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Spectrophotometric studies of the reaction of quercetin with peroxynitrite at different pH

Research Article

Lidia Gebicka*, Katarzyna Stawowska

Institute of Applied Radiation Chemistry,
Technical University of Łodz, Wroblewskiego 15, 93-590 Łodz, Poland

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Abstract: Peroxynitrite (ONOOH/ONOO-) which is formed in vivo under oxidative stress is a strong oxidizing and nitrating agent. It has been reported that several flavonoids, including quercetin, inhibit the peroxynitrite-induced oxidation and/or nitration of several molecules tested; however, the mechanism of their protective action against peroxynitrite is not univocally resolved. The kinetics of the reaction of quercetin with peroxynitrite was studied by stopped-flow as well as by conventional spectrophotometry under acidic, neutral and alkaline pH. The obtained results show that the protective mechanism of quercetin against peroxynitrite toxicity cannot be explained by direct scavenging of peroxynitrite. We propose that quercetin acts via scavenging intermediate radical products of peroxynitrite decomposition (it is an excellent scavenger of •NO2) and/or via reduction of target radicals formed in the reaction with peroxynitrite.

Keywords: *Autoxidation • Bicarbonate • Flavonoids • Peroxynitrite • Quercetin*

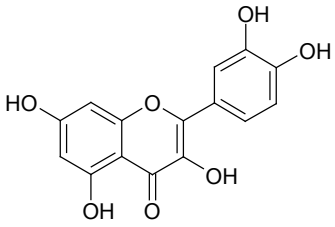
1. Introduction

Peroxynitrite is the product of the diffusion controlled reaction between nitric oxide ($^{\bullet}NO$) and the superoxide anion ($O_2^{-\bullet}$). The term peroxynitrite is used to refer to both peroxynitrite anion ($ONOO^-$) and peroxynitrous acid (ONOOH). The peroxynitrite anion is stable, but ONOOH (pK_a = 6.8) rapidly (k = 1.2 s^{-1} at 25°C [1]) isomerizes to nitrate, partially (about 30%) via intermediate radical products, $^{\bullet}NO_2$ and $^{\bullet}OH$ [2,3]. Peroxynitrite may oxidize biologically important molecules either directly or via intermediate radicals. It is also a strong nitrating agent. The participation of peroxynitrite in many pathological events has been reported [4 and references therein]. On the other hand, peroxynitrite has been identified as a key cytotoxic effector of immune system cells towards invading bacteria and parasites [5]. One of the major targets of peroxynitrite *in vivo* seems to be CO_2, present in biological media at millimolar concentration. This reaction leads to the formation of an adduct, $ONOOCO_2^-$ (k = 3×10^4 M^{-1} s^{-1} at 24°C [6]), which rapidly undergoes homolysis to form $CO_3^{-\bullet}$ and $^{\bullet}NO_2$ with the yield of about 30% [7 and references therein]. The $CO_3^{-\bullet}/^{\bullet}NO_2$ radical pair possesses a stronger nitration potential than the $^{\bullet}OH/^{\bullet}NO_2$ radical pair formed from the isomerization of peroxynitrous acid.

Flavonoids are plant polyphenols and are present as common components in human diet. Their antioxidant properties has been extensively studied [8 and references therein]. It has been reported that, among others, several flavonoids inhibit different peroxynitrite-mediated injuries [9 and references therein]. However, their protective mechanism of action against peroxynitrite is not univocally resolved. It has been suggested that flavonoids do not react directly with peroxynitrite but rather with reactive intermediates from peroxynitrite decomposition [10] or with radicals formed in the reaction of peroxynitrite with several molecules previously investigated (*e.g.* tyrosyl radicals) [11]. On the other hand, Kono *et al.* have concluded from a competitive kinetic analysis that peroxynitrite reacts directly with chlorogenic acid, a polyphenolic non-flavonoid compound [12].

