Tyrosine Oxidation by NOI 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 NO2⁻ by dimerization and disproportionation. 2,2'-Biphenolic tyrosine dimers and nitro-tyrosine were identified spectroscopically as stable products. The results suggest that NO2⁻ reacts with the peptide by electron abstraction, generating Gly-Tr phenoxyl radicals (PhoO') which terminate by dimerization (2PhoO' -> 2,2'-biphenol) and NO2-scavenging (PhoO' + NO2 -> 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]

\[
2\text{NO}_2^\cdot = \text{N}_2\text{O}_4
\]  
(1)

\[
\text{N}_2\text{O}_4 + \text{H}_2\text{O} \rightarrow 2\text{H}^+ + \text{NO}_2^2^- + \text{NO}_3^-(2)
\]

In competition with these reactions NO2⁻ was found capable of oxidizing Fe(CN)6³⁻ at pH 7 [2] thus the oxidation-reduction potential of the NO2⁻/NO3⁻ couple is probably well above 0.36 V, possibly in the order of 0.9 V [3]. The chemical behaviour of NO2⁻ in aqueous solutions containing organic compounds has however not been explored up to date, although NO2⁻ 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 NO2⁻-induced oxidation of tyrosine in dilute aqueous solution competes favourably with the natural decay of NO2⁻ by the reactions (1) and (2).

Experimental

NO2⁻ was generated by γ-irradiation of deaerated aqueous solutions containing 10⁻² m NaNO2 and

5 x 10⁻² m NH4NO2 at pH > 8 (unbuffered). The system involves reactions (3) to (6), [1, 5–7]

\[
\text{H}_2\text{O} \rightarrow \text{HO}^-, \text{e}_\text{aq}^-, \text{H}^+ \text{ etc.} \quad (3)
\]

\[
\text{OH}^- + \text{NO}_2^- \rightarrow \text{OH}^- + \text{NO}_3^- \quad (4)
\]

\[
\text{e}_\text{aq}^- + \text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{H}_2\text{O}^- \rightarrow 2\text{OH}^- + \text{NO}_2^- \quad (5, 6)
\]

\[k_4 \approx k_5 \approx 10^{10}\text{ M}^{-1}\text{s}^{-1}; k_6 = 5.5 \times 10^4\text{s}^{-1}\]

and avoids reactions of eaq⁻ with O₂ and NO3⁻ [6]. The yield of NO2⁻ is Y(NO2⁻) ~ 0.57 μM/Gy (G(NO2⁻) = 5.5 per 100 eV); reactions of NO₂⁻, formed by interaction of H⁺ with NO3⁻ [8], were not considered in view of the low yield (~ 0.06 μM/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 N₂ gas, the solutions were irradiated in the closed test tube at ambient temperature, using a 35 Gy/min 90Co-γ-source ("220 Gammacell"; Atomic Energy of Canada Ltd.).

Results and Discussion

γ-Irradiation of the deaerated NO2⁻/NO3⁻ system (see Experimental) in the absence of the model compound Gly-Tyr gave no detectable changes in the absorption spectra, indicating that NO2⁻ reverts quantitatively to the parent ions by the reactions (1) and (2). When Gly-Tyr was added to the NO2⁻/NO3⁻ system, stable products were formed upon γ-irradiation with characteristic pH-dependent absorption...
and fluorescence spectra, as shown in Figs. 1 and 2. The Gly-Tyr concentration in these experiments was sufficiently low to exclude reactions of $'OH$ and $e_aq$ with the peptide. It is evident thus that NO$_2^-$, generated by the reactions (4) to (6), is capable of reacting with Gly-Tyr in competition with the natural NO$_2^-$ 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$_3^-$) 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. 1A). The 290/428 nm absorption is characteristic for nitro-tyrosine, and an extinction coefficient of $\varepsilon_{428}$(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$_2^\cdot$, like N$_3^\cdot$ [9], is capable of electron (or H-atom) abstraction from tyrosine to form phenoxyl radicals (PheO$^\cdot$),

$$\text{Tyr} + \text{NO}_2^\cdot \rightarrow \text{NO}_2^- + \text{H}^+ + \text{PheO}' \quad (7)$$

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

$$2 \text{PheO}' \rightarrow 2,2'$'$-\text{biphenol (316 nm)} \quad (8)$$

Formation of nitro-tyrosine can conceivably arise from interactions of PheO$^\cdot$ with NO$_2^\cdot$,

$$\text{O} \quad \cdot \quad \text{NO}_2^\cdot \rightarrow \text{NO}_2^- \quad (428 \text{ nm}) \quad (9)$$

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(\text{nitro-Tyr}) \sim 0.1 \mu\text{M/Gy at } 0.25 \text{ mM Gly-Tyr, and} Y(2,2'$'$-\text{biphenol}) \sim 0.16 \mu\text{M/Gy at } 1 \text{ mM Gly-Tyr.}

Since two NO$_2^\cdot$ radicals (\(Y(\text{NO}_2^\cdot) \sim 0.57 \mu\text{M/Gy}\)) are required for each tyrosine dimer, this means that 56% of the NO$_2^\cdot$ end up in 2,2'$'$-biphenol at 1 mM Gly-Tyr. Comparing this value with the phenoxyl $\rightarrow$ 2,2'$'$-biphenol yield (62%) [9], it can be concluded that NO$_2^\cdot$ almost quantitatively oxidizes tyrosine under these conditions.

**Conclusions**

The results presented reveal that NO$_2^\cdot$ 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$_2^\cdot$ in water. It can be anticipated therefore that NO$_2^\cdot$ is capable also of inactivating proteins and other constituents of living cells such as thiols. Such direct action of NO$_2^\cdot$ may be generally involved in the deleterious effects initiated by NO$_2^\cdot$ 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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[1] M. Grätzel, A. Henglein, J. Lilie, and G. Beck, Ber. Bunsenges. phys. Chem. 73, 646 (1969). Since an incorrect relation \((k_{1b} = K \cdot k_{1f})\) was used by these authors, we have quoted \(k_{1b} = K \cdot 2k_{1f}.\)