Free Rad. Res., Vol. 29, pp. 13–16
Reprints available directly from the publisher
Photocopying permitted by license only

Published by license under
the Harwood Academic Publishers imprint,
part of The Gordon and Breach Publishing Group.
Printed in Singapore.

Mechanism of Reaction of Nitrogen Dioxide Radical With Hydroxycinnamic Acid Derivatives: A Pulse Radiolysis Study

ZHANG ZHOUEN*, YAO SIDE, LIN WEIZHEN, WANG WENFENG, JIN YIZUN† and LIN NIANYUN

Laboratory of Radiation Chemistry, Shanghai Institute of Nuclear Research, Academic Sinica, P.O. Box 800-204, Shanghai 201800, Peoples Republic of China

Accepted by Dr. M. Dizdaroglu

(Received 31 January 1997; In revised form 18 December 1997)

Nitrogen dioxide radical ($NO_2^\bullet$) is known as a toxic agent produced in the metabolism of nitrates and nitrites. By the use of the pulse radiolysis technique, the mechanism of the reaction of $NO_2^\bullet$ radical with hydroxycinnamic acid derivatives (HCA) was studied and the rate constants have been measured. The rate constants were found to be 7.4×10^8, 7.2×10^8, $8.6 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$ for ferulic acid, sinapic acid and caffeic acid, respectively. The reactions produce the corresponding phenoxyl radical.

Keywords: Nitrogen dioxide radical, pulse radiolysis, hydroxycinnamic acid derivatives

INTRODUCTION

Nitrogen dioxide radical ($NO_2^\bullet$), produced by combustion processes and by bacterial action, is a common atmospheric pollutant.[1,2] It is a constituent of tobacco smoke, and it can be generated in aqueous systems, e.g., by acid decomposition of nitrite and by exposure of nitrate or nitrite solutions to ionizing radiation. $NO_2^\bullet$ is a strongly oxidizing free radical (its one-electron oxidation–reduction potential in neutral aqueous solution, $E^0(NO_2^\bullet/NO_2^-) = 1.0\,V$[3]) and a toxic agent, and several modes of the deleterious action of $NO_2^\bullet$ in biological systems have been proposed. $NO_2^\bullet$ induces lipid peroxidation, as demonstrated by its reactions with alkene in nonaqueous solutions, and is believed to cause membrane damage and cell death.[4–6] $NO_2^\bullet$ also oxidizes cysteine and tyrosine residue in peptides[7,8] leading to loss of enzyme activity. Therefore, many studies have been focused on scavenging $NO_2^\bullet$. As natural antioxidants, hydroxycinnamic acid derivatives (HCA), including ferulic acid, sinapic acid and caffeic acid, have been shown to scavenge OH and $O_2^{-\bullet}$ radicals effectively and to repair the oxidizing OH adducts of DNA via electron transfer reactions.[9–13] In this work, the reaction mechanism of $NO_2^\bullet$ with HCA has been studied

---

* Corresponding author.
† Shanghai Institute of Radiation Medicine, Shanghai Medical University, Peoples Republic of China.

by use of pulse radiolysis. A series of rate constants for reactions with HCA were determined, $k = (7.2\text{–}8.6) \times 10^8\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$. It is shown that HCA are good scavengers of $NO_2^\bullet$ and possibly can be used as good protectants of cell membrane and enzymes against oxidation by nitrogen dioxide radical.

MATERIALS AND METHODS

Ferulic acid (3-methoxy-4-hydroxycinnamic acid), sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid), and caffeic acid (3,4-dihydroxycinnamic acid) (> 98%) purchased from Sigma Ltd. were used without any purification. Nitrite was recrystallized three times with triply distilled water. All solutions were prepared with triply distilled water and deoxygenated with high purity nitrous oxide just before the experiments.

