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Radiolytic One-Electron Reduction Characteristics of Tyrosine Derivative Caged by 2-Oxopropyl Group

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Abstract

We employed X-irradiation to activate a caged amino acid with a 2-oxoalkyl group. We designed and synthesized tyrosine derivative caged by a 2-oxoalkyl group (Tyr(Oxo)) to evaluate its radiolytic one-electron reduction characteristics in aqueous solution. Upon hypoxic X-irradiation, Tyr(Oxo) released a 2-oxopropyl group to form the corresponding uncaged tyrosine. In addition, radiolysis of dipeptides containing Tyr(Oxo) revealed that the efficiency of radiolytic removal of 2-oxopropyl group increased significantly by the presence of neighboring aromatic amino acids.

Caged amino acids and proteins, the functions of which can be regulated by external triggers, are applied widely to biological research.[1-5] The activities of these molecules can be blocked by chemical modification, while their intrinsic activities are recovered by external stimulation such as photoirradiation[6] and enzymatic treatment.[4] In view of a feature that their functions are easily regulated both temporally and spatially, these caged amino acids are useful for studying protein chemistry in biological systems.

Recently we have proposed caged drugs (prodrugs) that are activated by hypoxic X-irradiation.[7-11] Our studies illustrated that a 2-oxoalkyl group has an effective functionality for caging antitumor drugs such as 5-fluorouracil (5-FU)[8,9] and 5-fluorodeoxyuridine (5-FdUrd),[10] and for radiolytic activation of the resultant caged drugs. This class of radiation activated caged drugs release the 2-oxoalkyl group by reducing hydrated electrons, which are generated as the major active species along with hydrogen atoms and hydroxyl radicals upon radiolysis of an aqueous solution. An activation mechanism has been proposed by which the 2-oxoalkyl group undergoes one-electron reduction by hydrated electrons to form corresponding π^* anion radical, followed by thermal activation into the σ^* anion radical that has a weakened N-C bond between the 5-FU unit and the 2-oxoalkyl group and is readily hydrolyzed to release the 2-oxoalkyl group. In this study, we designed and synthesized an amino acid caged by a

2-oxopropyl group on tyrosine (Tyr), which has a high affinity for hydrated electrons.[12] Under hypoxic conditions, radiolytic one-electron reduction of tyrosine derivative possessing 2-oxopropyl group (Tyr(Oxo)) resulted exclusively in releasing of 2-oxopropyl group to recover the corresponding uncaged Tyr. In the case of dipeptides bearing Tyr(Oxo), the neighboring amino acid was identified to have a strong influence on the overall efficiency of radiolytic one-electron reductive release of the 2-oxopropyl group from Tyr(Oxo).

The synthetic procedure of Tyr(Oxo) is outlined in Scheme 1. Coupling of N-(tert-butoxycarbonyl)tyrosine methyl ester **1** with α-bromoacetone and deprotection of the terminal amino group gave Tyr(Oxo) (**3**).[13]

(Scheme 1)

We initially performed one-electron reduction of Tyr(Oxo) by the X-radiolysis of an argon-purged aqueous solution containing excess 2-methyl-2-propanol as the scavenger of oxidizing hydroxyl radicals.[14] Reducing hydrate electrons (e_{aq}^-) are generated as the major active species under these radiolysis conditions, in which the yield of reducing

hydrogen atoms is much less than those of hydrated electrons and oxidizing hydroxyl radicals.[15] Figure 1 shows the reaction profiles of the radiolytic reduction of Tyr(Oxo) under hypoxic conditions. The appearance of a single new peak in Figure 1 is attributable to the formation of uncaged Tyr as confirmed by the overlap injection of authentic samples in the HPLC analysis. The G values (the number of molecules produced or changed per 1 J of radiation energy absorbed by the reaction system) were 223 nmol/J for the decomposition of caged Tyr(Oxo) and 130 nmol/J for the formation of the corresponding uncaged Tyr.[16] These results clearly indicate that the 2-oxopropyl group on the amino acid can be similarly removed as in the case of prodrugs.[10]

