doi: 10.17221/611/2014-CJFS

Details of the Antioxidant Mechanism of Hydroxycinnamic Acids

Ya-Jing SHANG, Bao-Yong LIU and Meng-Meng ZHAO

School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou, P.R. China

Abstract

Shang Y.-J., Liu B.-Y., Zhao M.-M. (2015): **Details of the antioxidant mechanism of hydroxycinnamic acids**. Czech J. Food Sci., 33: 210–216.

Antioxidant activities and free radical-mediated DNA strand breakages of five hydroxycinnamic acids were examined. Kinetic analysis of a stable galvinoxy (GO•)-scavenging reaction of hydroxycinnamic acids demonstrated that the molecular structure and the reaction medium were two important factors affecting the antioxidant mechanism and activity. In methanol, the kinetic process of the compounds, which have electron-donating groups (-OH, -OCH_3) in the *ortho-* or *para*-position of 4-OH, was primarily governed by the sequential proton loss electron transfer (SPLET mechanism). While, in ethyl acetate, the reaction mechanism is predominantly direct hydrogen atom transfer (HAT mechanism). But for the compounds having only one hydroxyl, both in ethyl acetate and methanol, the reaction mechanism is only HAT. At the same time, the compound bearing *o*-diphenoxyl is not the most active one in our tested environment.

Keywords: radical-scavenging activity; DNA strand breakage; reaction mechanisms structure-activity relationship

Hydroxycinnamic acids (HCAs) are a group of naturally occurring phenolic compounds and highly abundant in the human diet, such as fruits, vegetables, and grains (Herrmann 1989; Clifford 1999; Pulido *et al*. 2000; Mattila & Hellstrom 2007). They represent one third of our total intake of phenolic compounds, and have a C_6C_3 carbon skeleton with a double bond in the side chain that may have *cis-* or *trans*-configuration. Meanwhile, they have been shown to have beneficial effects in various human diseases such as cancer (Mattila & Kompulainen 2002), foetal growth restriction and preeclampsia (Braekke *et al*. 2006; Hracsko *et al*. 2008), and several neurological disorders including Parkinson's and Alzheimer's diseases (Halliwell 2001; Butterfield 2002). This is due to the protective and preventive function against oxidative damage caused by an excess of reactive oxygen species (ROS), and which often involves reactions between free radicals and molecules of high biological importance such as lipids, proteins, and DNA. Therefore, the free radical scavenging activity is the most appealing property of these compounds.

The molecular basis for the antioxidant properties of phenolic compounds is recognised in four main mechanisms, arising from the direct reaction with free radicals, direct hydrogen atom transfer [HAT, Eq. (1)], electron transfer-proton transfer [ET-PT, Eq. (2)], proton-coupled electron transfer [PCET, Eq. (3)] and sequential proton loss electron transfer [SPLET, Eq. (4)] (Wenum *et al*. 2013).

In the HAT mechanism, a hydrogen atom of OH group is transferred from phenol (ArOH) to free radical X•, resulting in the formation of a phenoxyl radical (ArO•) and the X-H molecule [Eq. (1)]. Bond dissociation energy (BDE) has been used as an energetic parameter to evaluate the feasibility of this mechanism. Therefore the compound that has the weakest OH bond reacts more quickly with free radicals.

$$ArOH + X^{\bullet} \rightarrow ArO^{\bullet} + XH \quad (1)$$

Eq. (2) describes the ET-PT mechanism, the first step is the electron transfer from the aromatic alcohol to X• to form a radical cation and X anion, this process being characterised by calculating the ionization potential of the neutral molecule (IP). The second step consists in the proton transfer from a radical cation of alcohol to X anion giving the corresponding neutral radical.

Supported by the Youth Science Foundation of Lanzhou Jiaotong University, Project No. 2012007, and Gansu Province Natural Science Fund Project, Grant No. 310RJZA066.

doi: 10.17221/611/2014-CJFS

$$ArOH + X^{\bullet} \longrightarrow ArOH^{\bullet} + X^{-} \longrightarrow ArO^{\bullet} + XH \quad (2)$$

PCET mechanism cannot be experimentally differentiated from HAT. In the HAT mechanism, the proton together with one of its two bonding electrons is transferred to the radical. In the PCET mechanism, the proton and electron go to different acceptors. The proton moves between two electron pairs and the accompanying fifth electron moves between non-bonding orbitals.