Pulse radiolysis experiments were conducted by using a linear accelerator providing 8 MeV electron pulses with a duration of 8 ns. The dose of per pulse was determined by thiocyanate dosimety using $10\,\mathrm{mmol\,dm^{-3}}$ KCNS solution saturated with air and taking $\varepsilon = 7600\,\mathrm{dm^3\,mol^{-1}\,cm^{-1}}$ and $G = 2.8$ for $(SCN)_2^{\bullet-}$ at 480 nm. In this work, the average pulse dosage for the kinetic determination was 10 Gy and all the experiments for the spectra were taken in a flow cell with an optical path length of 2 cm. Detailed descriptions of the experimental set-up and conditions were given elsewhere.[11]

The $NO_2^\bullet$ radical was generated by oxidation of NO_2^- by OH radical produced from pulse radiolysis of water containing $4\,\mathrm{mmol\,dm^{-3}}$ sodium nitrite and saturated with N_2O,[2,8] by the following reactions:

$$H_2O \rightsquigarrow OH, e_{aq}^-, H, H_3O^+ \tag{1}$$

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

$$k_2 = 6.5 \times 10^9\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$$

$$OH + NO_2^- \rightarrow OH^- + NO_2^\bullet \tag{3}$$

$$k_3 = 1.0\times10^{10}\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$$

$$NO_2^\bullet + NO_2^\bullet \underset{k_{-4}}{\overset{k_{+4}}{\rightleftharpoons}} N_2O_4 \tag{4}$$

$$k_{+4} = 3.8 \times 10^7\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$$
$$k_{-4} = 5.8 \times 10^2\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$$

The initial concentration of $NO_2^\bullet$ can easily be derived from primary radical yields, while $Y(e_{aq}^-) = 0.28\,\mathrm{\mu mol\,J^{-1}}$, $Y(OH) = 0.28\,\mathrm{\mu mol\,J^{-1}}$, $Y(NO_2^\bullet) = 0.56\,\mathrm{\mu mol\,J^{-1}}$ in the above system.[8]

RESULTS

The reaction rate constants of OH radical with nitrite, ferulic acid, sinapic acid and caffeic acid have been determined as 1.0×10^{10}, 7.6×10^9, 9.6×10^9, $7.4 \times 10^9\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ at pH 7, respectively. Thus, the fraction of OH radical oxidizing of nitrite were calculated to be 0.990, 0.998, 0.991 for $4\,\mathrm{mmol\,dm^{-3}}$ sodium nitrite aqueous solutions containing $0.05\,\mathrm{mmol\,dm^{-3}}$ ferulic acid, sinapic acid or caffeic acid, respectively.

In the pulse radiolysis of N_2O saturated neutral aqueous solution containing $4\,\mathrm{mmol\,dm^{-3}}$ sodium nitrite and $0.05\,\mathrm{mmol\,dm^{-3}}$ ferulic acid, an absorption spectrum with a maximum at 350 nm was observed at 20 μs after the electron pulse (Figure 1(A)). This spectrum is in good agreement with the results obtained in the oxidation of ferulic acid by $N_3^\bullet$ radical,[12] and can be assigned to the phenoxyl radical produced by reaction of ferulic acid with nitrogen dioxide radical.

$$HCA + NO_2^\bullet \rightarrow HCA(-H)^\bullet + H^+ + NO_2^- \tag{5}$$

Figure 1(B) shows the change of absorption at 350 nm with time after the pulse, showing formation of the phenoxyl radical. The rate constant for the reaction of ferulic acid with nitrogen dioxide radical (reaction 5) at pH 7, was determined to be $7.4 \times 10^8\,\mathrm{dm^3\,mol^{-1}\,s^{-1}}$ by plotting the observed first-order rate constants deduced from the formation of phenoxyl radical