(Figure 1)

To assess the effect of molecular oxygen on the radiolysis of Tyr(Oxo), we performed similar one-electron reduction under aerobic conditions. In contrast to the hypoxic X-radiolysis, the removal of the 2-oxopropyl group to convert Tyr(Oxo) into Tyr became considerably less efficient in the aerobic X-radiolysis (Figure 2). The G values were estimated as 81 nmol/J for the decomposition of Tyr(Oxo) and 51 nmol/J for the formation of Tyr. In view of well-documented evidence that molecular oxygen

efficiently inhibits radiolytic reduction due to the trapping of e_{aq}^- to form a superoxide anion radical,[17] the one-electron reduction of Tyr(Oxo) by e_{aq}^- is likely to occur in a hypoxia-selective manner.

(Figure 2)

To identify the effect of the neighboring amino acid on the radiolytic reduction of Tyr(Oxo), we compared one-electron reduction reactivity of dipeptides **7–13** comprising Tyr(Oxo) and seven types of natural amino acids upon hypoxic or aerobic X-irradiation. Dipeptides **7–13** were prepared from **3** (Ac-Tyr-OMe) as outlined in Scheme 2. The phenol group of **4** was alkylated by α-bromoacetone, and subsequent hydrolysis gave acid **6**. Coupling of **6** with the corresponding amino acids furnished the synthesis of dipeptides **7–13**.[18] Figure 3 shows representative reaction profiles of the radiolytic reduction of dipeptide Tyr(Oxo)-Gly under hypoxic conditions. Similar to the radiolysis of monomeric Tyr(Oxo), reductive removal of the 2-oxopropyl group occurred commonly for all dipeptides in a hypoxia-selective manner. The dipeptides **10–13**, in which Tyr(Oxo) is linked to aromatic amino acids, showed higher G values than dipeptides **7–9** with a linkage between Tyr(Oxo) and aliphatic amino acids, as listed in

Table 1. Thus, the neighboring amino acid has a marked effect on the one-electron reduction of Tyr(Oxo) to generate uncaged Tyr. It has been reported that aromatic amino acids are reduced by e_{aq}^- faster than aliphatic amino acids.[12] These reduction characteristics of e_{aq}^- could be responsible for the accelerated reduction of dipeptides bearing aromatic amino acids. It is most likely that capturing of e_{aq}^- by the aromatic amino acid residues in the caged dipeptides occurred more efficiently followed by rapid intramolecular electron transfer to Tyr(Oxo), thereby resulting in the efficient formation of an uncaged dipeptide.

(Scheme 2)

(Figure 3)

(Table 1)

In summary, we have demonstrated the activation of caged Tyr possessing a 2-oxoalkyl group by X-radiolytic one-electron reduction. Hypoxic X-irradiation caused the efficient removal of the 2-oxopropyl group on Tyr(Oxo), whereas the reaction efficiency decreased dramatically upon aerobic irradiation. More remarkable was that the neighboring aromatic amino acids increased the radiolytic reduction efficiency of

Tyr(Oxo), presumably due to their higher electron affinity. These results strongly indicate that functions of amino acids and proteins may be regulated by means of radiolytic reduction of 2-oxoalkyl group. Further exploration of the one-electron reduction of longer and higher-order peptides bearing Tyr(Oxo) by X-radiolysis is in progress.

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12. Rate constants for the reactions of hydrated electrons in aqueous solution around pH 7 was estimated as 8.8×10^6, 4.2×10^6, 5.0×10^6, 1.4×10^8, 3.4×10^8, 2.9×10^8, 6.0×10^7 and 1.8×10^{10} L mol^{-1} s^{-1} for Gly, Ala, Val, Phe, Tyr, Trp, His and O_2, respectively. See Buxton, G. V.; Greenstock, C. V.; Helman, W. P.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1988**, *17*, 513.

