bullet$OH or other oxidizing radicals at pH < 3.5, H$^\bullet$ was scavenged with O_2; the resulting $HO_2^\bullet$ radicals did not react with ITyOH. In highly alkaline pH, the $^\bullet$OH radical is converted into $^\bullet O^-$ ($pK_a = 11.9$). Selected secondary radicals were generated by the reactions of the primary radicals with appropriate solutes according to well-established reaction schemes.[10−12]

TABLE 1: Transient Kinetic and Spectral Data for Oxidation of ITyOH/ITyO$^-$

oxidizing radical	pH	k_f (±10%) (M^{-1} s^{-1})	$\epsilon_{405/275}$ (M^{-1} cm^{-1})	$2k/\epsilon$ (±10%) 405 nm (s^{-1})
$Cl_2^{\bullet-}$	1.5−2	2.4×10^9	3304/8300	4.6×10^5
$Br_2^{\bullet-}$	6.7	8.1×10^7	3410/8360	5.1×10^5
	11.7	5.0×10^8		
$I_2^{\bullet-}$	9.5	1.6×10^7	3600/8410	4.0×10^5
	11.5	5.8×10^7		
$(SCN)_2^{\bullet-}$	6.8	2.4×10^7		
	11.5	4.1×10^8	3300/8280	6.8×10^5
$N_3^\bullet$	5	1×10^9	3275/8360	6.2×10^5
	12	6×10^9	3350/8350	7.2×10^5
$Br^\bullet$	7	5.0×10^9	3300/8270	3×10^5
$CCl_3O_2^\bullet$	7	5.0×10^8	3180/8100	1×10^5
Tl^{2+}	2	1.1×10^9	3350/8320	4.8×10^5
$O_3^{\bullet-}$	12	6.5×10^8	3200/8600	8×10^5
$CO_3^{\bullet-}$	12	3.9×10^8	3320/8590	1×10^6
$NO_2^\bullet$	12	3.0×10^7	2940/7900	1×10^6
$^\bullet OH$	1−7	1.5×10^{10}	3200/9600[a]	4.7×10^5
	>10	2.3×10^{10}		
	6	1.8×10^{10} [b]		
	10	2×10^{10} [b]		
	5		6000[c]	4.3×10^4
$^\bullet O^-$	13	2.7×10^9 [d]	3190/8150	6.5×10^5

[a] Higher value due to the additional 30% contribution of the −OH adduct. [b] Competetion kinetics, with respect to SCN^-, measured at 500 nm. [c] At 305 nm, assuming 30% yield and correcting for the contribution from the 275 nm peak comparing with transient absorption spectrum obtained with $N_3^\bullet$ radical. [d] Corrected for $^\bullet$OH contribution.

Results and Discussions

In spectrophotometric measurements, the absorption peak of ITyOH at 280 nm at neutral pH shifted to 305 nm at pH > 10. These measurements gave a pK_a value of 8.3 ± 0.05 for the phenolic OH. Three pK_a values estimated by titrimetric method were 2.1 ± 0.1, 8.30 ± 0.1, and 10.0 ± 0.1. The first value has been assigned to the $-CO_2H$ group and the last to the $-NH_3^+$ group. Thus, at or near the physiological pH (7.4), the matrix of concern in mammalian thyroid, virtually all ITyOH molecules exist in the phenolic form. However, to complement and compare the redox reactions of ITyOH, a wider pH range has been covered in this study.

Reactions with Secondary Oxidizing Radicals. The appropriate $X_2^{\bullet-}$ radicals were generated by the reaction of $^\bullet$OH radical with Cl^- (0.05 M), Br^- (0.02 M), I^- (0.02 M), and SCN^- (0.02 M). The $N_3^\bullet$ radical was produced by $^\bullet$OH/$^\bullet O^-$ with N_3^- (0.01 M). The spectral parameters and kinetic constants of the transients formed in reactions of ITyOH with different oxidizing radicals are listed in Table 1. Some representative transient spectra are shown in Figure 1. These spectra have been corrected for ITyOH bleaching between 250 and 330 nm (and absorption due to the $ITyOH_2^\bullet$ radical formed by H$^\bullet$ reaction, between 330 and 430 nm, discussed below). In these experiments, the radical−radical decay was assumed to be negligible, and the yield of the resulting transient was taken equal to the initiating radical yield. The two peaks observed at 275 and 405 nm are similar to those of the phenoxyl radical obtained from tyrosine.[13,14] For Cl_2^-, Br_2^-, I_2^-, and $(SCN)_2^-$ as the oxidizing radicals, due to their intense optical absorption in the near-UV and visible range,[11] the kinetics of their reaction with ITyOH were measured near their peak absorption (λ_{max}) in the