In the present study the kinetics of the reaction of peroxynitrite with quercetin at neutral and alkaline pH is investigated by stopped-flow as well as by conventional spectroscopy. We have selected quercetin (3,3,'4', 5,7 –

* *E-mail: lgebicka@mitr.p.lodz.pl*



Scheme 1. Structure of quercetin (3,3,'4', 5,7 – pentahydroxyflavone).

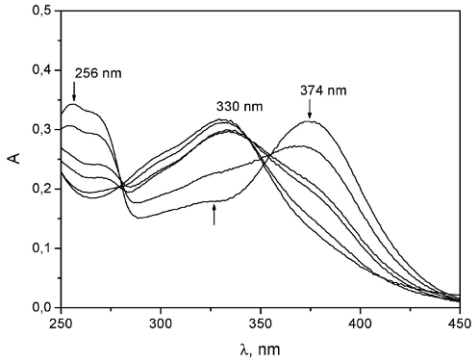


Figure 1. Absorption spectra of 17 μM quercetin taken after 1 min incubation with peroxynitrite at concentrations: 0, 25, 50, 75, 100 and 150 μM, at pH 7.2.

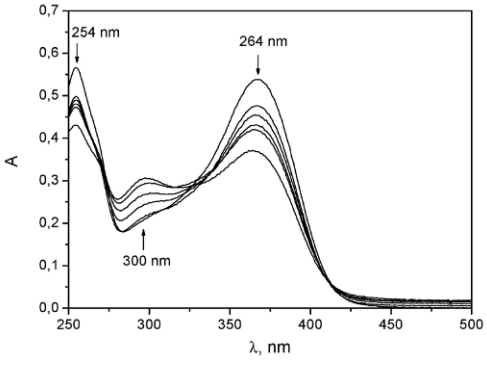


Figure 2. Absorption spectra of 20 μM quercetin taken after 0.5 min incubation with peroxynitrite at concentrations: 0, 50, 100, 150, 200 and 250 μM, at pH 5.0.

pentahydroxyflavone, Scheme 1) as a model flavonoid, because it is one of the most efficient flavonoids for inhibiting peroxynitrite-induced oxidation and/or nitration of previously tested molecules [13-16].

2. Experimental procedure

A stock solution of quercetin (Sigma) was prepared in ethanol. The concentration of quercetin was determined spectrophotometrically using the extinction coefficient ε_{374} = 2.84×10^4 M^{-1} cm^{-1} [17]. Peroxynitrite was synthesized by the reaction of nitrite with hydrogen peroxide under acidic conditions [18]. Unreacted hydrogen peroxide was removed from peroxynitrite solution by treatment with solid manganese dioxide whereas nitrite remained as a contaminant. The final concentration of peroxynitrite was equal to 25-50 mM as determined spectrophotometrically using the extinction coefficient ε_{302} = 1670 M^{-1} cm^{-1} [19]. The stock solutions of $ONOO^-$ at pH 12 were stored at -25° C and used within 3-4 weeks of synthesis. Quercetin, nitrite and hydrogen peroxide (30%) were obtained from Sigma. All others chemicals were of analytical grade. Nano-pure water from MilliQ (Millipore) was used throughout.

The reactions of peroxynitrite with quercetin were studied at pH 5.0, 6.0, 7.2 and 11. Peroxynitrite solutions were brought to the desired concentrations by diluting the stock solution with 0.01 M NaOH. Quercetin solutions were prepared in 0.2 M acetate or phosphate buffers of a desired pH and were bubbled with nitrogen in order to remove traces of CO_2. In some experiments 40 mM bicarbonate was added to quercetin solution. Solutions used in experiments taken at pH 11.0 were saturated with argon for 2 h in a glove box.

Kinetic measurements on millisecond- and second-time scales were performed using the SX-20 MV Applied Photophysics stopped-flow spectrophotometer with 1-cm cell and with a mixing time < 1 ms. In some experiments, the photodiode-array accessory (PDA1, covering the spectral range 325-700 nm) attached to the stopped flow machine was used.

Kinetic measurements in the long-time regime were recorded using a Specord S600 diode-array spectrophotometer.