13. **Tyr(Oxo) (3)**: mp 159–160 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.56 (s, 3H), 7.13 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 4.79 (s, 2H), 4.22 (t, J = 6.0 Hz, 1H), 3.68 (s, 3H), 3.07 (m, 2H), 2.15 (s, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.1, 169.3, 157.0, 130.5, 126.8, 114.5, 72.0, 53.3, 52.5, 34.9, 26.2; FAB-MS: m/e 252 [$(M+H)^+$]; HRMS calcd for $C_{13}H_{18}NO_4$ [$(M+H)^+$] 252.1236, found 252.1228.

14. **General procedure for radiolytic reduction:** To establish hypoxia, aqueous solutions of Tyr(Oxo) (**3**) (95 μM) containing 10 mM 2-methyl-2-propanol (20 % 2-methyl-2-propanol in the case of Tyr(Oxo)-Phe, Tyr(Oxo)-Tyr and Tyr(Oxo)-Trp for their insolublities) were purged with argon for 15 min and then irradiated in a sealed Pyrex sample tube at ambient temperature with X-ray source (Rigaku RADIOFLEX-350, 5.0 Gy min^{-1}). After the irradiation,

the solution was subjected to HPLC analysis.

15. Radiolysis of a diluted aqueous solution at around pH 7.0 produces primary water radicals such as oxidizing hydroxyl radical (•OH), reducing hydrated electrons (e_{aq}^-) and reducing hydrogen atoms (•H) with the G values of G(•OH) = 280 nmol/J, G(e_{aq}^-) = 280 nmol/J, and G(•H) = 60 nmol/J, respectively.

16. Reductive degradation of peptide mainchain may lead to a small G values for the formation of uncaged Tyr. See Garrison, W. M. *Chem. Rev.* **1987**, *87*, 381.

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18. **Tyr(Oxo)-Gly (7)**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.43 (t, J = 6.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.14 (d, J = 8.8 Hz, 2H), 6.78 (d, J = 8.8 Hz, 2H), 4.75 (s, 2H), 4.47 (m, 1H), 3.85 (d, J = 6.1 Hz, 2H), 3.63 (s, 3H), 2.94 (m, 1H), 2.65 (m, 1H), 2.14 (s, 3H), 1.75 (s, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 172.0, 170.2, 169.0, 156.2, 130.4, 130.0, 114.0, 72.1, 53.9, 51.7, 38.2, 36.7, 26.2, 22.5; FAB-MS: m/e 351 [$(M+H)^+$]; HRMS calcd. for $C_{17}H_{23}N_2O_6$ [$(M+H)^+$] 351.1556, found 351.1545.; **Tyr(Oxo)-Ala (8)**: mp 158–159 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.45 (d, J = 7.1 Hz, 1H), 8.03 (d, J = 8.6 Hz,

1H), 7.15 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 4.75 (s, 2H), 4.47 (m, 1H), 4.27 (m, 1H), 3.61 (s, 3H), 2.92 (m, 1H), 2.63 (m, 1H), 2.14 (s, 3H), 1.73 (s, 3H), 1.29 (d, *J* = 7.3 Hz, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 172.9, 171.5, 169.0, 156.2, 130.3, 130.1, 114.0, 72.1, 53.7, 51.8, 47.5, 36.9, 26.2, 22.4, 16.8; FAB-MS: m/e 365 [$(M+H)^+$]; HRMS calcd. for $C_{18}H_{25}N_2O_6$ [$(M+H)^+$] 365.1713, found 365.1726.; **Tyr(Oxo)-Val (9)**: mp 142–143 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.21 (d, *J* = 8.0 Hz, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.16 (d, *J* = 8.6 Hz, 2H), 6.79 (d, *J* = 8.6 Hz, 2H), 4.75 (s, 2H), 4.56 (m, 1H), 4.17 (m, 1H), 3.62 (s, 3H), 2.89 (m, 1H), 2.64 (m, 1H), 2.14 (s, 3H), 2.04 (m, 1H), 1.74 (s, 3H), 0.88 (t, *J* = 6.9 Hz, 6H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 171.9, 171.8, 169.1, 156.2, 130.3, 130.1, 114.0, 72.1, 57.4, 53.7, 51.6, 36.6, 29.9, 26.2, 22.4, 18.9, 18.2; FAB-MS :m/e 393 [$(M+H)^+$]; HRMS calcd for $C_{20}H_{29}N_2O_6$ [$(M+H)^+$] 393.2026, found 393.2027.; **Tyr(Oxo)-Phe (10)**: mp 107–108 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.40 (d, *J* = 7.6 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.24 (m, 5H), 7.12 (d, *J* = 8.8 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, 2H), 4.73 (s, 2H), 4.47 (m, 2H), 3.58 (s, 3H), 2.95 (m, 3H), 2.61 (m, 1H), 2.14 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 171.7, 171.6, 168.9, 137.0, 130.2, 130.1, 129.0, 128.2, 128.1, 126.5, 114.0,