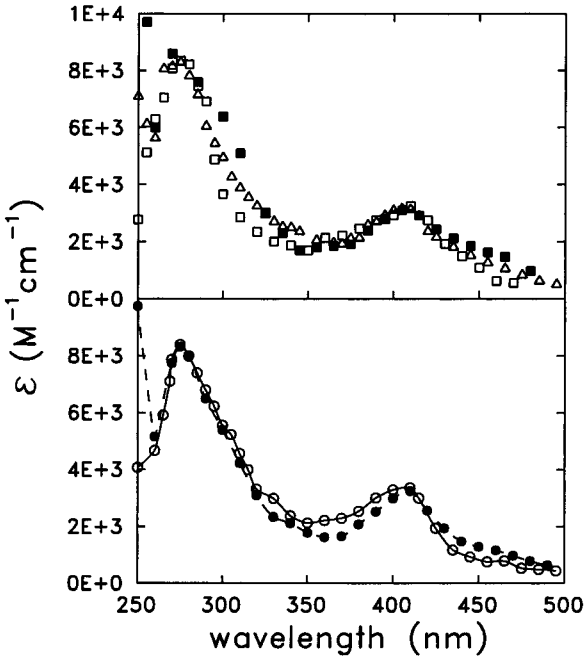


Figure 1. Absorption spectra of semioxidized ITyOH/ITyO$^-$. (top) Oxidizing radical (□) Cl_2^-, 8 μs after pulse, pH = 1.6, O_2 saturated, [Cl^-] = 0.05 M; (△) $CCl_3O_2^•$, 20 μs after pulse, pH = 7, O_2 saturated, 4% CCl_4, 48% *i*-PrOH; (■) CO_3^-, 12 μs after pulse, pH = 12, N_2O saturated, [CO_3^{2-}] = 20 mM. **(**bottom) Oxidizing radical $N_3^•$, [N_3^-] = 10 mM, 8 μs after pulse, pH 5 (○), pH 12 (●), both N_2O saturated. Absorbed dose in each case 15 Gy and [ITyOH] = 0.5 mM.

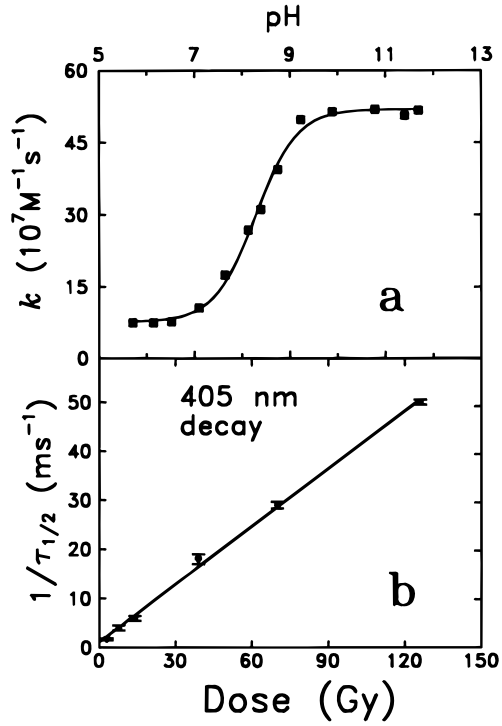


Figure 2. (a**)** Variation of the bimolecular rate constant *k* with matrix pH for the oxidation of ITyOH/ITyO$^-$ by Br_2^-. Matrix: 10 mM phosphate, [Br^-] = 10 mM, N_2O saturated, dose 5 Gy. **(**b) Variation of $(1/\tau_{1/2})_{first}$ for ITyO$^•$ decay vs [ITyO$^•$]$_{max}$ (proportional to the absorbed dose). Matrix: [N_3^-] = 10 mM, pH = 6.5, N_2O saturated, [ITyOH] = 1 mM.

presence of increasing amounts of ITyOH. For $N_3^•$ as the oxidizing radical, the rate constant was measured from the formation rate at 405 nm. With Br_2^- as the oxidizing radical, an increase in the rate constant was observed in the alkaline pH. A plot of *k* vs pH (Figure 2a) gave a pK_a value of 8.3 ± 0.1. The variation of the *k* values against pH for $N_3^•$ and I_2^- as the oxidizing radicals (not shown) also gave identical pK_a values. This confirmed the assignment of the value of 8.3 for the phenolic −OH since phenoxides are known to be oxidized more rapidly than the respective phenols. With I_2^-, in acidic pH, the low rate constant probably indicates that the reduction potential of the ITyO$^•$,H^+/ITyOH couple is close to that of the $I_2^-/2I^-$ couple (1.04 V vs NHE).

The linear plot of the inverse of the first $\tau_{1/2}$ of ITyO$^•$ decay against the absorbed dose (Figure 2b) indicates a radical−radical reaction. The second-order decay remained unaffected with increasing [ITyOH] or [O_2]. Low reactivity of tyrosine phenoxyl radical with oxygen has been observed earlier.[15] The ITyO$^•$ radicals probably decay by dimerization as reported for tyrosine[16−18] and phenol[19] and postulated for 3,5-diiodotyrosine.[20] Other secondary oxidizing radicals used (Table 1) include the bromine atom generated by the reaction of $^•$OH with 1,2-dibromoethane (DBE, saturated) at pH 7;[21] trichloromethylperoxyl radical ($CCl_3O_2^•$ from 0.4 M CCl_4, 5 M isopropyl alcohol and saturated with O_2 with $G = 0.62$);[22] Tl^{2+} generated from a solution of Tl^+ (5 mM) at pH 2 saturated with oxygen;[23] CO_3^- generated in the presence of CO_3^{2-} (0.02 M) in N_2O-saturated matrix; and O_3^- radicals generated in solutions containing a mixture of N_2O and O_2 ([N_2O]/[O_2] ⩾ 20) at pH 12.[10] In all these cases, the resulting transient characteristics (Table 1 and Figure 1) revealed that the oxidation of ITyO$^-$ produced only the phenoxyl radical.