$$ArOH + X^{\bullet} \rightarrow [ArOH^{\bullet} \cdots X^{\bullet}] \rightarrow [ArO^{\bullet +} \cdots X^{\bullet}] \rightarrow ArO^{\bullet} + XH \quad (3)$$

SPLET mechanism occurs while phenols lose a proton to a phenolate anion by an acid-base equilibrium, following an electron transfer to form the phenoxyl radical and X anion [Eq. (4)]. Recent experimental evidence suggests that the contribution of one of the pathways depends on the nature of the solvent, trace of acid or base (Litwinienko & Ingold 2003, 2004, 2007; Foti *et al*. 2004; Musialik & Litwinienko 2005; Wenum *et al*. 2013).

$$ArOH \overset{H^+}{\rightarrow} ArO^{-} \overset{X^{\bullet}}{\rightarrow} ArO^{\bullet} + X^{-} \overset{H^+}{\rightarrow} ArO^{\bullet} + XH \quad (4)$$

In previous studies, we reported a very detailed study of radica Comparison of mercury distribution between liver and muscle – a biomonitoring of fish from lightly and heavily contaminated localities. Sensors l scavenging activity and antioxidant mechanism of chalcones and resveratrol analogues by studying the influences of solvents, radicals, and substitutions on the antioxidant activity (Shang *et al*. 2009; Qian *et al*. 2011). Hydroxycinnamic acids are organic acids, with pK_a values ranging from 4 to 5 (Beltrán *et al*. 2003). What mechanism is responsible for the antioxidant reaction of these compounds? How do the polarity of a solvent and the molecular structure of cinnamic acids influence the antioxidant mechanisms? With respect to the antioxidant mechanisms of the HCAs, it has been reported that the HAT from the phenolic OH is the key mechanism (Kong *et al*. 2004; Amorati *et al*. 2006; Pino *et al*. 2006; Leon-Carmona *et al*. 2012). On the other hand, the electron transfer (ET) mechanisms have been proposed as the most important pathways for the reactions with various radicals in the aqueous phase (Lin *et al*. 1998; Pan *et al*. 1999; Amorati *et al*. 2006; Zhu *et al*. 2006). However, there has been little systematic investigation on the antioxidant mechanism and structure-activity relationship of hydroxycinnamic acids in organic solvents. Thus, this study was undertaken to compare several hydroxycinnamic acids (Figure 1) scavenging the galvinoxyl radical (GO$^{\bullet}$) efficiency in methanol and ethyl acetate. Besides, the inhibition of 2,2'-azobis(2-amidinopropane dihydrochloride) (AAPH)-induced DNA strand breakage was also tested to compare the activity in different microenvironments.

MATERIAL AND METHODS

Caffeic acid (CA, > 98%), sinapic acid (SA, > 98%), ferulic acid (FA, > 98%), 3-hydroxycinnamic acid (3-HCA, > 98%), and 4-hydroxycinnamic acid (4-HCA, > 98%) were purchased from Aldrich (Merch, Germany). Galvinoxyl radical (GO$^{\bullet}$), 2,2'-azobis(2-amidinopropane dihydrochloride) (AAPH), and pBR322 DNA were obtained from Sigma-Aldrich with the highest purity available. All solvents were of spectrographic grade.

Spectral and kinetic measurement. The rates of hydrogen transfer reactions from HCAs to GO$^{\bullet}$ were determined as follows (Shang *et al*. 2009).

The absorbance change of GO$^{\bullet}$ reaction was monitored by UV-vis spectrophotometer at 428 nm in the quartz cell at 25°C. Typically, the loss of GO$^{\bullet}$ (5×10^{-6} M in ethyl acetate or methanol) absorbance in the presence of an excess of HCAs follows pseudo-first-order kinetics.

AAPH-induced oxidative DNA strand breakage. The inhibition of AAPH-induced DNA strand breakage by HCAs was assessed by measuring the conversion of the supercoiled pBR322 plasmid DNA into open circular and linear forms by gel electrophoresis (Rahman *et al*. 1992). The procedure for

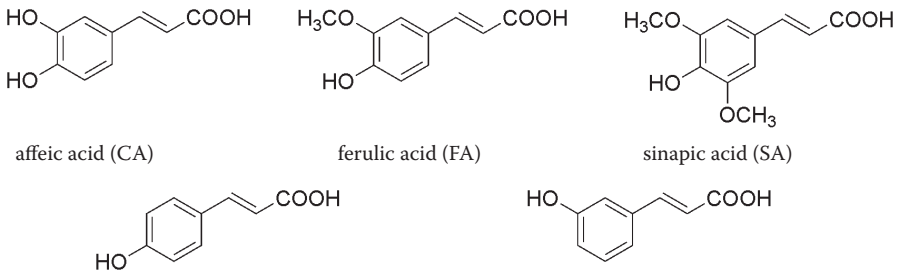


Figure 1. Molecular structures of tested hydroxycinnamic acids

doi: 10.17221/611/2014-CJFS

pBR322 DNA strand breakage assay was described in our previous works (Qian *et al*. 2011).

