Tyrosine Oxidation by $NO_2^\bullet$ in Aqueous Solution

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Nitrogen dioxide, formed by γ-radiolysis in deaerated aqueous nitrate/nitrite solutions, is capable of oxidizing Gly-Tyr in favourable competition with the natural decay of $NO_2^\bullet$ by dimerization and disproportionation. 2,2′-Biphenolic tyrosine dimers and nitro-tyrosine were identified spectroscopically as stable products. The results suggest that $NO_2^\bullet$ reacts with the peptide by electron abstraction, generating Gly-Tr phenoxyl radicals (PheO$^\bullet$) which terminate by dimerization (2 PheO$^\bullet$ → 2,2′-biphenol) and $NO_2^\bullet$-scavenging (PheO$^\bullet$ + $NO_2^\bullet$ → Nitro-Tyr).

Introduction

The kinetics of the elementary chemical processes of nitrogen dioxide in aqueous solution have been resolved by application of pulse radiolysis techniques [1]

$$2\,NO_2^\bullet \rightleftharpoons N_2O_4 \qquad (1)$$
$$(2k_{1f} = 9.0 \times 10^8\ \text{M}^{-1}\,\text{s}^{-1};\ k_{1b} = 1.4 \times 10^4\ \text{s}^{-1})\,.$$

$$N_2O_4 + H_2O \rightarrow 2\,H^+ + NO_2^- + NO_3^- \qquad (2)$$
$$(k_2 = 1.0 \times 10^3\ \text{s}^{-1})\,.$$

In competition with these reactions $NO_2^\bullet$ was found capable of oxidizing $Fe(CN)_6^{4-}$ at pH 7 [2] thus the oxidation-reduction potential of the $NO_2^\bullet/NO_2^-$ couple is probably well above 0.36 V, possibly in the order of 0.9 V [3]. The chemical behaviour of $NO_2^\bullet$ in aqueous solutions containing organic compounds has however not been explored up to date, although $NO_2^\bullet$ is supposed to be a highly deleterious agent in biological systems [4]. In the present study, using Gly-Tyr as model compound, it is shown that $NO_2^\bullet$-induced oxidation of tyrosine in dilute aqueous solution competes favourably with the natural decay of $NO_2^\bullet$ by the reactions (1) and (2).

Experimental

$NO_2^\bullet$ was generated by γ-irradiation of deaerated aqueous solutions containing 10^{-2} M $NaNO_2$ and 5×10^{-2} M NH_4NO_3 at pH > 8 (unbuffered). The system involves reactions (3) to (6), [1, 5–7]

$$H_2O \xrightarrow{\gamma} {}^\bullet OH,\ e_{aq}^-,\ H^\bullet \text{ etc.} \qquad (3)$$

$$^\bullet OH + NO_2^- \rightarrow OH^- + NO_2^\bullet\,. \qquad (4)$$

$$e_{aq}^- + NO_3^- \rightarrow NO_3^{\bullet 2-} \xrightarrow{H_2O} 2\,OH^- + NO_2^\bullet \qquad (5, 6)$$
$$(k_4 \sim k_5 \sim 10^{10}\ \text{M}^{-1}\,\text{s}^{-1};\ k_6 = 5.5 \times 10^4\ \text{s}^{-1})$$

and avoids reactions of e_{aq}^- with O_2 and NO_2^- [6]. The yield of $NO_2^\bullet$ is $Y(NO_2^\bullet) \sim 0.57\,\mu\text{M/Gy}$ ($G(NO_2^\bullet) = 5.5$ per 100 eV)[1]; reactions of NO$^\bullet$, formed by interaction of H$^\bullet$ with NO_2^- [8], were not considered in view of the low yield ($\sim 0.06\ \mu$M/Gy).

Solutions were prepared shortly before each experiment with redistilled water, Gly-Tyr from Serva (Heidelberg) and A.R. grade inorganic chemicals. After deaeration, by 30 min bubbling with N_2 gas, the solutions were irradiated in the closed test tube at ambient temperature, using a 35 Gy/min ^{60}Co-γ-source ("220 Gammacell", Atomic Energy of Canada Ltd.).