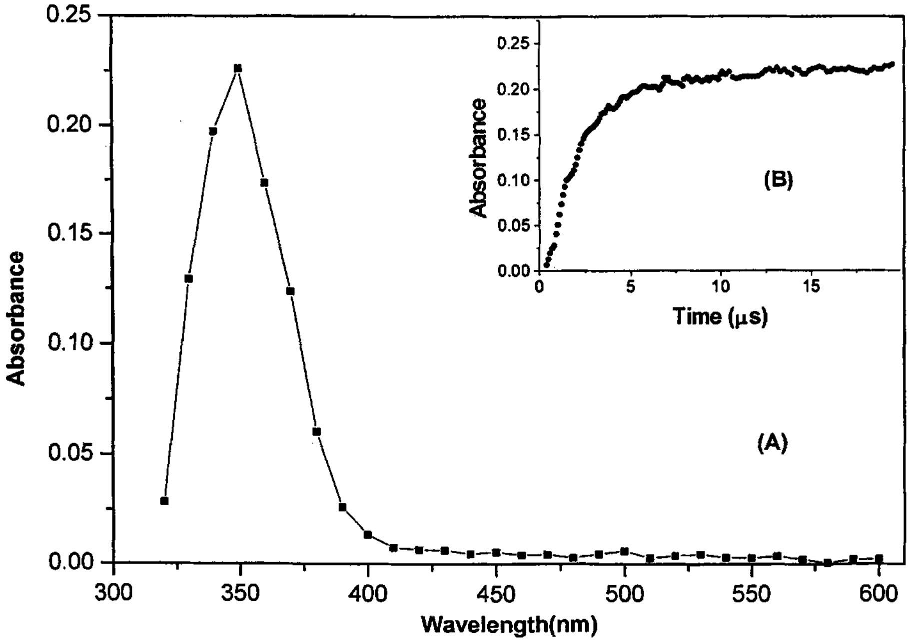

FIGURE 1 (A) Absorption spectrum of transient species formed in reaction of $NO_2^\bullet$ with ferulic acid, which corresponds to the phenoxyl radical $PhO^\bullet$, was observed at 20 μs in pulse radiolysis (average pulse dosage was 9 Gy) of the N_2O saturated aqueous solution containing 0.05 mmol dm^{-3} ferulic acid and 4 mmol dm^{-3} $NaNO_2$ at pH = 7. (B) The inset shows the absorption–time profile recorded at 350 nm.

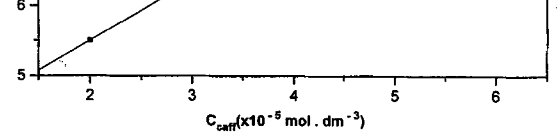

FIGURE 2 The rate costant for the first-order reaction of $NO_2^\bullet$ with ferulic acid, which was determined from the rate of build-up of $PhO^\bullet$ absorption at 350 nm after pulse radiolysis of N_2O saturated aqueous solution contain 4 mmol dm^{-3} $NaNO_2$ and the ferulic acid at five different concentrations from 0.02 to 0.06 mmol dm^{-3}.

observed at 350 nm vs concentration of ferulic acid (in the range of 0.02 ~ 0.06 mmol dm^{-3}) as shown in Figure 2. The rate constants for reaction of electron transfer from sinapic acid or caffeic acid to nitrogen dioxide radical have also been determined by the same way as listed in Table I.

TABLE I The rate constants *k* for reaction of $NO_2^\bullet$ with HCA

HCA	k (10^8 dm^3 mol^{-1} s^{-1})
Caffeic acid	8.6
Ferulic acid	7.4
Sinapic acid	7.2

The molar extinction coefficients of the phenoxyl radicals of HCA derivatives have been determined[13] to be around 24 000, 59 000 and 9000 dm^3 mol^{-1} cm^{-1} for ferulic acid, sinapic acid and caffeic acid observed at 350, 360 and 360 nm by use of KSCN as reference, respectively. Thus, the *G* value of the phenoxyl radicals generated from ferulic acid, sinapic acid and caffeic acid oxidized by nitrogen dioxide radical, was calculated to be 4.96, 4.90 and 4.28 respectively. So HCA derivatives can effectively scavenge nitrogen dioxide radical via electron transfer.

DISCUSSION

The rate constants for reactions of $NO_2^{\bullet}$ with HCA derivatives are much higher than those with phenol ($8.6 \times 10^6\,dm^3\,mol^{-1}\,s^{-1}$ at pH = 12.1) and *p*-methoxyphenol ($1.4 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$ at pH = 12),[14] indicating that the extended conjugation in HCA derivatives makes them easier to oxidize. Therefore HCA derivatives can repair the oxidizing OH radical adduct of dGMP with rate constants of electron transfer reactions for ferulic acid, sinapic acid and caffeic acid determined as 2.2×10^9, 2.5×10^9, and $2.5 \times 10^9\,dm^3\,mol^{-1}\,s^{-1}$, respectively.[13]