Reactions with $^•$OH/$^•O^-$ Radicals. These reactions were studied over a wide pH range of 1−13.5. From pulse radiolysis studies, the time-resolved transient spectra at pH 5 are presented

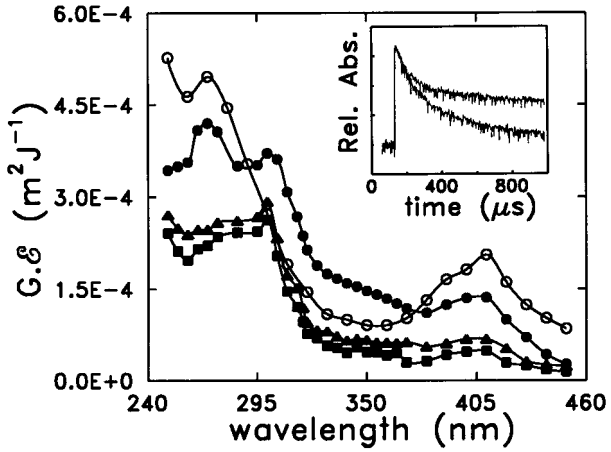


Figure 3. Time-resolved transient spectrum with$^•$OH radical as the oxidant: (●) 7 ms after pulse; (▲) 100 ms after pulse and (■) 250 ms after pulse. Absorbed dose 11.5 Gy, pH 5.5, N_2O saturated; [ITyOH] = 0.3 mM. Oxidation of ITyO$^-$ with$^•O^-$ at pH 13.5 shown for comparison (○) for the same absorbed dose in a similar matrix. Inset: decay of absorbance at 305 nm in the absence (upper trace) and presence of 0.5 mM O_2 (lower trace) at pH 5.5.

in Figure 3. While, the peaks at 275 and 405 nm indicate the formation of ITyO$^•$, another prominent peak observed at 305 nm showed different decay kinetics as compared to the transient decays at 275/405 nm (Table 1). The peak at 305 nm was absent when $^•O^-$ was the oxidizing radical at pH 13.5 (Figure 3, contribution from the $^•$OH radical <5%). The probable transients giving rise to this peak at 305 nm are **(**i**)** the $^•$OH adduct radical (ITy(OH)$_2^•$) since $^•$OH is known to add to phenolic rings[24] or **(**ii**)** a radical cation as observed earlier under similar conditions for 3,5-diiodotyrosine.[20] The nature of the transient absorbing at 305 nm was confirmed to be the adduct

$ITy(OH)_2^{\bullet}$ from its reactivity with O_2 (Figure 3 inset). The rate constant for oxygen addition to $ITy(OH)_2^{\bullet}$ was 1×10^7 M^{-1} s^{-1} (decay of a cation is expected to remain unaffected).[25] The difference in A_{405nm} values obtained from a comparative evaluation of the transient spectra obtained with $N_3^{\bullet}$/$^{\bullet}OH$ radicals suggests the following reaction mechanism: after the initial addition of the $^{\bullet}OH$ radical **(**a**)** 70% of the adducts eliminate H_2O in nanosecond time scale, and the resulting $ITyO^{\bullet}$ is detected as one of the transients; **(**b**)** the remaining 30% of the $^{\bullet}OH$ adducts have longer lifetime and are detected as the $ITy(OH)_2^{\bullet}$ transient. Differences in the nature of the $^{\bullet}OH$ adducts arise because ITyOH offers addition sites of dissimilar nature on the ring. Near diffusion-controlled rates (Table 1) suggest the addition process is of random nature, possibly influenced by the available electron density at various C atoms. The longer lived adducts are likely to be those where the $^{\bullet}OH$ is added at the *meta* position relative to the phenolic −OH, where concurrent loss of a molecule of water is not favored. The $ITy(OH)_2^{\bullet}$ decays by radical dimerization reaction ($2k = 2.5 \times 10^8$ M^{-1} s^{-1}) but, unlike the $^{\bullet}OH$ adduct of 3,5-diiodotyrosine,[20] remains unaffected by increasing amounts of the parent compound. The marginal increase in the formation rate constant of $ITyO^{\bullet}$ with pH in the alkaline medium (pH 7−11, Table 1) reflected the same pattern as observed for Br_2^-, presented above in Figure 2. This suggests that the rate of loss of a molecule of water from the *o*- and *p*-OH adducts, discussed above, is probably faster or equal to the rate of $^{\bullet}OH$ addition. Neither the phenoxyl nor the adduct radical showed a pK_a in the pH range 1−13.5. Although the pK_a of phenoxyl radicals arising from phenols are close to pH = 0,[26] no measurements have been reported for the pK_a of the $^{\bullet}OH$ adducts of phenols.

Reactions with these primary and secondary radicals indicate that oxidation of 3-iodotyrosine proceeds mainly via the phenoxyl radical. Comparison of the absorption spectrum in different cases (Table 1) suggests that extents of other reactions, such as abstraction of an aliphatic hydrogen atom, are negligible. Although only some of these radicals may take part in the cellular reactions in the thyroid, other radicals were also included in this study **(**i**)** to estimate the propensity of possible reaction channels and **(**ii**)** to compare 3-iodotyrosine reactivities with that of tyrosine and 3,5-diiodotyrosine toward these radicals.[12,20] In Figure 4, the rate constants with different radicals are compared for tyrosine, 3-iodotyrosine, and 3,5-diiodotyrosine. Close matching of these values for the latter two and their almost 1 order of magnitude higher than the former (especially in those cases where the oxidizing radicals are moderate) indicates **(**i**)** the initiation of oxidations of iodo-substituted tyrosines will not be affected appreciably in the presence of each other in the thyroid where these are reported to coexist and **(**ii**)** first iodine substitution on the ring enhances the electron density appreciably to override any opposing steric effect due to the presence of bulky iodine substitution. However, similar values for both the substituted tyrosines suggest that, with the second iodine substitution, these opposite effects become balanced. Since the oxidation is initiated mainly by an electron transfer, inside the thyroid cells, the extent of ITyOH depletion under an oxidative stress can be better judged from its one-electron reduction potential.