RESULT AND DISCUSSION

GO•-scavenging reaction kinetics of HCAs. GO• is a relatively stable oxygen-centred radical, like the prototypic models for peroxyl radicals, and it is widely used for quick evaluation of the polyphenol ability to transfer labile hydrogen atoms to radicals (Watanabe *et al*. 2000). The advantages of utilising this radical are as follows: it is commercially available, air stable and strongly coloured, allowing the course of the reaction to be monitored using UV-vis spectroscopy. The GO•-scavenging property of the HCAs was tested in ethyl acetate and methanol. The reason for selection of the two solvents is based on: (1) their polarity and hydrogen-bonding ability, leading to a dipolar, aprotic solution environment (ethyl acetate), and a polar, protic one (methanol), ethyl acetate has a much lower dielectric constant (ε = 6.02) (Litwinienko & Ingold 2004) than methanol (ε = 32.63) (Litwinienko & Ingold 2004), (2) the reactants easily dissolve in them, (3) they are stable towards radical attacks. Meanwhile, the kinetic solvent effect which leads to a modification of the phenol reactivity is independent of the nature of the radical. This test aims at determining the rate constant of hydrogen transfer from phenols to a yellow stable radical (GO•) by following its absorbance since its reduced form is colourless (Figure 2).

The kinetic rate constants (k) were carried out with pseudo-first-order kinetics: GO•-scavenging reactions of HCAs at 25°C were measured by monitoring

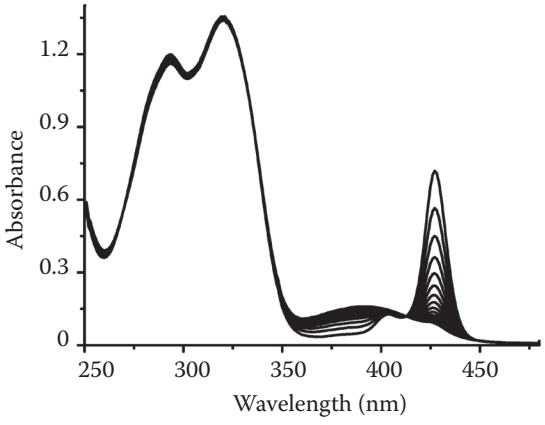


Figure 2. Spectral changes observed upon addition of caffei acid (5×10^{-5} M) to an ethyl acetate solution of galvinoxyl radikal GO• (5×10^{-6} M) at 25°C (interval 2 min)

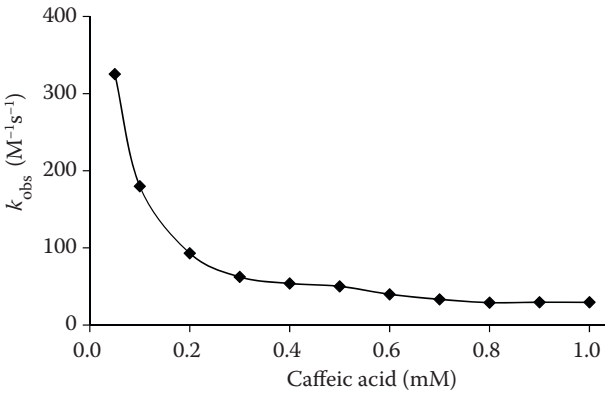


Figure 3. Relation/Plot of rate constants of reaction of caffeic acid (CA) with GO• vs. [CA]

the decrease in absorbance at λ = 428 nm using UV–vis spectroscopy [Figure 2, Eq. (5)]. Plotting this pseudo-first-order rate constant (k_{obs}) versus the concentration of compounds gave a straight line, from which the second-order rate constant (k) for the GO•-scavenging reaction by compounds could be obtained. In methanol, the k values decreased remarkably with the increasing substrate concentrations and reached the limiting values (Figure 3 and Table 1), which agreed with the reaction rates of HCAs with DPPH• in alcoholic solution reported by Foti *et al*. (2004). Suggesting that GO• and DPPH• belong to different radical models (GO• is an oxygen radical model and DPPH• is a nitrogen radical model) and also have different reduction potentials [GO• (E^0_{red} vs. SEC = 0.05 V), DPPH• (E^0_{red} vs. SEC = 0.18 V) (Nakanishi *et al*. 2002)], but the reaction result does not change.