All kinetic measurements were carried out at 23±0.5°C and were repeated at least three times.

Kinetic simulations were performed with the use of Chemical Kinetic Simulator (CKS) 1.01 (IBM Almaden Research Center).

3. Results and discussion

The absorption spectra obtained after mixing quercetin with peroxynitrite at pH 7.2 and 5.0 are shown in Figs. 1 and 2, respectively. The addition of peroxynitrite to a solution of quercetin at pH 7.2 causes a decrease in the quercetin absorption bands at 256 and 374 nm and build up of a new band at 330 nm. The latter is probably connected with the formation of a quercetin quinone-water adduct [20]. Similar absorption spectra have been reported for enzymatically oxidized quercetin [21,22]. The addition of peroxynitrite to a solution of quercetin at pH 5.0 and 6.0 (data not shown) causes a decrease in the quercetin absorption bands at 254 and 364 nm and a build up of a new band around 300 nm. It should be

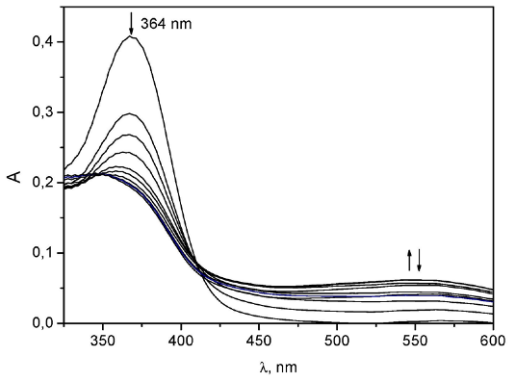


Figure 3. Time-resolved spectra of 15 μM quercetin taken 0, 0.2, 0.6, 1, 1.6, 2, 3, 5, 7, and 9 seconds after mixing with 100 μM peroxynitrite at pH 6.0.

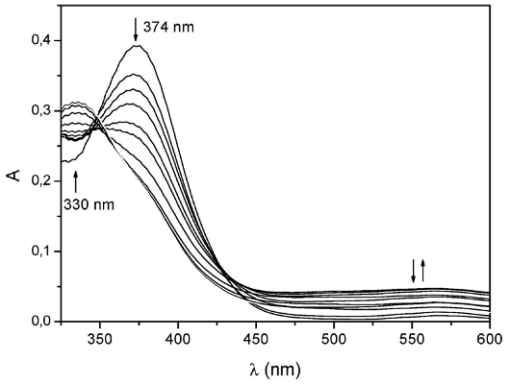


Figure 4. Time-resolved spectra of 15 μM quercetin taken 0, 0.2, 0.6, 1, 1.6, 2, 3, 5, 7, and 9 seconds after mixing with 100 μM peroxynitrite at pH 7.0.

stressed that at pH below 7, quercetin is fully protonated [23], which affects its absorption spectrum. The pH variations can also modify the nature of quercetin oxidation products. For example, under acidic conditions the absorption spectrum of enzymatically oxidized quercetin exhibits a maximum at 293 nm [24,25].

The kinetics of the reaction of quercetin with peroxynitrite at different pH was studied by a stopped-flow method. We did not observe measurable changes in the absorption spectrum of quercetin during the reaction with peroxynitrite under conditions when quercetin (15-20 μM) was in excess or at comparable concentration with that of peroxynitrite. Thus, we studied the kinetics of this reaction under conditions at which peroxynitrite was in excess. The rates and yields of peroxynitrite-induced absorption changes in quercetin were dependent on peroxynitrite concentration. The time-resolved absorption spectra taken after mixing quercetin with a 5-fold excess of peroxynitrite at pH 6.0 and 7.2 are shown in Figs. 3 and 4, respectively. Characteristic absorption peaks of quercetin at 364 nm (pH 6.0) and 374 nm (pH 7.2) disappear and new bands in the UV (with a maximum dependent on pH) and visible regions (very broad one, with a maximum around 550 nm) are formed. The latter band disappears in a matter of seconds (Fig. 5). The kinetics of the decay of 364 or 374 nm absorption bands is complex and we are not able to resolve it. The higher peroxynitrite concentration, the faster and the higher were the observed absorption changes of quercetin. Surprisingly, the fastest absorption changes were detected at pH 6.0. The obtained results may be explained by the known facts that a variety of products are formed as a result of quercetin oxidation depending on pH [22,25,26]. On the other hand, both stages of kinetic traces (an absorption increase was followed by an absorption decrease) recorded at 550 nm could be fitted with a single exponential equation and the obtained rate constants (k_i and k_d) depend on peroxynitrite concentration. The values of these rate constants determined at different pH are summarized in Table 1. These absorption changes are probably connected with the formation and decay of an unstable free quinone [20].