One-Electron Reduction Potential. The reactivity of ITyOH with the secondary oxidants and the reported one-electron reduction potentials of tyrosine ($E = 0.93$ at pH 7) and 3,5-diiodotyrosine ($E = 0.78$ at pH = 7.4)[20,27,28] suggest that reference couples with $E \leq 1.0$ V vs NHE may be suitable for estimation of E_{ITyOH} at different pH; the parent phenolic pK_a of

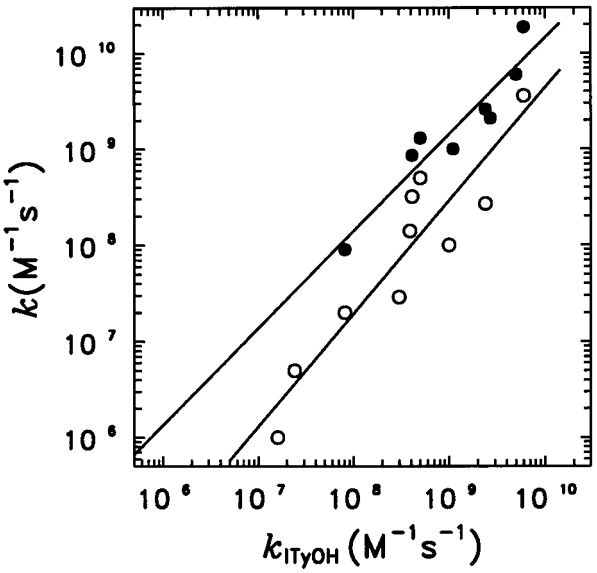


Figure 4. Comparison of bimolecular rate constants for oxidation of ITyOH/$ITyO^-$ with secondary oxidizing radicals and $^{\bullet}O^-$ against bimolecular rate constants for oxidation of tyrosine (○) and 3,5-diiodotyrosine (●).

8.3 indicates a linear increase of E for the $ITyO^{\bullet},H^+$/ITyOH couple in the acidic pH.[27] To estimate E over the pH range 3−12, the following secondary standards were found suitable: SO_3^-/SO_3^{2-} couple,[29] $CyS^{\bullet}/CyS^-$ couple[30] where $CyS^{\bullet}$ is the thiyl radical derived from oxidation of cysteine, $I_2^-/2I^-$ and $NO_2^{\bullet}/NO_2^-$ couples.[27] Briefly, these radicals were prepared as follows: $NO_2^{\bullet}$ was generated employing either **(**a**)** N_2-saturated solution containing NO_2^- (and NO_3^- to scavenge the e_{aq}^-) or **(**b**)** N_2O-saturated solution with NO_2^-. The $G(NO_2^{\bullet})$ was 0.56 in both these cases.[12] $CyS^{\bullet}$ was produced in N_2O-saturated solution by H-abstraction reaction of $^{\bullet}OH$ radical; SO_3^- was produced by oxidation of SO_3^{2-} in N_2O-saturated solution. From the equilibrium reaction 5 with $S^{\bullet}$ as the appropriate oxidizing radical, the equilibrium constant K in eq 6 was estimated.

$$S^{\bullet} + ITyO^- \text{ (or ITyOH)} \rightleftharpoons S^- + ITyO^{\bullet} (+H^+) \quad (5)$$

$$K = \frac{[S^-]}{[ITyO^-]} \frac{[ITyO^{\bullet}]}{[S^{\bullet}]} \quad (6)$$

These studies were carried out at a low absorbed dose of ≤4 Gy, and by keeping [solute]/[radical] >100, radical−radical reactions were minimized. It was assumed that any other mode of reaction was negligible within the time scale of equilibration. Depending on the absorption characteristics of the radicals involved, either the formation or decay of the $ITyO^{\bullet}$ radical at 275/405 nm or the decay of $S^{\bullet}$ at appropriate wavelength was measured. In the case of cysteine, the error due to the absorption of $CyS^{\bullet}$ or its dimer cation radical ($CyS^{\bullet+}SCy$, formed during its reaction with excess parent cysteine) was <10% of the absorption due to the $ITyO^{\bullet}$ radical at 275 nm. For the SO_3^- and $NO_2^{\bullet}$ radicals, measurements were made at 405 nm. To estimate K in relation 6, radical concentrations were estimated from the absorbance values using the relation $c = A/\epsilon l$ ($l = 1$ cm in the present case; ϵ was corrected for bleaching or mixed absorption) and taking $[S^{\bullet}] + [ITyO^{\bullet}] = [\text{initiating radical}]$. The methodology has been described in the literature.[27]

The results are presented in Table 2 and in Figure 5; the E values measured at different pH are shown. From this plot, the E for $ITyO^{\bullet},H^+$/ITyOH and $ITyO^{\bullet}/ITyO^-$ couples are 1.26