$$-\frac{d[GO^{\bullet}]}{dt} = k_{GO}\,[GO^{\bullet}][ArOH] = k_{obs}\,[GO^{\bullet}] \quad (5)$$

It is worth noting that the reaction rate constants of GO• with HCAs in ethyl acetate are lower than in methanol, and that there is a strong linear correla-

Table 1. Rate constants for GO•-scavenging reactions of hydroxycinnamic acids (HCA) at 25°C

HCAs	k_{obs}(M^{-1} s^{-1}) (GO•)	
	methanol	ethyl acetate
CA	325.30 ± 30.23–29.32 ± 2.60	55.84 ± 1.95
SA	21.00 ± 23.76–39.17 ± 1.89	104.72 ± 1.99
FA	20.09 ± 1.08–3.45 ± 0.01	5.43 ± 0.16
3-HCA	1.63 ± 0.02–0.31 ± 0.00	0.87 ± 0.01
4-HCA	1.27 ± 0.06–0.24 ± 0.00	0.63 ± 0.01

Data are expressed as the mean ± SD for three determinations

doi: 10.17221/611/2014-CJFS

Table 2. Summary of the first two pK_a values and bond dissociation energy (BDE) of studied HCAs reported in literatures.

HCAs	pK_{a1}		pK_{a2}		BDE(kcal/mol)	
	water	methanol/water (20% v/v)	water	methanol/water (20% v/v)	ethanol	*n*-heptane
CA	4.47[a]	4.86[a]	8.32[a]	8.87[a]	79.23[c]	71.91[c]
SA	4.40[a]	4.60[a]	9.21[a]	9.58[a]	75.22[c]	74.75[c]
FA	4.56[a]	4.78[a]	8.65[a]	8.89[a]	81.31[c]	79.22[c]
4-HCA	4.39[a]	4.62[a]	8.37[a]	9.45[a]	82.19[c]	79.60[c]
3-HCA	4.44[b]	–	–	–	–	–

[a]Erdemgil *et al.* (2007); [b]Ray *et al.* (1996); [c]Lithoxoidou and Bakalbassis (2004)

tion between the k_{obs} and the concentrations. All HCAs gave the same second-order kinetics (Figure 4) and their second-order rate constants are shown in Table 1. Nevertheless, the trends of the second-order rate constants of HCAs are SA > CA > FA > 3-HCA > 4-HCA, both in methanol and ethyl acetate, suggesting that the number and position of hydroxyl groups on the aromatic ring significantly affect the ability of these compounds to scavenge GO• in organic solvents. It is clear that the introduction of electron-donating groups, such as methoxyl and hydroxyl, into the *ortho-* or *para-*position of 4-OH decreases the BDE value (Table 2), due to the formation of a strong intramolecular bond between the hydroxyl proton of the OH or OCH_3 groups and the oxygen radical centre, which facilitates hydrogen abstraction, as a result, the GO•-scavenging activity remarkably increases. SA, which possesses two methoxy groups besides 4-OH, is the most active one among the selected HCAs and its *k* value is 165 times higher than that of 4-HCA. The reaction of hydrogen transfer from phenol to GO• is fairly well correlated with the reported BDE values of HCAs (Table 2). On the other hand, the reaction rate of 3-HCA is slightly larger than

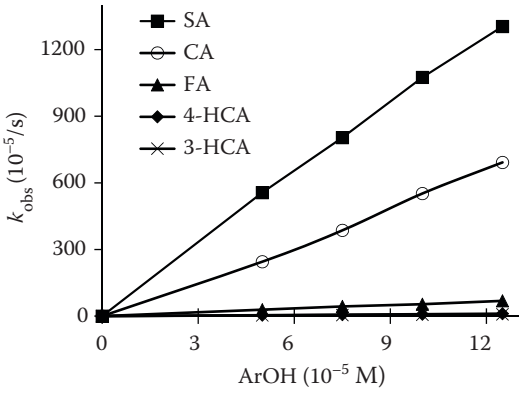


Figure 4. Plot of the pseudo-first-order rate constants (k_{obs}) vs. [HCAs] for the reaction of hydroxycinnamic acids (HCA) with GO• in ethyl acetate at 25°C

that of 4-HCA, it may express that the ethylenic side chain causes an extended conjugation that allows the propagation of the electron-withdrawing effects of the terminal carboxylic group.