Moreover, the rate constants for oxidation of tyrosine and cysteine residues in peptides, arachidonic acid and linoleate by $NO_2^{\bullet}$ radical were determined as $3.2 \times 10^5\,dm^3\,mol^{-1}\,s^{-1}$ (pH = 7.5), $2.4 \times 10^8\,dm^3\,mol^{-1}\,s^{-1}$ (pH = 9.2), $1 \times 10^6\,dm^3\,mol^{-1}\,s^{-1}$ (pH = 9.0), $2 \times 10^6\,dm^3\,mol^{-1}\,s^{-1}$ (pH = 9.4),[7,8] respectively. They are all far lower than those determined with HCA. It is expected that HCA should be a good protectant of cell membrane and enzymes against oxidation by nitrogen dioxide radical.

Acknowledgment

This work was supported by the National Natural Science Foundation of China.

References

[1] P. Urone (1976). I. Air Pollutants, their transformation and transport. In *Air Pollution* (Stern, A.C., ed.), Academic Press, New York, pp. 23–75.

[2] A. Treinin and E. Hayon (1970). Absorption spectra and reaction kinetics of NO_2, N_2O_3, and N_2O_4 in aqueous solution. *Journal of The American Chemistry Society*, **92**, 521–5828.

[3] A.J. Frank and M. Gratzel (1982). Sensitized photoreduction of nitrate in homogeneous and micellar solutions. *Inorganic Chemistry*, **21**, 3834–3438.

[4] W.A. Pryor and J.W. Lightsey (1981). Mechanisms of nitrogen dioxide reaction: initiation of lipid peroxidation and the production of nitrous acid. *Science*, **214**, 435–437.

[5] W.A. Pryor, J.W. Lightsey and D.F. Church (1982). Reaction of nitrogen dioxide with alkenes and polyunsaturated fatty acids: addition and hydrogen abstraction mechanisms. *Journal of The American Chemistry Society*, **104**, 6685–6692.

[6] A.S. Csallany and K.L. Ayaz (1978). Long-term NO_2 exposure of mice in the presence and absence of vitamin E. I. Effect on body weights and lipofuscin in pigments. *Archives of Environmental Health*, **6**, 285–291.

[7] L.G. Forni, V.O. Mora-Arellano, J.E. Packer and R.L. Willson (1986). Nitrogen dioxide and related free radicals: electron-transfer reactions with organic compounds in solutions containing nitrite or nitrate. *Journal of The Chemistry Society. Perkin Trans. II*, **1**, 1–6.

[8] W.A. Prutz, H. Monig, J. Butler and E.J. Land (1985). Reactions of nitrogen dioxide in aqueous model systems: oxidation of tyrosine units in peptides and proteins. *Archives of Biochemistry and Biophysics*, **243**, 125–134.

[9] N.Y. Lin (1990). Radiation induced long range electron transfer in biological systems. *Research Chemistry Intermedium*, **14**, 209–221.

[10] W.F. Wang, J. Luo, S.D. Yao, Z.R. Lian, Z.H. Zuo, J.S. Zhang and N.Y. Lin (1993). Interaction of phenolic antioxidants and hydroxyl radical. *Radiation Physics and Chemistry*, **42**, 985–988.

[11] S.D. Yao, S.G. Sheng, J.H. Cai, J.S. Zhang and N.Y. Lin (1995). Nanosecond pulse radiolysis study in China. *Radiation Physics and Chemistry*, **46**, 105–110.

[12] W.F. Wang, J. Luo, S.D. Yao, Z.R. Lian, Z.H. Zuo, J.S. Zhang and N.Y. Lin (1995). Pulse radiolysis studies of the interruption of hydroxycinnamic acid derivatives with oxidizing OH adducts of pyrimidine. *Radiation Physics and Chemistry*, **46**, 41–45.

[13] Y. Jiang, S.D. Yao and N.Y. Lin (1997). Fast repair of oxidizing OH radical adduct of dGMP by hydroxycinnamic acid derivatives: a pulse radiolytic study. *Radiation Physics and Chemistry*, **49**, 447–450.

[14] R.E. Hue and P. Neta (1986). Kinetics of one-electron transfer reactions involving ClO_2 and NO_2. *The Journal of Physics and Chemistry*, **90**, 1193–1198.