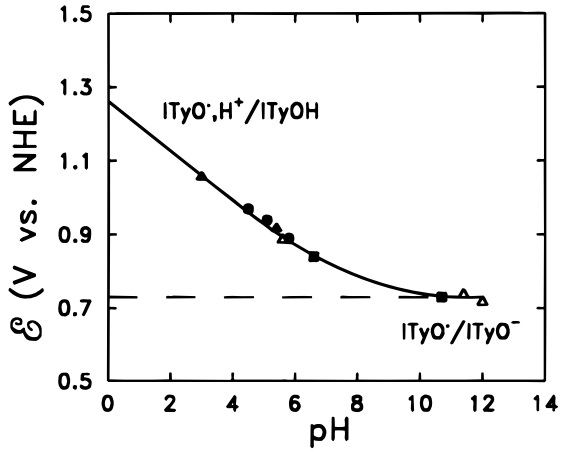


Figure 5. Plot of measured one-electron reduction potential (E) vs matrix pH. Reference couples: (▲) $I_2^{\bullet-}/2I^-$; (●) $NO_2^{\bullet}/NO_2^-$; (■) $CyS^{\bullet}/CyS^-$, and (△) $SO_3^{\bullet-}/SO_3^{2-}$. Absorbed dose 5 Gy in each case. Solid line represents the variation of E taking into account the pK_a of 8.3 of the phenolic group. From plots $E^0 = 0.73$ V for ITyO•/ITyO$^-$ and 1.26 V for ITyO•,H$^+$/ITyOH couples.

TABLE 2: One-Electron Reduction Potentials of ITyO•,H$^+$/ITyO$^-$ and ITyO•/ITyO$^-$ Couples

oxidizing radical	pH	K	E_{ref} (V)	E (±0.03 V)
$I_2^{\bullet-}$	3	0.46	1.04	1.06
	5.3	110	1.04	0.92
$NO_2^{\bullet}$	4.3	12.6	1.04	0.975
	5	40.8	1.04	0.945
	5.8	276	1.04	0.896
$CyS^{\bullet}$	6.5	22	0.92	0.841
	10.7	1120	0.72	0.740
$SO_3^{\bullet-}$	5.6	0.14	0.84	0.89
	11.3	0.011	0.63	0.745
	12	0.026	0.63	0.723

V at pH = 0 and 0.73 V at pH = 11.5, respectively. The solid line represents the calculated value based upon $E = 0.73$ V at pH 11.5 taking into account the ITyOH pK_a of 8.3. Close matching of different data sets with the continuous line indicates that the assumptions have been supported by the experimental conditions. These results indicate that, at low pH, the oxidation of ITyOH is expected to be more difficult than at alkaline pH. However, near physiological pH of 7.4 (E = 0.82 V), the oxidation of ITyOH is expected to be fast. A marginally smaller value of E for 3,5-diiodotyrosine at pH ⩾ 7[20] suggests an equilibrium may be established between the semioxidized iodo-substituted tyrosines inside the thyroid cells.

Reactions with Reducing Radicals. To study the reactions of e_{aq}^- and H• with ITyOH, the •OH radicals were scavenged by *tert*-butyl alcohol. The resulting radical, $(CH_3)_2C(OH)^{\bullet}CH_2$, did not show any reactivity toward ITyOH.

Reaction with e_{aq}^-. These reactions were studied over the pH range 4−13. The rate constant for reaction of e_{aq}^- with ITyOH was measured by pulse radiolysis by following the decay of the e_{aq}^- absorption at 700 nm. The values varied from 5×10^9 M^{-1} s^{-1} at pH 12 to 12.2×10^9 M^{-1} s^{-1} at pH 6.5 (Table 3). These rate constants are more than 1 order of magnitude higher than that for tyrosine and reflect the effect of the iodine on the electron affinity of the compound. In previous studies with iodo-aromatics it has been reported that dissociative electron capture reactions take place to form I^- and the appropriate aromatic carbon centered (σ-type) radical transient.[16,17,31] Indeed, at high pH, all the e_{aq}^- reacting with ITyO$^-$ were found to lead to deiodination. The higher rate constant measured at lower pH values may be due to the decreased

TABLE 3: Transient Kinetic and Spectral Data for Reduction of ITyOH/ITyO$^-$

reducing radical	pH	k_f (±10%) (M^{-1} s^{-1})	ϵ (nm) (M^{-1} cm^{-1})	$2k/\epsilon$ (±10%) (s^{-1})
e_{aq}^-	6.5	12.2×10^9	6400 (280)	8.3×10^5
	10	7.5×10^9		
	12	5.0×10^9		1×10^8 [a]
H•	1.2			6.1×10^9 [b]
	1.4	4×10^9 [c]		
	1.5	4×10^9	1370 (360)	3.1×10^6
	5.0	4.8×10^9	1370 (360)	2.8×10^6
$(CH_3)_2C^{\bullet}OH$	7	$<1 \times 10^6$ [d]		
$(CH_3)_2CO^{\bullet-}$	12	$<1 \times 10^6$ [d]		
$CO_2^{\bullet-}$	7, 12	$<1 \times 10^6$ [d]		
$O_2^{\bullet-}$		no reaction		

[a] Reacts with ITyO$^-$ forming TyO$^-$ and ITyO•. [b] Competetion kinetics with thionine measured at 770 nm. [c] Competetion kinetics, with methylviologen, measured at 595 nm. [d] Estimated from $G(I^-)$ in γ-radiolysis assuming competition from radical dimerization reaction.