The reaction rates of HCAs with GO• decreased monotonically with the increasing concentration in methanol, and they were different from those obtained in ethyl acetate. This remarkable kinetic difference between methanol and ethyl acetate should arise from different mechanisms. There are excellent studies indicating that the reaction rates of different phenols towards DPPH• were modified by adding different concentrations of acetic acid to the system, the reaction being generally slower (Litwinienko & Ingold 2007). This modification can be explained based on the fact that a proton is first lost by an acid-base equilibrium to give the phenoxide anion followed by a very fast electron transfer from these species to radical (SPLET). According to their approach, we also confirm by studying the effect of added acetic acid on the measured rate constant that the radical-scavenging reaction of resveratrol analogues in ethanol primarily involved the SPLET mechanism (Shang *et al.* 2009). Hydroxycinnamic acid derivatives, which are relatively strong acids, are in equilibrium with the carboxylic anion and H^+ in methanol that supports ionisation. With respect to the reaction mechanisms, it may express that in methanol the acidity of the medium rises with an increasing HCA concentration, however, the acid can suppress the ionisation of ArOH to phenolate anion, which is a much stronger electron donor as compared to the parent ArOH. The rates of the GO•-scavenging reaction decrease and reach different limiting values until the CA concentration of 0.8 mM (Figure 3), suggesting that in a lower HCA concentration, the electron donor is a phenolate anion and SPLET step dose occur in methanol and with the increasing acidity the rates are reduced by eliminating SPLET

doi: 10.17221/611/2014-CJFS

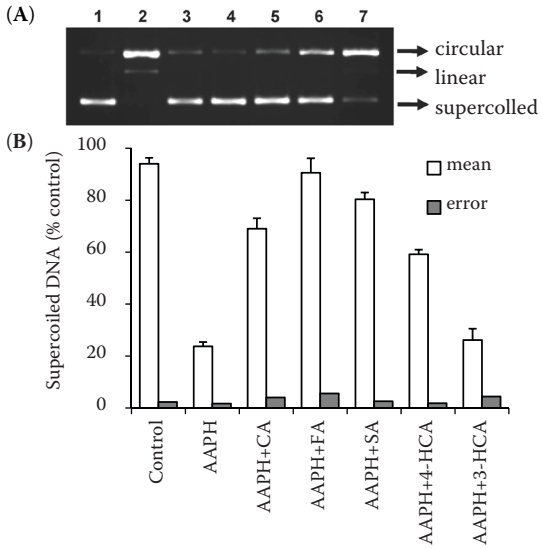
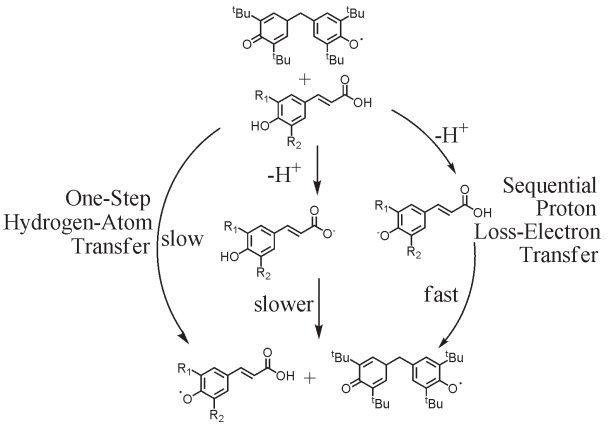


Figure 5. Agarose gel electrophoresis pattern of pBR322 DNA strand breakage induced by AAPH and inhibited by HCAs: (A) inhibitory effects of HCAs (10 μM) against AAPH (5 mM)-induced DNA strand breakage: (lane 1 – control; lane 2 – 5 mM AAPH alone; lanes 3–7 – CA, FA, SA, 4-HCA, 3-HCA); (B) quantitative analysis of protective effects of HCAs against AAPH-induced DNA strand breakage (DNA damage is represented by the percentage of supercoiled DNA to native DNA)

to leave only HAT. In dipolar aprotic ethyl acetate there is no acid-base equilibrium and low ability to ionise HCAs, so the rate constants correspond to the HAT reaction, and are near to the limiting rates in methanol. In general, the electron transfer rate is much faster than the rate of hydrogen atom transfer. Therefore, even a very low concentration of ArO^- can produce a huge increase in reaction rate and activity. Meanwhile, it should be pointed out that we did not find the correlation between the radical scavenging activity and pK_a values of HCAs (Table 2) in this study.