72.1, 53.6, 53.5, 51.8, 36.5, 26.2, 22.4; FAB-MS: m/e 441 [$(M+H)^+$]; HRMS calcd. for $C_{24}H_{29}N_2O_6$ [$(M+H)^+$] 441.2026, found 441.2012.; **Tyr(Oxo)-Tyr (11)**: mp 77–78 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.21 (s, 1H), 8.32 (d, J = 7.5 Hz, 1H), 7.99 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 6.78 (d, J = 8.6 Hz, 2H), 6.65 (d, J = 8.6 Hz, 2H), 4.74 (s, 2H), 4.42 (m, 2H), 3.57 (s, 3H), 2.89–2.27 (m, 4H), 2.14 (s, 3H), 1.72 (s, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 171.8, 171.5, 168.9, 156.2, 156.0, 130.3, 130.1, 130.0, 126.9, 115.0, 114.0, 72.1, 53.9, 53.6, 51.7, 36.6, 35.9, 26.2, 22.4; FAB-MS: m/e 457 [$(M+H)^+$]; HRMS calcd. for $C_{24}H_{29}N_2O_7$ [$(M+H)^+$] 457.1975, found 457.1981.; **Tyr(Oxo)-Trp (12)**: mp 80–81 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.89 (s, 1H), 8.38 (d, J = 7.3 Hz, 1H), 8.00 (d, J = 8.6 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 7.7 Hz, 1H), 7.17–6.94 (m, 5H), 6.77 (d, J = 8.6 Hz, 2H), 4.74 (s, 2H), 4.51 (m, 2H), 3.56 (s, 3H), 3.12 (m, 2H), 2.91 (m, 1H), 2.63 (m, 1H), 2.14 (s, 3H), 1.73 (s, 3H); ^{13}C NMR (DMSO-d_6, 75.5 MHz) δ 204.3, 172.1, 171.6, 169.0, 156.2, 136.0, 130.3, 130.1, 127.0, 123.7, 120.9, 118.4, 117.9, 113.9, 111.4, 109.2, 72.1, 53.7, 53.0, 51.8, 36.6, 26.9, 26.2, 22.4; FAB-MS: m/e 480 [$(M+H)^+$]; HRMS calcd. for $C_{26}H_{30}N_3O_6$ [$(M+H)^+$] 480.2135, found 480.2126.; **Tyr(Oxo)-His(1-Me) (13)**:

^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.42 (d, J = 7.3 Hz, 1H), 8.07 (d, J = 7.3 Hz, 1H), 7.13 (d, J = 8.3 Hz, 2H), 6.77 (d, J = 8.3 Hz, 2H), 4.73 (s, 2H), 4.47–4.38 (2H), 3.58 (s, 3H), 3.56 (s, 3H), 2.96–2.53 (6H), 2.13 (s, 3H), 1.74 (s, 3H) ; ^{13}C NMR (DMSO-d_6, 100 MHz) δ 204.3, 171.8, 171.5, 169.0, 156.2, 137.3, 136.7, 130.1, 117.9, 114.0, 79.1, 72.1, 53.8, 52.3, 51.8, 36.4, 32.7, 29.7, 26.2, 22.4; FAB-MS: m/e 445 [$(M+H)^+$]; HRMS calcd. for $C_{22}H_{29}N_4O_6$ [$(M+H)^+$] 445.2087, found 445.2089.