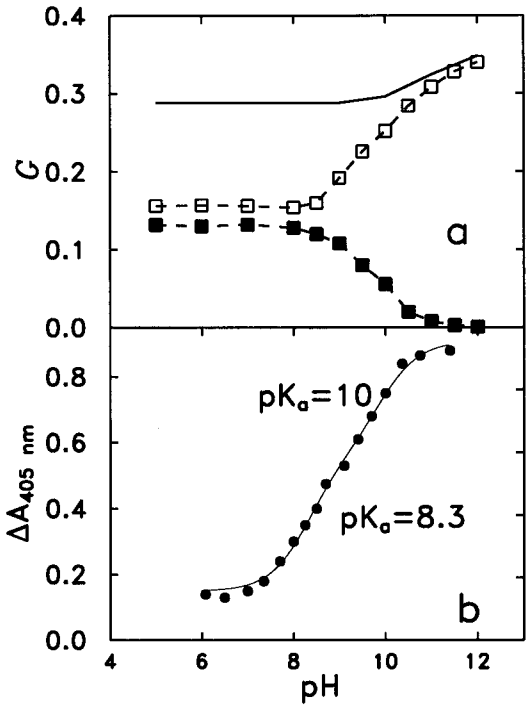


Figure 6. (a) Variation of $G(NH_3)$ (■) and $G(I^-)$ (□) with pH for e_{aq}^- reaction. $G(e_{aq}^-)$ shown for comparison (—). **(b)** Transient absorption ($\Delta A \times 100$) at 405 nm in the similar matrix vs the pH.

electrostatic repulsion between the molecule and e_{aq}^- upon going from the dianion to the species in neutral solution, which has no overall charge. Furthermore, as the pH is decreased below 12, the yield of I^- was found to decrease to a limiting value of $\approx 0.55G(e_{aq}^-)$ at pH < 8. This decrease in the yield of I^- was found to be accompanied by an increasing yield of NH_3 (Figure 6) to a limiting value of $\approx 0.45G(e_{aq}^-)$ at pH < 8. This result suggests that the initial electron adduct to ITyOH has a finite lifetime which permits the electron to go either to the iodine site resulting in the release of I^- or to the positively charged NH_3^+ site and form NH_3. In fact, NH_3 is known to be a major product from the reaction of e_{aq}^- with amino acids (with NH_3^+ group), although the rate constants are considerably lower in the absence of iodine.[10,17] The analytical methods employed in this study to estimate NH_3 and I^- have been discussed earlier.[31]

The transient spectrum at neutral pH from pulse radiolysis experiments is shown in Figure 7. The two possible radicals

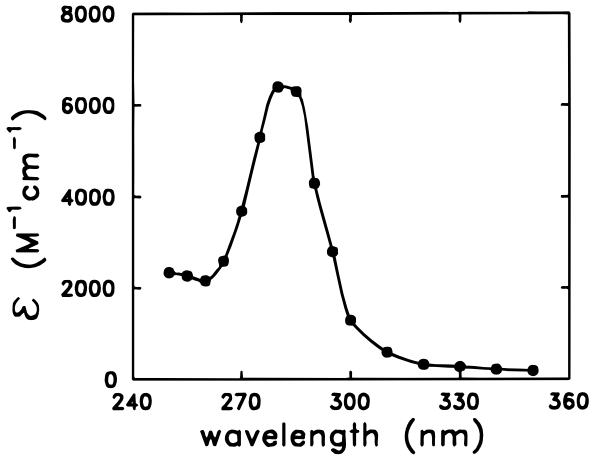


Figure 7. Absorption spectrum of •TyOH transient at pH 7, 6 μs after pulse; absorbed dose 10 Gy. Matrix: 0.1 M *t*-BuOH, N_2 saturated, [ITyOH] = 0.8 mM.

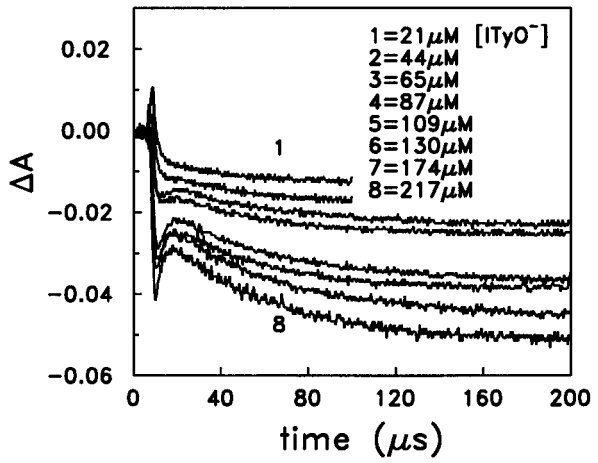


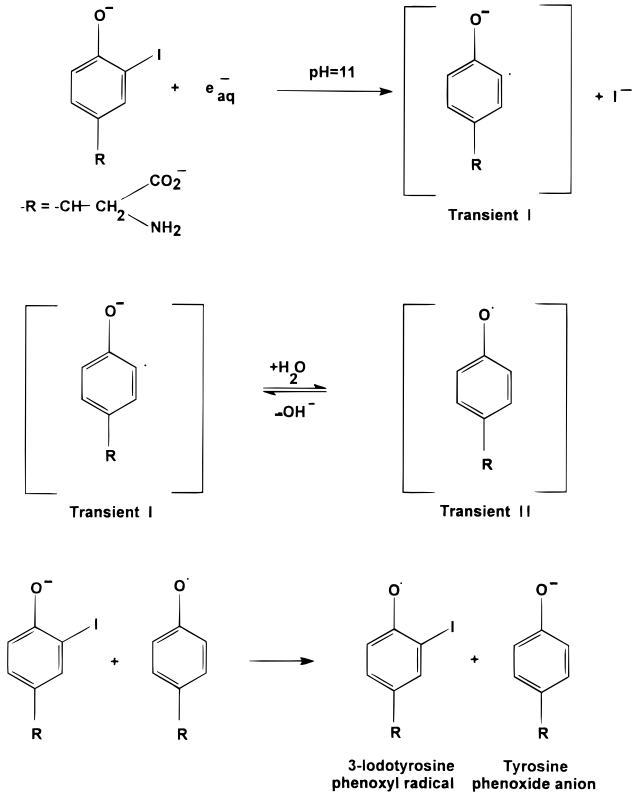
Figure 8. Bleaching signal observed at 310 nm at pH 12 with increasing [ITyO$^-$] in the matrix (10 mM phosphate buffer, N_2 saturated, 0.1 M *t*-BuOH, and absorbed dose = 50 Gy; $G(e_{aq}^-) = 0.34$.