To simulate the kinetic traces obtained from stopped-flow measurements, the following processes were included:

pH-dependent peroxynitrous acid/peroxynitrite anion ratio:

$$ONOO^- + H^+ \rightarrow ONOOH \quad (1)$$
$k_1 = 1.0 \times 10^{10}\ M^{-1} s^{-1}$ [27]

$$ONOOH \rightarrow ONOO^- + H^+ \quad (2)$$
$k_2 = 1578\ s^{-1}$ (calculated from pK_a of ONOOH)

isomerization of peroxynitrite including radical formation and decay:

$$ONOOH \rightarrow NO_3^- + H^+ \quad (3)$$
$k_3 = 0.84\ s^{-1}$ (calculated from $k_{izo} = 1.2\ s^{-1}$ ([1]) taking into account that 30% of peroxynitrite isomerizes *via* radical products [2,3].

$$ONOOH \rightarrow {}^{\bullet}NO_2 + {}^{\bullet}OH \quad (4)$$
$k_4 = 0.36\ s^{-1}$ (calculated as above)

$$2\ {}^{\bullet}NO_2 \rightarrow N_2O_4 \quad (5)$$
$2k_5 = 4.5 \times 10^8\ M^{-1} s^{-1}$ [28]

$$N_2O_4 + H_2O \rightarrow NO_3^- + NO_2^- + H^+ \quad (6)$$
$k_6 = 1 \times 10^3\ s^{-1}$ [29]

$${}^{\bullet}OH + NO_2^-\ \text{(contaminant in peroxynitrite solution)} \rightarrow$$
$$\rightarrow NO_2^{\bullet} + OH \quad (7)$$
$k_7 = 5.3 \times 10^9\ M^{-1} s^{-1}$ [30]

$^{\bullet}OH + ONOO^{-} \rightarrow {}^{\bullet}NO + O_2 + OH^{-}$ (8)
$k_8 = 4.8 \times 10^9\ M^{-1}\ s^{-1}$ [30]

$^{\bullet}NO + {}^{\bullet}NO_2 \rightarrow N_2O_3$ (9)
$k_9 = 1.1 \times 10^9\ M^{-1}\ s^{-1}$ [30]

$N_2O_3 + ONOO^{-} \rightarrow 2\ {}^{\bullet}NO_2 + NO_2^{-}$ (10)
$k_{10} = 3.1 \times 10^8\ M^{-1}\ s^{-1}$ [30]

reactions of quercetin with $^{\bullet}OH$ and $^{\bullet}NO_2$ and disproportionation of quercetin radicals:

quercetin (Q) + $^{\bullet}OH \rightarrow$ Q($^{\bullet}OH$) adduct (11)
$k_{11} = 2 \times 10^9\ M^{-1}\ s^{-1}$ [31]

Q($^{\bullet}OH$) adduct $\rightarrow$ Qphenoxyl radical (QPhe$^{\bullet}$) (12)
$k_{12} = 3 \times 10^5\ s^{-1}$ [31]

Q + $^{\bullet}NO_2 \rightarrow$ QPhe$^{\bullet}$ (13)
$k_{13} = 1.9 \times 10^8\ M^{-1}\ s^{-1}$ [32]