19. We carried out one-electron reduction of **13** in aqueous solution containing 10% or 20% 2-methyl-2-propanol. We compared one-electron reactivity and confirmed that both reactions showed similar profiles.

Captions:

Scheme 1[a]

[a]Reagents and conditions: (a) K_2CO_3, KI, bromoacetone, acetone, reflux, quant.; (b) HCl in Et_2O, room temperature, 78%.

Scheme 2[a]

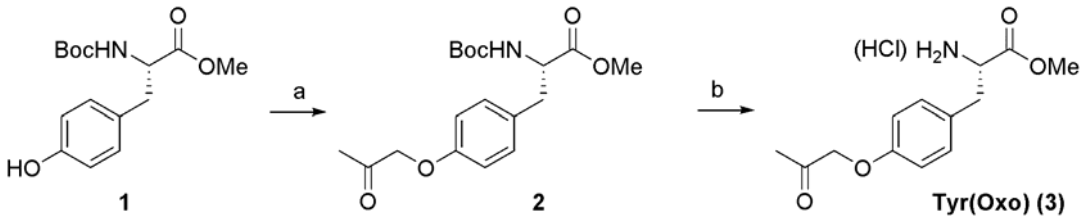
[a]Reagents and conditions: (a) K_2CO_3, KI, bromoacetone, acetone, reflux, 77%; (b) LiOH, MeOH-H_2O, room temperature, 76%; (c) aminoacid methyl ester hydrochloride, HBTU, DIEA, THF, room temperature, 7-34% (for **7**-**12**); (d) 1-methylhistidine methyl ester hydrochloride, EDCI, triethylamine, DMF, room temperature, 28% (for **13**).

Figure 1. HPLC profiles for the one-electron reduction of Tyr(Oxo) (**3**) (95 μM) upon hypoxic X-radiolysis (0, 150, 400 and 700 Gy) of aqueous solution containing 10 mM 2-methyl-2-propanol.

Figure 2. Decomposition of Tyr(Oxo) (**3**) (open symbol) and release of Tyr (filled symbol) in the hypoxic (circle) or aerobic (triangle) radiolysis of aqueous solution containing 10 mM 2-methyl-2-propanol. Each error bar represents the SE calculated from three experimental results.

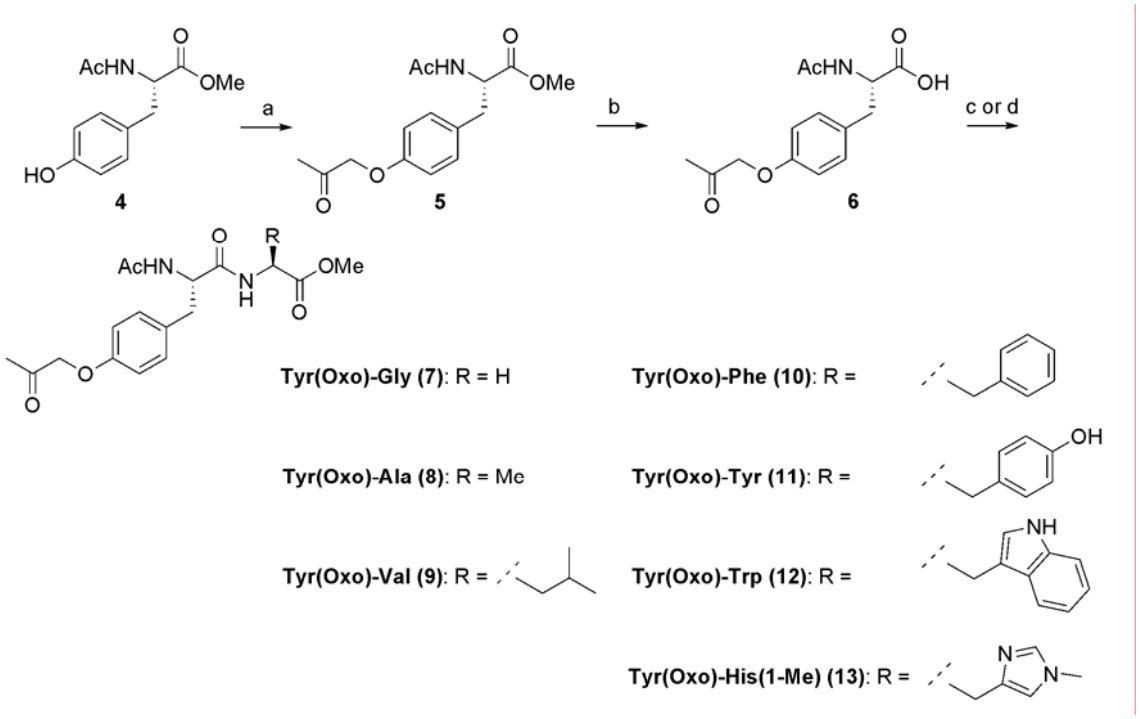
Figure 3. HPLC profiles for the one-electron reduction of Tyr(Oxo)-Gly (**7**) (100 μM) upon hypoxic X-radiolysis (0, 100, 300 and 500 Gy) of aqueous solution containing 10 mM 2-methyl-2-propanol.