giving rise to this spectrum are (i) the deiodinated •TyOH with the radical center on the ring or (ii) the deaminated aliphatic radical with the radical center on the α-carbon bearing the CO_2^- group. To eliminate the contribution from the latter, spectral studies were conducted at pH > 11. However, due to intense absorption by the parent ITyO$^-$ and opening up of other secondary reaction channels (see below), no conclusive evidence could be obtained. Since the hydroxyphenyl radical has been shown to absorb at 275 nm,[32] it is likely that the spectrum in Figure 7 is due to the •TyOH. The other radical present in solution is an aliphatic radical at position β to the ring, which is likely to absorb mainly below 260 nm. Assuming that the spectrum obtained is that of •TyOH only, and taking into account that the yield of this radical corresponds to only 55% of $G(e_{aq}^-)$, the molar absorptivity was estimated (Table 3 and Figure 7).

The reactivity of oxygen with •TyOH was measured by pulse radiolysis. For these studies, at 7 Gy absorbed dose, $[O_2]$ was restricted within a narrow range of $(4-10) \times 10^{-5}$ M while [ITyOH] was maintained at 4×10^{-3} M, and 1 M *tert*-butyl alcohol was used to scavenge the •OH radicals. Due to these severe restrictions in matrix preparation, the rate constant value of reaction between O_2 and TyOH• radical was estimated to lie in the range 10^7-10^8 M^{-1} s^{-1}.

In pulse radiolysis experiments, at pH > 11, it was observed that e_{aq}^- reactions produced the phenoxyl radical (ITyO•) as the secondary transient with a rate constant close to 1×10^8 M^{-1} s^{-1} and G(ITyO•) ≈ $G(e_{aq}^-)$ (Figure 6). At pH < 11, the G(ITyO•) decreased rapidly and was negligible below pH 7. As shown in Figure 8, at pH 12, during the progress of this reaction, the initial fast bleaching signal (in nanosecond time scale) at 305 nm at first showed a partial recovery that was masked by enhanced bleaching in a slower (microsecond) time scale. In alkaline pH, tyrosine (TyOH) was recovered as an end product. From fluorescence measurements of the irradiated samples, its yield was found to be better than 80% of the $G(e_{aq}^-)$ at pH 11. For these measurements, calibration was performed **(a)** using authentic samples of tyrosine in the presence of ITyOH in the matrix and **(b)** employing the standard addition technique and estimating the amount of radiolytically generated tyrosine in the irradiated samples from the linear plot of [TyOH] vs I_{fl}. These observations can be rationalized by the mechanism shown in Scheme 1. After the initial deiodination, transient I (•TyO$^-$ in this case) rearranges in the presence of water to give the tyrosine phenoxyl radical (transient II which also shows substantial absorption near 305 nm[13] and effects the fast recovery

SCHEME 1



of bleaching in the microsecond time scale). Similar rearrangements have been postulated earlier during reduction of *p*-bromophenol in aqueous medium.[33] The tyrosine phenoxyl radical oxidizes another molecule of 3-iodotyrosine to generate the 3-iodotyrosine phenoxyl radical (ITyO•) as the tertiary transient and a molecule of tyrosine. During radiolysis, although two molecules of ITyOH were destroyed for each e_{aq}^- (Scheme 1), the observed bleaching of only $1.2G(e_{aq}^-)$ was supported by the TyO$^-$ absorption characteristics at 305 nm where the remaining 80% absorption reappeared due to its creation. This mechanism does not operate at lower pH where the phenolic OH group is not dissociated. Negligible G(tyrosine) from γ-radiolysis experiments and a second-order radical decay kinetics even in the presence of excess ITyOH in pulse radiolysis experiments instead suggest a radical dimerization reaction, probably forming a bityrosine type of product. The higher value

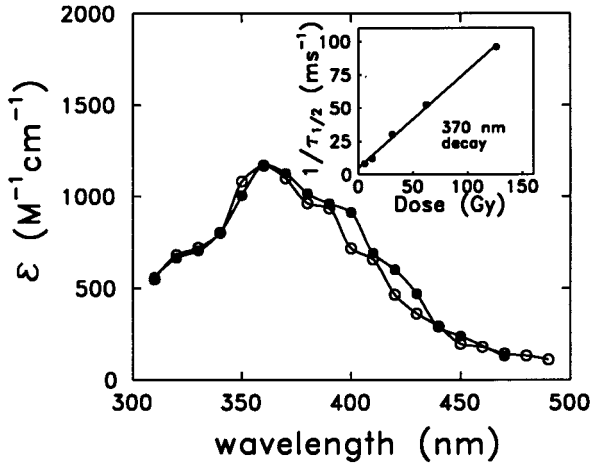


Figure 9. Absorption spectrum of $ITyOH_2^{\bullet}$ transient 8 μs after pulse; (●) pH 1.5 with $HClO_4$ and (○) pH 5 with 1 M KH_2PO_4, absorbed dose 20 Gy. Matrix: 0.2 M *t*-BuOH, N_2 saturated, [ITyOH] = 0.8 mM. Inset: variation of $(1/\tau_{1/2})_{first}$ for $ITyOH_2^{\bullet}$ decay vs $[ITyOH_2^{\bullet}]_{max}$ (proportional to the absorbed dose).

of the one-electron reduction potential of the tyrosyl phenoxyl radical as compared to that of the 3-iodotyrosyl phenoxyl radical at pH > 11 supports the electron-transfer mechanism in Scheme 1.