2 QPhe$^{\bullet} \rightarrow$ Qquinone + Q (14)
$2k_{14} = 6 \times 10^7\ M^{-1}\ s^{-1}$ [33]

and reaction of quercetin with peroxynitrous acid:

Q + ONOOH $\rightarrow$ product (15)
k_{15} – to be elucidated

The simulations showed that introducing the rate constant of the reaction of 20 μM quercetin with equimolar amounts of peroxynitrite at pH 7.2 equal to $10^3\ M^{-1}\ s^{-1}$ yielded a concentration of the product for this reaction of about 0.3 μM, which corresponds to absorption changes at 374 nm of < 0.01. Recalling the fact that we did not detect measurable changes in the absorption spectrum of 20 μM quercetin after mixing it with peroxynitrite at comparable concentration, it seems that the value of $10^3\ M^{-1}\ s^{-1}$ represents the upper limit for the rate constant of this reaction, if it takes place at all.

In order to check whether peroxynitrite anion is involved in the reaction with quercetin, we studied this reaction at pH 11.0, where peroxynitrite exists exclusively in its anionic form. As quercetin in alkaline solution, in the presence of air, is very unstable due to autoxidation process [20,34], all reagents were deoxygenated prior to mixing. An absorption spectrum taken immediately after alkalization of quercetin solution up to pH 11.0 shows two absorption peaks, at 275 and 422 nm, characteristic for deprotonated quercetin [34]. In spite of deoxygenation, this spectrum decays in tens of minutes and a new spectrum of oxidized

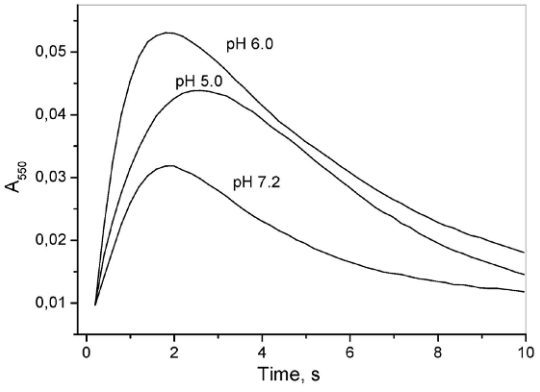


Figure 5. Kinetic traces observed at 550 nm after mixing 15 μM quercetin with 100 μM peroxynitrite at pH 5.0, 6.0 and 7.2.

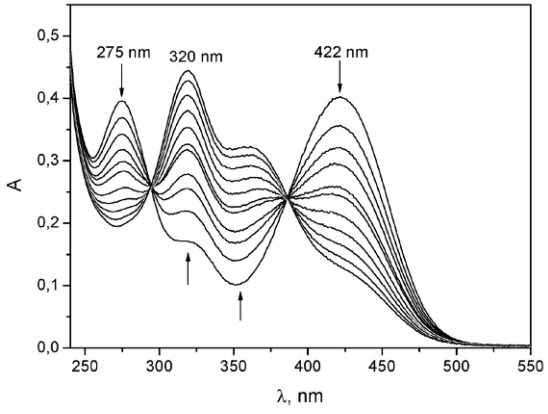


Figure 6. Absorption spectra of 20 μM quercetin at pH 11.0 taken every 2 min after alkalization of quercetin solution.

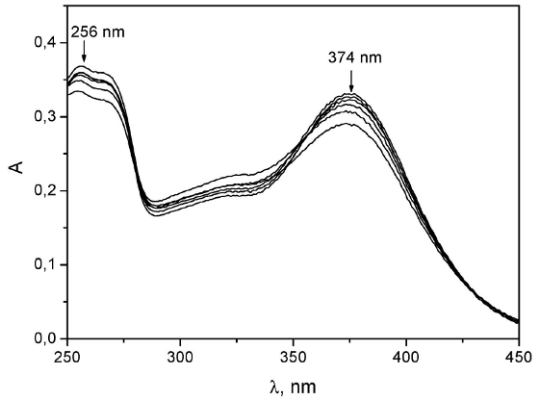


Figure 7. Absorption spectra of 15 μM quercetin taken after 1 min incubation with 40 mM bicarbonate and peroxynitrite at concentrations: 0, 25, 50, 75, 100 and 150 μM, at pH 7.2.