Results and Discussion

γ-Irradiation of the deaerated NO_2^-/NO_3^- system (see Experimental) in the absence of the model compound Gly-Tyr gave no detectable changes in the absoption spectra, indicating that $NO_2^\bullet$ reverts quantitatively to the parent ions by the reactions (1) and (2). When Gly-Tyr was added to the NO_2^-/NO_3^- system, stable products were formed upon γ-irradiation with characteristic pH-dependent absorption

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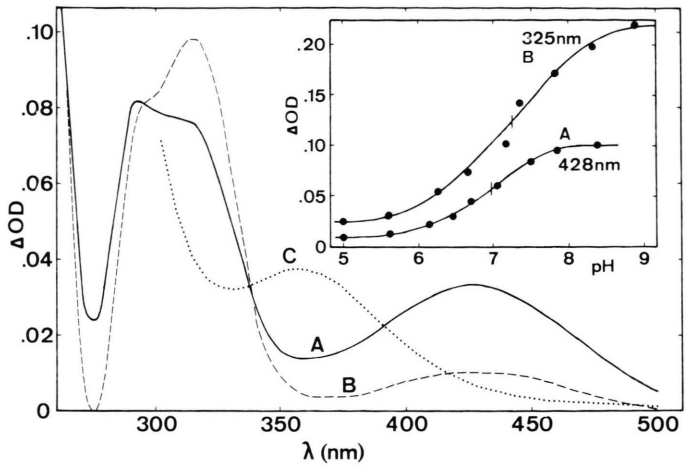


Fig. 1. Absorptions detected after γ-irradiation ($D = 90$ Gy) of deaerated aqueous solutions containing 10 mM $NaNO_2$ and 50 mM NH_4NO_3 at pH 8.4 (unbuffered), (A) in the presence of 0.25 mM Gly-Tyr, (B) in the presence of 1 mM Gly-Tyr. All absorbances were measured against the corresponding unirradiated solution, using 1 or 5 cm cells; the optical densities (ΔOD) in the spectra A and B are normalized to 1 cm optical path. The pH dependences (insert), obtained by 1:1 dilution of the irradiated solutions A (428 nm) and B (325 nm) with buffers, refer to 5 cm optical path; the spectrum C is that of the diluted (1:1) solution A at pH 5.7.

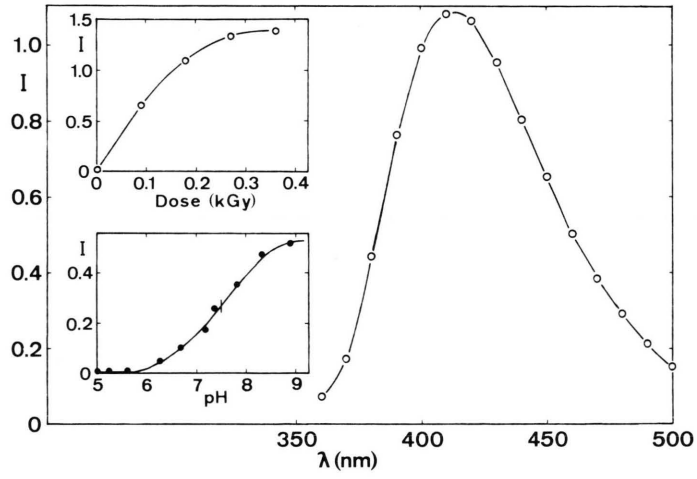


Fig. 2. Fluorescence of products detected after γ-irradiation of a deaerated solution containing 10 mM $NaNO_2$, 50 mM NH_4NO_3 and 1 mM Gly-Tyr at pH 8.4. The fluorescence was excited at 325 nm and the intensity (I) was measured relative to a 0.5 mM Na-salicylate reference solution. The spectrum refers to $D = 180$ Gy, and the pH dependence refers to $D = 90$ Gy and subsequent dilution (1:1) with buffers (*cf.* Fig. 1 B). 410 nm fluorescence intensities are given in the inserts.

and fluorescence spectra, as shown in Figs. 1 and 2. The Gly-Tyr concentration in these experiments was sufficiently low to exclude reactions of $^{\bullet}OH$ and e_{aq}^- with the peptide. It is evident thus that $NO_2^{\bullet}$, generated by the reactions (4) to (6), is capable of reacting with Gly-Tyr in competition with the natural $NO_2^{\bullet}$ decay by reactions (1) and (2), even at low peptide concentrations (0.25 mM).