Reaction with H$^{\bullet}$. Near pH 5, the H$^{\bullet}$ radical was generated in deoxygenated solutions containing 0.2 M *tert*-butyl alcohol and 1 M KH_2PO_4.[34] Below pH 2, H$^{\bullet}$reactions were studied in deoxygenated solutions; $G(H^{\bullet}) = 0.34$ in both cases. Transient spectra obtained from H$^{\bullet}$ reactions are presented in Figure 9. The spectral and kinetic parameters are listed in Table 3. The rate constants for the reaction of H$^{\bullet}$ with ITyOH were measured **(**i**)** directly from the formation kinetics at 360 nm and also from **(**ii**)** competition kinetics with respect to methylviologen and thionine by following the kinetics of the formation of the appropriate transients arising out of these solutes.[7,10] The rate constant values indicate that H$^{\bullet}$ reaction with ITyOH is independent of pH. From the reported reactions of H$^{\bullet}$ with tyrosine[17] and 3,5-diiodotyrosine[31] and the close matching of the transient spectra at both pH 1.5 and 5, formation of the H-adduct, i.e., $ITyOH_2^{\bullet}$, is indicated. In γ-radiolysis experiments, neither I^- nor NH_3 was detected as end products after radiolysis. The second-order transient decay characteristics (Figure 9 inset) indicated that it does not react with ITyOH, and instead a radical−radical route is favored. The possible reactions are the dimerization and the reaction between $ITyOH_2^{\bullet}$ and the β-hydroxy radical derived from *tert*-butyl alcohol used to scavenge the $^{\bullet}$OH radicals. The effect of O_2 on $ITyOH_2^{\bullet}$ could not be quantified in this study.

Reactions with Other Reducing Radicals. These include the superoxide anion, O_2^- (an important intermediate in living systems formed mainly by enzymatic processes),[16] generated in O_2-saturated 0.1 M HCO_2^- solution at pH > 6; the carboxyl radical anion (CO_2^-), produced from N_2O-saturated 0.1 M HCO_2^- solution; and the 1-hydroxy-1-methylethyl radical (or its anion) from N_2O-saturated 1.0 M isopropyl alcohol. ITyOH was found to be unreactive toward O_2^- radical. However, its reduction via the deiodination route was confirmed in γ-radiolysis studies with the latter two radicals. From the yields of I^- and assuming competition between the reduction process and the radical−radical reaction, the rate constants with both the radicals were estimated to be $<10^6$ M^{-1} s^{-1}. Ammonia was not detected as an end product, indicating the absence of the deamination reaction.

Conclusions

These results indicate that the oxidation of ITyOH follows the trend for phenolic compounds; formation of the phenoxyl radical followed by its dimerization that remain unaffected in the presence of O_2 or excess parent compound. For the $^{\bullet}$OH radical, although the rate constant for the reaction is similar for tyrosine and 3,5-diiodotyrosine, the transient yields suggest different pathways. It appears that, with each additional iodine substitution, the extent of formation of the cyclohexadienyl radical is increasingly disfavored, and instead, simultaneous loss of a molecule of water is favored. Thus, going from tyrosine to 3-iodotyrosine and 3,5-diiodotyrosine, the extent of formation of the $^{\bullet}$OH adduct decreases from >50%[13] to ≈30% and ≈15%,[20] respectively. This decrease may result from addition of $^{\bullet}$OH onto the iodine atoms, as is known to occur in aliphatic iodine compounds,[23] followed by rapid loss of water to give the phenoxyl radical. The reaction of $^{\bullet}$OH with iodobenzene has been shown to yield a radical cation in acidic solutions.[35] If such a species is formed from ITyOH, it is likely to lose the −OH proton rapidly and form the phenoxyl radical. The reduction potential value of ITyOH at physiological pH also suggests cellular oxidation to be fast and irreversible, resulting in loss of active ITyOH under oxidative stress.

Reduction of ITyOH, although very rapid with the primary reducing radicals, is quite slow with secondary reducing radicals. Since, in living cells, the primary reducing radicals are efficiently scavenged by other chemical species present in the surrounding (e.g., omnipresent O_2), the chance of survival for ITyOH under a reductive stress is high. For e_{aq}^- reactions, although the *k* values are of similar order of magnitude for 3-iodo- and 3,5-diiodotyrosines, only in the case of 3-iodotyrosine is the semioxidized transient formed following reduction (Scheme 1). Since the initial deiodination step occurs with both these species, the subsequent reactions of the hydroxy−iodo−phenyl radical anion from 3,5-diiodotyrosine are also expected to produce the ITyO$^{\bullet}$ as the secondary transient (following similar reactions as in Scheme 1). Probably, due to a small difference between reduction potentials of the respective phenoxyl radicals (ITyO$^{\bullet}$ and $I_2TyO^{\bullet}$),[20] further oxidation of 3,5-diiodotyrosine by ITyO$^{\bullet}$ was not observed earlier.[31] Thus, favorable energetics in aqueous medium makes this mode of reaction unique for 3-iodotyrosine between the iodine-substituted tyrosines. Mimicking a situation where both 3-iodo- and 3,5-diiodotyrosines are present in the same matrix, as reported during hormone synthesis in the thyroid cells, e_{aq}^- reactions may show interesting results.

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