Biochimie 94 (2012) 961–967

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Research paper

Alkyl esters of hydroxycinnamic acids with improved antioxidant activity and lipophilicity protect PC12 cells against oxidative stress

Jorge Garrido [a,b], Alexandra Gaspar [a], E. Manuela Garrido [a,b], Ramin Miri [c], Marjan Tavakkoli [c], Samaneh Pourali [c], Luciano Saso [d], Fernanda Borges [a,**], Omidreza Firuzi [c,*]

[a] CIQ/Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 4169-007 Porto, Portugal
[b] Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto, 4200-072 Porto, Portugal
[c] Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[d] Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Rome, Italy

ARTICLE INFO

Article history:
Received 6 November 2011
Accepted 16 December 2011
Available online 23 December 2011

Keywords:
Hydroxycinnamic acid
Caffeic acid
Ferulic acid
Alkyl esters
Antioxidant
Redox properties
PC12 cells
Neuroprotection

ABSTRACT

Hydroxycinnamic acids (HCAs) are phenolic compounds present in dietary plants, which possess considerable antioxidant activity. In order to increase the lipophilicity of HCAs, with the aim of improving their cellular absorption and expansion of their use in lipophilic media, methyl, ethyl, propyl and butyl esters of caffeic acid and ferulic acid have been synthesized. All caffeate esters had a slightly lower DPPH IC_{50} (13.5–14.5 μM) and higher ferric reducing antioxidant power (FRAP) values (1490–1588 mM quercetin/mole [mMQ/mole]) compared to caffeic acid (16.6 μM and 1398 mMQ/mole, respectively) in antioxidant assays. In contrast, ferulate esters were less active in DPPH (56.3–74.7 μM) and FRAP assays (193–262 mMQ/mole) compared to ferulic acid (44.6 μM and 324 mMQ/mole, respectively). Redox properties of HCAs were in line with their antioxidant capacities, so that compounds with higher antioxidant activities had lower oxidation potentials. Measurement of partition coefficients disclosed the higher lipophilicity of the esters compared to parent compounds. All esters of caffeic acid significantly inhibited hydrogen peroxide-induced neuronal PC12 cell death assessed by MTT assay at 5 and 25 μM. However, caffeic acid, ferulic acid and ferulate esters were not able to protect the cells. In conclusion, these findings suggest that alkyl esterification of some HCAs augments their antioxidant properties as well as their lipophilicity and as a consequence, improves their cell protective activity against oxidative stress. These compounds could have useful applications in conditions where oxidative stress plays a pathogenic role.

© 2011 Elsevier Masson SAS. All rights reserved.

1. Introduction

Oxidative stress is caused by the action of reactive oxygen species (ROS) and is implicated in the pathogenesis of several human diseases such as cancer and neurodegenerative diseases [1,2]. Antioxidants are believed to counteract the harmful effects of ROS and regulate the physiological defence systems and therefore could be useful for prevention or treatment of oxidative stress-related diseases [3,4].

Hydroxycinnamic acids (HCAs, including caffeic acid, ferulic acid and sinapic acid among others) are phenolic compounds widely distributed in plants and prevalently found in cereals, fruits, vegetables and beverages [5]. These compounds have shown considerable *in vitro* antioxidant activities against many ROS [5,6]. Several recent reports have also shown that HCAs isolated from plants or as purified compounds protect neuronal cells against various types of oxidative damage [7–10]. Furthermore, a number of studies have shown that these compounds may play a protective role against ischemic brain injury in rats [11] and can be effective neuroprotective agents in mice [12].

The lipophilicity of a compound is a crucial factor that determines its efficiency as an antioxidant in lipophilic compartments and has a great impact on its ability in crossing the cell membranes and reaching its target [13]. Therefore, various esters of HCAs that are generally more lipophilic compared to their parent compounds have been synthesized in the recent years and some of them have shown some advantages over their precursor compounds [14,15]. For example, the influence of esterification on the *in vitro* antioxidant efficiency [16,17] and neuroprotective activity [18] of ferulic acid has been reported. Likewise, caffeic acid phenethyl ester (CAPE) has shown promise in cell [19] and animal models of

* Corresponding author. Tel.: +98 711 230 3872; fax: +98 711 233 2225.
** Corresponding author. Tel.: +351 220 402 560; fax: +351 220 402 009.
E-mail addresses: fborges@fc.up.pt (F. Borges), firuzio@sums.ac.ir, foomid@yahoo.com (O. Firuzi).

0300-9084/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2011.12.015

neurodegeneration [20], in which oxidative stress is involved. We have recently shown that alkyl esterification of sinapic acid has a positive impact on its partition coefficient and can improve its antioxidant efficacy in more lipophilic media [21].