Inhibition of AAPH-initiated DNA strand breakage. Free radical-mediated oxidative damage of DNA might play a major role in cancer development. Thus, the supercoiled plasmid pBR322 DNA was used as a simple model for the measurement of strand break formed at DNA damage by agarose gel electrophoresis analysis (Moon & Terao 1998). We assessed the ability of HCAs to inhibit the AAPH-initiated oxidative damage to plasmid DNA (Figure 5). The treatment of plasmid DNA with 0.5 mM AAPH produced a major open circular and a minor linear form of DNA (indication of a double-strand breakage). The inhibition effects of HCAs depended on the specific compound used (Figure 5A). On the basis of the percentage of intact supercoiled DNA, the relative activity order for the inhibition of DNA strand breakage was as follows: FA > SA > CA > 4-HCA > 3-HCA (Figure 5B), which is different from that obtained by kinetic measurement in GO•-scavenging reaction. This indicates that the microenvironment of the reaction media has a significant influence on the antioxidant activity order, which supports the proposal of Moon *et al.* (1998) that this aspect may affect the antioxidant activity of HCAs.

Mechanism and structure-activity relationship. HCAs are abundant in edible plants, and exert multiple biological actions including the cancer chemopreventive activity; this activity could be linked with the antioxidant ability to eliminate free radicals and to reduce oxidative stress, thus, the structural basis and mechanism for antioxidant activity still need to be further explored. The acidity of HCAs makes their partial dissociation possible in methanol and water that supports ionisation. Moreover, the dramatic decreases of their rate constants in methanol were produced (Figure 3 and Table 1), and the GO•-scavenging activity order is SA > CA > FA > 3-HCA > 4-HCA in methanol and ethyl acetate (Table 1). Therefore the SPLET and the HAT reactions are possible. For the compounds CA, SA, FA, their GO•-scavenging maximum rate constants in methanol are 4 times higher than those in ethyl acetate (Table 1). The above facts confirm that in methanol their antioxidant reactions proceed primarily via the SPLET mechanism, but with the increasing concentration the systemic acidity rises, resulting in eliminating SPLET to leave only HAT. The decay rate of GO• is given by Eq. (6). The cooperation between HAT and SPLET processes in antioxidant reactions by HCAs is shown in Scheme 1. Recently, Litwinienko and co-workers have determined the acid



Scheme 1. Two possible pathways for the GO•-scavenging reaction of hydroxycinnamic acids

doi: 10.17221/611/2014-CJFS

dissociation constants (pK_a) and DPPH•-scavenging rate of flavonoids and demonstrated that the kinetics of the DPPH•-scavenging reactions in methanol and ethanol is noticeably affected by the acidity of a phenol, that is, the amount of accessible phenolate anions (Wenum *et al*. 2013). Although we did not determine the pK_a values of HCAs, the 4-OH of HCAs should be more acidic than the 3-OH due to the presence of the strong electron withdrawing α,β-unsaturated carbonyl group in the *para*-position of 4-OH. Therefore, the 4-OH first loses a proton to form the phenolate anion, followed by electron transfer between the anion and GO•, giving the corresponding phenoxyl radical (Scheme 1).

It should be pointed out that the relative contribution of SPLET and HAT is sensitive to both the reaction medium and the structure of the molecules. For example, the rate constants of CA, SA, FA are not decreasing in ethyl acetate (Figure 4 and Table 1), suggesting that, in ethyl acetate, the reaction is mainly a HAT mechanism. The extremely low and near rate constants for the reactions of the compounds 3-HCA and 4-HCA with GO• in methanol and ethyl acetate (Table 1) prove that, in both methanol and ethyl acetate, the reaction mechanism of the compounds 3-HCA, 4-HCA is only HAT.