quercetin, with absorption maximum at 320 nm and a shoulder around 365 nm is formed. Two isosbestic points at 294 and 386 nm are observed (Fig. 6). Most probably, the concentration of residual oxygen was enough to initiate the oxidation of quercetin. When peroxynitrite

Table 1. The observed first-order rate constants (s^{-1}) of quercetin absorbance changes at 550 nm induced by peroxynitrite at different pH as a function of peroxynitrite concentration. [quercetin] = 20 μM.

[peroxynitrite], mM	pH 5.0		pH 6.0		pH 7.2	
	k_i	k_d	k_i	k_d	k_i	k_d
0.05	0.90	0.19	1.32	0.15	0.84	0.07
0.1	1.22	0.25	1.72	0.20	1.12	0.08
0.15	1.25	0.30	3.20	2.16	1.63	0.43
0.2	1.32	0.34	7.20	2.97	2.67	1.12

anion was added to an alkaline quercetin solution, the rate of quercetin oxidation increased with an increase of the concentration of peroxynitrite. In order to verify whether an increased rate of quercetin oxidation is the consequence of the quercetin reaction with peroxynitrite anion or results only from the increased ionic strength [20], we obtained time-resolved spectra of quercetin at pH 11.0 in the presence of $NaNO_2$ (peroxynitrite contaminant) or NaCl at the same ionic strength as that of peroxynitrite solution. The rate of quercetin oxidation measured at 422 nm did depend on ionic strength in an identical manner as for $NaNO_2$, NaCl and peroxynitrite. Isosbestic points (one near the maximum of the absorption spectrum of peroxynitrite anion) were detected in all cases. The above observations suggest that the peroxynitrite anion does not react with quercetin.

The results obtained in these studies do not allow for a definitive conclusion regarding whether quercetin reacts directly with peroxynitrous acid or not. We can only speculate, based on the kinetic simulation (see above), that even if peroxynitrite reacts directly with quercetin, the rate constant of this reaction is not higher than 10^3 M^{-1} s^{-1}. If this is the case, the combination of such a small rate constant with the fact that the concentration of quercetin is relatively low *in vivo* [35], indicates that this reaction is practically insignificant.

Studies *in vitro* have shown that quercetin is very effective in protecting against peroxynitrite-induced tyrosine nitration and oxidation of dihydrorhodamine 123 (RhH_2) [13-16]. For example, the yield of oxidation of 25 μM RhH_2 by 1.2 μM peroxynitrite at pH 7.4 is lowered by almost a factor of two in the presence of 1 μM quercetin [15]. It is quite likely that RhH_2 is oxidized by peroxynitrite indirectly, *i.e.*, *via* the radical products [36]. Under such conditions, the protective mechanism of quercetin against peroxynitrite toxicity cannot be explained by the direct scavenging of peroxynitrite (see discussion above). Both RhH_2 and quercetin react with hydroxyl radicals with rate constants close to the diffusion-controlled limit [31,37]. The rate constant of the reaction of quercetin with $^{\bullet}NO_2$ (reaction 15) is, however, significantly higher than that reported for RhH_2 (~4-7×10^5 M^{-1} s^{-1} [38]). Thus quercetin may protect dihydrorhodamine against peroxynitrite via scavenging $^{\bullet}NO_2$ radicals. It should be noted that the rate constants of the reactions of some other flavonoids with $^{\bullet}NO_2$ are also significantly higher than those reported for other molecules [31,32,39,40]. In an aerated solution, dihydrorhodamine radicals ($RhH^{\bullet}$) react with oxygen with a rate constant of ~ 7×10^8 M^{-1} s^{-1} [38] to form unstable peroxyl radicals ($RhHOO^{\bullet}$). It has been suggested that these radicals eliminate $O_2^{-\bullet}$ which reacts with $^{\bullet}NO_2$ to form peroxynitrate at a diffusion controlled rate [38]. Most likely, under conditions described by Santos and Mira [15], quercetin also scavenges $RhHOO^{\bullet}$ radicals before they eliminate $O_2^{-\bullet}$. Quercetin and some others flavonoids are very efficient scavengers of peroxyl radicals [41].