In an attempt at product identification we recall that phenoxyl radicals formed by 1-electron oxidation of Gly-Tyr (*e.g.* by $N_3^{\bullet}$) efficiently dimerize to form the Gly-Tyr 2,2′-biphenol [9], which deprotonates at pH ~ 7.4; the alkaline form of 2,2′-biphenol (not the acid form) absorbs with a maximum at 316 nm ($\varepsilon_{316} = 5790\ M^{-1}\,cm^{-1}$) [10] and exhibits a strong fluorescence with a peak at 410 nm. The characteristic 316 nm absorption and the 410 nm fluorescence (excited at 325 nm) is clearly seen at pH 8.4 in Fig. 1 B and Fig. 2, respectively, and it was also confirmed that this species disappears at lower pH with pK ~ 7.4 (see OD (325 nm) and I (410 nm) titration curves in the Figures). The 2,2′-biphenol does, however, not exhibit absorption peaks at 290 and 428 nm, as indicated particularly in the spectrum at low peptide concentration (Fig. 1 A). The 290/428 nm absorption is characteristic for nitro-tyrosine, and an extinction coefficient of $\varepsilon_{428}(\text{Nitro-Tyr}) = 4100\ M^{-1}\,cm^{-1}$ has been reported [11]. Nitro-tyrosine deprotonates at pK ~ 7, with an accompanying shift in the absorption maximum to 360 nm [11]. This behaviour is clearly demonstrated (Fig. 1) by the 428 nm titration curve and the spectrum C at pH 5.7.

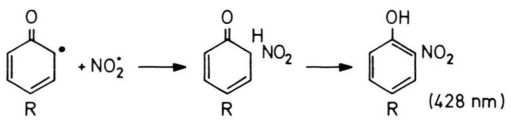
The above observations suggest that $NO_2^{\bullet}$, like $N_3^{\bullet}$ [9], is capable of electron (or H-atom) abstraction from tyrosine to form phenoxyl radicals (PheO$^{\bullet}$),

$$\text{Tyr} + NO_2^{\bullet} \rightarrow NO_2^- + H^+ + \text{PheO}^{\bullet} . \qquad (7)$$

About 62% of the phenoxyls dimerize in the absence of other reagents to form 2,2′-biphenols [9],

$$2\,\text{PheO}^{\bullet} \rightarrow \text{2,2′-biphenol (316 nm)} . \qquad (8)$$

Formation of nitro-tyrosine can conceivably arise from interactions of PheO$^{\bullet}$ with $NO_2^{\bullet}$,



(9)

Reaction (8), *i.e.* the 316 nm species, predominates at high tyrosyl concentration (Fig. 1 B) and reaction (9), *i.e.* the 428 nm species, contributed particularly at low tyrosyl concentration (Fig. 1 A), where reaction (7) is slower.

From Fig. 1 the product yields, $Y = OD/(\varepsilon \cdot D)$, can be roughly estimated. By assuming that OD(428) = 0.036 (Fig. 1 A) is due to nitro-Tyr only, and OD(316) = 0.099 (Fig. 1 B) is pertinent to 2,2′-biphenol in 85% (since nitro-Tyr contributes), we find Y(nitro-Tyr) ~ 0.1 μM/Gy at 0.25 mM Gly-Tyr, and Y(2,2′-biphenol) ~ 0.16 μM/Gy at 1 mM Gly-Tyr. Since two $NO_2^{\bullet}$ radicals ($Y(NO_2^{\bullet}) \sim 0.57$ μM/Gy) are required for each tyrosine dimer, this means that 56% of the $NO_2^{\bullet}$ end up in 2,2′-biphenol at 1 mM Gly-Tyr. Comparing this value with the phenoxyl → 2,2′-biphenol yield (62%) [9], it can be concluded that $NO_2^{\bullet}$ almost quantitatively oxidizes tyrosine under these conditions.

Conclusions

The results presented reveal that $NO_2^{\bullet}$ can act as a strong one-electron oxidant in aqueous environment; oxidation of the tyrosyl model compound competes favourably, even at low concentrations, with the disproportionation of $NO_2^{\bullet}$ in water. It can be anticipated therefore that $NO_2^{\bullet}$ is capable also of inactivating proteins and other constituents of living cells such as thiols. Such direct action of $NO_2^{\bullet}$ may be generally involved in the deleterious effects initiated by $NO_2^{\bullet}$ in biological systems. It should be noted also that oxidation (reaction (7)), as compared to disproportionation (reaction (2)), generates up to twice the yield of harmful nitrite.

Acknowledgements

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