In the previous studies, the most active compounds had a hydroxyl group in the *ortho*-position of 4-OH. However, in this study, we found that SA is more active than CA. The results are fully consistent with the HAT reaction mechanism, which is preferred in aprotic solvents, well characterised by the O-H bond-dissociation enthalpy (BDE) of ArOH (Ray *et al*. 1996; Erdemgil *et al*. 2007). However, in a non-polar aprotic solvent *n*-heptane (ε =1.92), the antioxidant activity trend may be CA > SA (Ray *et al*. 1996; Erdemgil *et al*. 2007). But in the inhibition of AAPH-induced DNA strand breakage, FA is the best one, SA and CA in sequence, which may be caused by the binding between $-OCH_3$ and DNA, and CA without $-OCH_3$. Additionally, 4-HCA is more active than 3-HCA. It clearly indicates that the microenvironment of the reaction media is an important effect on antioxidant activity.

CONCLUSION

In conclusion, the present work reveals that the SPLET and HAT mechanisms are responsible for the antioxidant reactions of hydroxycinnamic acids and the relative contribution depends on the reaction medium and the structure of the molecules. The antioxidant activity of HCAs depends significantly on the position and number of the hydroxyl groups, O-H BDE, and the reaction medium. The detailed antioxidant mechanism and structure-activity relationship also provide the necessary groundwork for understanding biological activities of HCAs

Reference

Amorati R., Pedulli G.F., Cabrini L., Zambonin L., Landi L. (2006): Solvent and pH effects on the antioxidant activity of caffeic and other phenolic acids. Journal of Agricultural and Food Chemistry, 54: 2932–2937.

Braekke K., Harsem N.K., Staff A.C. (2006): Oxidative stress and antioxidant status in fetal circulation in preeclampsia. Pediatric Research, 60: 560–564.

Butterfield D.A. (2002): Amyloid β-peptide (1-42)-induced oxidative stress and neurotoxicity: Implications for neurodegeneration in Alzheimer's disease brain. a review. Free Radical Research, 36: 1307–1313.

Beltrán J.L., Sanli N., Fonrodona G., Barrón D., Özkan G., Barbos J. (2003): Spectrophotometric, potentiometric and chromatographic pK_a values of polyphenolic acids in water and acetonitrile-water media. Analytica Chimica Acta, 484: 253–264.

Clifford M.N. (1999): Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture, 79: 362–372.

Erdemgil F.Z., Sanli S., Sanli N., Beltrán J.L. (2007): Determination of pK_a values of some hydroxylated benzoic acids in methanol-water binary mixtures by LC methodology and potentiometry. Talanta, 72: 489–496.

Foti M.C., Daquino C., Ceraci C. (2004): Electron-transfer reaction of cinnamic acids and their methyl esters with the DPPH• radical in alcoholic solutions. Journal of Organic Chemistry, 69: 2309–2314.

Halliwell B. (2001): Role of free radicals in the neurodegenerative diseases. Drug Aging, 18: 685–716.

Herrmann K. (1989): Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. Critical Reviews in Food Science and Nutrition, 28: 315–347.

Hracsko Z., Orvos H., Novak Z., Pal A., Varga I.S. (2008): Evaluation of oxidative stress markers in neonates with intra-uterine growth retardation. Redox Report, 13: 11–16.

Kong L., Sun Z.-L., Wang L.-F., Zhang H.-Y., Yao S.-D. (2004): Theoretical elucidation of the radical-scavenging-activity difference of hydroxycinnamic acid derivatives. Helvetica Chimica Acta, 87: 511–515.

Leon-Carmona J.R., Alvare-Idaboy J.R., Galano A. (2012): On the peroxyl scavenging activity of hydroxycinnamic acid derivatives: Mechanisms, kinetics, and importance

doi: 10.17221/611/2014-CJFS

of the acid–base equilibrium. Physical Chemistry Chemical Physics, 14: 12534–12543.

Lin W., Navaratnam S., Yao S., Lin N. (1998): Antioxidative properties of hydroxycinnamic acid derivatives and a phenylpropanoid glycoside. A pulse radiolysis study. Radiation Physics and Chemistry, 53: 425–430.

Lithoxoidu A.T., Bakalbassis E.G. (2004): Liquid-phase theoretical antioxidant activity trend of some cinnamic acid antioxidants. Journal of the American Oil Chemists Society, 81: 799–802.

Litwinienko G., Ingold K.U. (2003): Abnormal solvent effects on hydrogen atom abstractions. 1. The reactions of phenols with 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) in alcohols. Journal of Organic Chemistry, 68: 3433–3438.