Considering the physiological relevance of the reaction between carbon dioxide and peroxynitrite we also studied the absorption spectra of quercetin incubated with bicarbonate and peroxynitrite. Under such reaction conditions peroxynitrite was scavenged by CO_2 and $^{\bullet}NO_2$ and $CO_3^{-\bullet}$ radicals were formed as intermediate products. These radicals could decay via biradical reactions or via reaction with quercetin. Taking into account all the possible reaction routes, their rate constants [32,39,42] and concentrations of reagents, we concluded that some of $^{\bullet}NO_2$ and $CO_3^{-\bullet}$ radicals could react with quercetin. Fig. 7 shows the absorption spectra of quercetin incubated with peroxynitrite and bicarbonate. The characteristic quercetin bands at 256 and 374 nm decrease with an increase of peroxynitrite concentration, but the yield of quinone formation is very low, if any. This could mean that other stable products of quercetin oxidation are formed under such conditions (*e.g.* nitrated quercetin) [16]. It has been shown that the protective effect of quercetin and some other flavonoids against the peroxynitrite-mediated nitration of tyrosine and oxidation of dihydrorhodamine diminishes in the presence of physiological concentrations of bicarbonate [15,43].

4. Conclusions

The results presented above indicate that the protective mechanism of quercetin against peroxynitrite toxicity cannot be explained by the direct scavenging of peroxynitrite. We propose that quercetin acts via scavenging the intermediate radical products of peroxynitrite decomposition (it is an excellent scavenger of $^{\bullet}NO_2$) and/or *via* the reduction of target radicals formed in the reaction with peroxynitrite.