Scheme 1[a]



[a]Reagents and conditions: (a) K_2CO_3, KI, bromoacetone, acetone, reflux, quant.; (b) HCl in Et_2O, room temperature, 78%.

Scheme 2[a]



[a]Reagents and conditions: (a) K_2CO_3, KI, bromoacetone, acetone, reflux, 77%; (b) LiOH, MeOH-H_2O, room temperature, 76%; (c) aminoacid methyl ester hydrochloride, HBTU, DIEA, THF, room temperature, 7-34% (for **7**-**12**); (d) 1-methylhistidine methyl ester hydrochloride, EDCI, triethylamine, DMF, room temperature, 28% (for **13**).

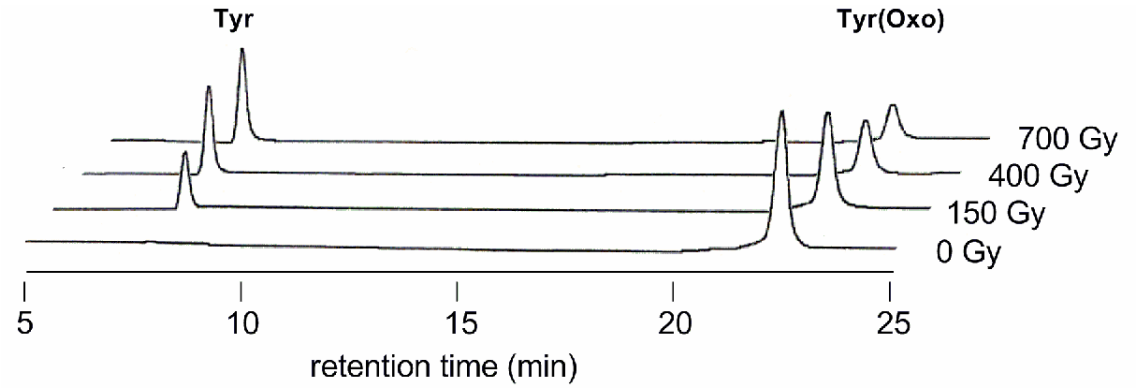


Figure 1. HPLC profiles for the one-electron reduction of Tyr(Oxo) (**3**) (95 μM) upon hypoxic X-radiolysis (0, 150, 400 and 700 Gy) of aqueous solution containing 10 mM 2-methyl-2-propanol.