Litwinienko G., Ingold K.U. (2004): Abnormal solvent effects on hydrogen atom abstraction. 2. Resolution of the curcumin antioxidant controversy. The role of sequential proton loss electron transfer. Journal of Organic Chemistry, 69: 5888–5896.

Litwinienko G., Ingold K.U. (2007): Solvent effects on the rates and mechanisms of reaction of phenols with free radicals. Accounts of Chemical Research, 40: 222–230.

Mattila P., Hellstrom J. (2007): Phenolic acids in potatoes, vegetables, and some of their products. Journal of Food Composition and Analysis, 20: 152–160.

Mattila P., Kumpulainen J. (2002): Determination of free and total phenolic acids in plant-derived foods by HPLC with diode-array detection. Journal of Agricultural and Food Chemistry, 50: 3660–3667.

Moon J.H., Terao J. (1998): Antioxidant activity of caffeic acid and dihydrocaffeic acid in lard and human low-density lipoprotein. Journal of Agricultural and Food Chemistry, 46: 5062–5065.

Musialik M., Litwinienko G. (2005): Scavenging of DPPH$^{\bullet}$ radicals by vitamin E is accelerated by its partial ionization: The role of sequential proton loss electron transfer. Organic Letters, 7: 4951–4954.

Nakanishi I., Kawashima T., Ohkubo K., Iizuka Y., Inami K., Mochizuki M., Urano S., Miyata N., Fukuzumi S. (2002): Effects of magnesium ion on kinetic stability and spin distribution of phenoxyl radical derived from a vitamin E analogue: mechanistic insight into antioxidative hydrogen-transfer reaction of vitamin E. Journal of the Chemical Society, Perkin Transaction, 2: 1520–1524.

Pan J.-X., Wang W.-F., Lin W.-Z., Lu C.-Y., Han Z.-H., Yao S.-D., Lin N.-Y. (1999): Interaction of hydroxycinnamic acid derivatives with the Cl_3COO radical: A pulse radiolysis study. Free Radical Research, 30: 241–245.

Pino E., Campos A.M., Lopez-Alarcon C., Aspee A., Lissi E. (2006): Free radical scavenging capacity of hydroxycinnamic acids and related compounds. Journal of Physical Organic Chemistry, 19: 759–764.

Pulido R., Bravo L., Saura-Calixto F. (2000): Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry, 48: 3396–3402.

Qian Y.-P., Shang Y.-J., Teng Q.-F., Zhou B. (2011): Hydroxychalcones as potent antioxidants: Structure-activity relationship analysis and mechanism considerations. Food Chemistry, 126: 241–248.

Rahman A., Fazel F., Greensill J., Ainley K., Parish J.H., Hadi S.M. (1992): Strand scission in DNA induced by dietary flavonoids: Role of Cu(I) and oxygen free radicals and biological consequences of scission. Molecular and Cellular Biochemistry, 111: 3–9.

Ray P.C., Munichandraiah N., Das P.K. (1996): Dissociation constants of some substituted cinnamic acids in protic solvents: measurements by hyper-Rayleigh scattering and potentiometric techniques. Chemical Physics, 211: 499–505.

Shang Y.-J., Qian Y.-P., Liu X.-D., Dai F., Zhou B. (2009): Radical-scavenging activity and mechanism of resveratrol-oriented analogues: Influence of the solvent, radical, and substitution. Journal of Organic Chemistry, 74: 5025–5031.

Watanabe A., Noguchi N., Fujisawa A., Kodama T., Tamura K., Cynshi O. (2000): Stability and reactivity of aryloxyl radicals derived from a novel antioxidant BO-653 and related compounds. Effects of substituent and side chain in solution and membranes. Journal of the American Chemical Society, 122: 5438–5424.

Wenum van E., Jurczakowski R., Litwinienko G. (2013): Media effects on the mechanism of antioxidant action of silybin and 2,3-dehydrosilybin: Role of the enol group. Journal of Organic Chemistry, 78: 9102–9112.

Zhu H., Zhao H., Zhang Z., Wang W., Yao S. (2006): Laser flash photolysis study on antioxidant properties of hydroxycinnamic acid derivatives. Radiation and Environmental Biophysics, 45: 73–77.

Received: 2014–10–23
Accepted after corrections: 2014–12–03

Corresponding author:

Doc Ya-Jing Shang, Lanzhou Jiaotong University, School of Chemical and Biological Engineering, Lanzhou, Gansu, 730070, P.R. China; E-mail: shangyaj@mail.lzjtu.cn