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References

[1] W.H. Koppenol, R. Kissner, Chem. Res. Toxicol. 11, 87 (1998)
[2] O.V. Gerasimov, S.V. Lymar, Inorg. Chem. 38, 4317 (1999)
[3] G.R. Hodges, K.U. Ingold, J. Am. Chem. Soc. 121, 10695 (1999)
[4] P. Pacher, J.S. Beckman, L. Liaudet, Physiol. Rev. 87, 315 (2007)
[5] G. Ferrer-Sueta, R. Radi, ACS Chem. Biol. 4, 161 (2009)
[6] S.V. Lymar, J.K. Hurst, J. Am. Chem. Soc. 117, 8867 (1995)
[7] O. Augusto, M.G. Bonini, A.M. Amanso, E. Linares, C.C.X. Santos, S.L. de Menezes, Free Radic. Biol. Med. 32, 841 (2002)
[8] K.E. Heim, A.R. Tagliaferro, D.J. Bobilya, J. Nutr. Biochem. 13, 572 (2002)
[9] M. Trujillo, G. Ferrer-Sueta, R. Radi, Antiox. Redox. Sign. 10, 1 (2008)
[10] G.E. Arteel, P. Schroeder, H. Sies, J. Nutr. 130, 2100S (2000)
[11] P. Schroeder, H. Zhang, L-O. Klotz, B. Kalyanaraman, H. Sies, Biochim. Biophys. Res. Commun. 289, 1334 (2001)
[12] Y. Kono, K. Kobayashi, S. Tagawa, K. Adachi, A. Ueda, Y. Sawa, H. Shibata, Biochim. Biophys. Acta 1335, 335 (1997)
[13] G.R.M.M. Haenen, J.B.G. Paquay, R.E.M. Korthouwer, A. Bast, Biochem. Biophys. Res. Commun. 236, 591 (1997)
[14] J.S. Choi, H.Y. Chung, S.S. Kang, M.J. Jung, J.W. Kim, J.K. No, H.A. Jung, Phytother. Res. 16, 232 (2002)
[15] M.R. Santos, L. Mira, Free Rad. Res. 38, 1011 (2004)
[16] M. Sadeghipour, R. Terreux, J. Phipps, Toxicol. in Vitro 19, 155 (2005)
[17] X. Chen, D.U. Ahn, J. Am. Oil Chem. Soc. 75, 1717 (1998)
[18] J.S. Beckman, J. Chen, H. Ischiropoulos, J.P. Crow, Methods Enzymol. 233, 229 (1994)
[19] M.N. Huges, H.G. Nicklin, J. Chem. Soc. 450 (1968)
[20] O. Dangles, C. Dufour, S. Bret, J. Chem. Soc. PerkinTrans 2, 737 (1999)
[21] D. Metodiewa, A.K. Jaiswal, N. Cenas, E. Dickancaite, J. Segura-Aguilar, Free Radic. Biol. Med. 26, 107 (1999)
[22] A. Zhou, O.A. Sadik, J. Agric. Food Chem. 56, 12081 (2008)
[23] J.M. Herrero-Martinez, M. Sanmartin, M. Roses, E. Bosch, C. Rafols, Electrophoresis 26, 1886 (2005)
[24] U. Takahama, T. Oniki, S. Sirota, J. Agric. Food Chem. 50, 4317 (2002)
[25] S. Hirota, U. Takahama, T.N. Ly, R. Yamauchi, J. Agric. Food Chem. 53, 3265 (2005)
[26] H.M. Awad, M.G. Boersma, J. Vervoort, I.M.C.M. Rietjens, Arch. Biochem. Biophys. 32, 224 (2000)
[27] R. Kissner, T. Nauser, C. Kurz, W.H. Koppenol, IUMBM Life 55, 567 (2003)
[28] R.K. Broszkiewicz, Bull. Acad. Sci. Sedr. Sci. Chim. 24, 221 (1976)
[29] S.E. Schwartz, W.H. White, Adv. Environ. Sci. Technol. 12, 1 (1983)
[30] S. Goldstein, J. Lind, G. Merenyi, Chem. Rev. 105, 2457 (2005)
[31] A. Torreggiani, A. Trinchero, M. Tamba, P. Taddei, J. Raman Spectrosc. 36, 380 (2005)
[32] C.Y. Zhao, Y.M. Shi, S.D. Yao, Z.J. Jia, B.T. Fan, W.F. Wang, W.Z. Lin, N.Y. Lin, R.L. Zheng, Pharmazie 58, 742 (2003)
[33] W. Bors, C. Michel, S. Schikora, Free Rad. Biol. Med. 19, 45 (1995)
[34] L-M. Lin, H-Y. Wu, W-S. Li, W-L. Chen, Y-J. Lee, D.C. Wu, P. Li, A. Yeh, Inorg. Chem. Commun. 13, 633 (2010)

[35] S.K. Nicholson, G.A. Tucker, J.M. Brameld, Br. J. Nutr. 103, 1398 (2010)
[36] J. Glebska, W.H. Koppenol, Free Rad. Biol. Med. 35, 676 (2003)
[37] M. Wrona, K. Patel, P. Wardman, Free Rad. Biol. Med. 38, 262 (2005)
[38] L.K. Folkes, K.B. Patel, P. Wardman, M. Wrona, Arch. Biochem. Biophys. 484, 122 (2009)
[39] P. Neta, R.E. Huie, A.B. Ross, J. Phys. Chem. Ref. Data 17, 1027 (1988)
[40] J.-L. Miao, W.-F. Wang, J.-X. Pan, C.-Y. Liu, R.-Q. Li, S.-D. Yao, Radiat. Phys. Chem. 60, 163 (2001)
[41] W. Bors, C. Michel, M. Saran, Methods Enzymol. 234, 420 (1994)
[42] S. Herold, M. Exner, F. Boccini, Chem. Res. Toxicol. 16, 390 (2003)
[43] U. Ketsawatsakul, M. Whiteman, B. Halliwell, Biochem. Biophys. Res. Commun. 279, 692 (2000)