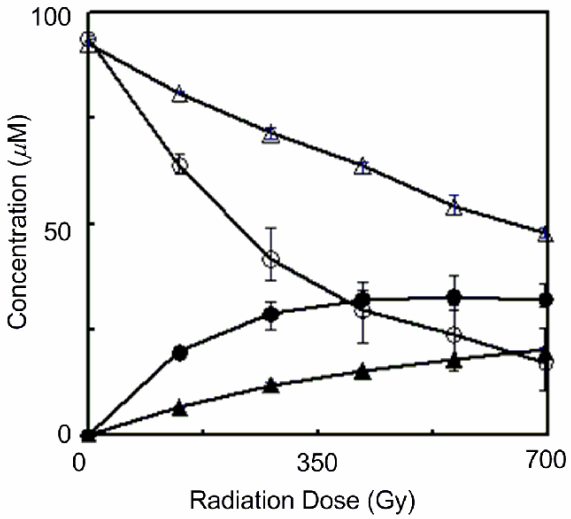


Figure 2. Decomposition of Tyr(Oxo) (**3**) (open symbol) and release of Tyr (filled symbol) in the hypoxic (circle) or aerobic (triangle) radiolysis of aqueous solution containing 10 mM 2-methyl-2-propanol. Each error bar represents the SE calculated from three experimental results.

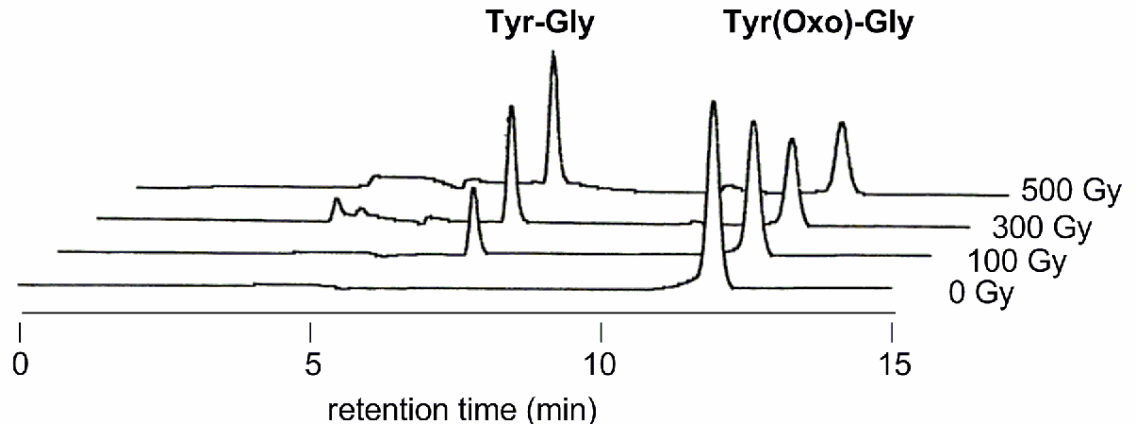


Figure 3. HPLC profiles for the one-electron reduction of Tyr(Oxo)-Gly (**7**) (100 μM) upon hypoxic X-radiolysis (0, 100, 300 and 500 Gy) of aqueous solution containing 10 mM 2-methyl-2-propanol.

Table 1. G-values (nmol/J) for the Decomposition of Tyr(Oxo) (**3**) and Dipeptides Bearing Tyr(Oxo) and the Formation of Corresponding Uncaged Tyr and Dipeptides upon X-radiolysis.[a]

	Hypoxic Conditions		Aerobic Conditions	
	Formation	Decomposition	Formation	Decomposition
Tyr(Oxo)(**3**)	130	223	51	81
Tyr(Oxo)-Gly(**7**)	55	212	11	52
Tyr(Oxo)-Ala(**8**)	96	232	20	72
Tyr(Oxo)-Val(**9**)	72	209	18	83
Tyr(Oxo)-Phe(**10**)	155	299	30	45
Tyr(Oxo)-Tyr(**11**)	196	258	37	52
Tyr(Oxo)-Trp(**12**)	131	295	24	55
Tyr(Oxo)-His(1-Me)(**13**)	158	282	42	96

[a] Aqueous solution of Tyr(Oxo) (**3**) and dipeptides (95-320 μM) containing excess amount of 2-methyl-2-propanol[19] were irradiated at ambient temperature with X-ray source (5 Gy min^{-1}).