In this study, we have evaluated the antioxidant activity of 4 different esters of caffeic acid (3,4-dihydroxycinnamic acid) and ferulic acid (4-hydroxy-3-methoxycinnamic acid). In addition, we have measured the redox potentials and partition coefficients of these compounds as important determinants of their physico-chemical properties and have assessed the ability of these derivatives in protecting neuronal PC12 cells against oxidative stress.

2. Materials and methods

2.1. General

Quercetin and Trolox C were purchased from Acros Organics (Geel, Belgium). Fetal bovine serum (FBS), penicillin/streptomycin, RPMI 1640, sterile phosphate buffered saline (PBS) and trypsin EDTA 0.05% were from Biosera (Ringmer, UK) and horse serum was acquired from Invitrogen (San Diego, CA, USA). Acetic acid glacial, hydrochloric acid 32%, dimethyl sulfoxide, ferrous sulfate heptahydrate, ferric chloride, methanol, sodium acetate and 2,4,6-tripyridyl-*s*-triazine (tptz) were obtained from Merck (Darmstadt, Germany). Caffeic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferulic acid, hydrogen peroxide and N-acetyl-L-cysteine were purchased from Sigma–Aldrich (San Louis, MO, USA). All reagents and solvents were *pro analysis* grade and were used without further purification. Deionised water (conductivity < 0.1 μS cm^{-1}) was used in all the experiments.

Thin layer chromatography (TLC) analyses were performed on aluminium silica gel sheets 60 F254 plates (Merck, Darmstadt, Germany) and spots were detected using a UV lamp at 254 nm.

2.2. Apparatus

^1H and ^{13}C NMR data were acquired, at room temperature, on a Brüker BioSpin GmbH 400 spectrometer operating at 400.15 and 100.62 MHz, respectively. Chemical shifts are expressed in δ (ppm) values relative to tetramethylsilane (TMS) as internal reference; coupling constants (J) are given in Hz. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (underlined values).

Microwave-assisted peptide synthesis was performed in Biotage® Initiator Microwave Synthesizer.

Voltammetric studies were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (Eco-Chemie, Netherlands) and a one-compartment glass electrochemical cell. Voltammetric curves were recorded at room temperature using a three-electrode system. A glassy carbon working electrode (GCE) ($d = 2$ mm), a platinum wire counter electrode and an Ag/AgCl saturated KCl reference electrode were used (Metrohm, Switzerland). A Crison pH-meter with glass electrode was used for the pH measurements (Crison, Spain).

2.3. Synthesis

The cinnamic acid (1 g), the appropriate alcohol (30 mmol) and concentrated sulfuric acid (2 drops) were put together in a glass vial (2–5 ml) sealed with a cap and heated in the MW reactor cavity under mechanical stirring for 5 min. The temperature was set at 20 °C above the boiling point of the alcohol. After cooling to room temperature, the crude material was purified by flash chromatography using silica gel as stationary phase and dichloromethane or dichloromethane/methanol as eluent.

The structural data of methyl, ethyl and propyl caffeates and methyl, ethyl and propyl ferulates were described elsewhere [22–25].

2.3.1. Trans-butyl-3-(4-dihydroxy)propenoate

Recrystallized of dichloromethane/light petroleum ether; Yield 70%. ^1H NMR δ(DMSO): 0.90 (3H, t, $J = 7.5$, CH_3), 1.32–1.42 (2H, m, CH_2), 1.56–1.65 (2H, m, CH_2), 4.11 (2H, t, $J = 6.6$, CH_2), 6.27 (1H, d, $J = 15.9$, H (β)), 6.76 (1H, d, $J = 8.1$, H(5)), 7.00 (1H, dd, $J = 2.0$,8.13, H(6)), 7.05 (1H, d, $J = 2.0$, H(2)), 7.46 (1H, d, $J = 15.9$, H(α)), 9.60 (2H, s, 3, 4-OH); ^{13}C NMR δ(DMSO): 23.3 (CH_3), 28.4 (CH_2), 40.0 (CH_2), 73.2 (CH_2), 123.8 (C5), 124.3 (C2), 125.5 (Cα), 131.2 (C6), 135.2 (C1), 154.7 (Cβ), 155.3 (C–OH), 158.1 (C–OH), 176.5 (COOH).

2.3.2. Trans-butyl-3-(3,4-hydroxy-3-methoxyphenyl)propenoate

Recrystallized of dichloromethane/light petroleum ether; Yield 75%. ^1H NMR δ($CDCl_3$): 0.97 (3H, t, $J = 7.4$, CH_3), 1.39–1.49 (2H, m, CH_2), 1.65–1.72 (2H, m, CH_2), 3.92 (3H, s, OCH_3), 4.20 (2H, t, $J = 6.7$, CH_2), 5.97 (1H, s, OH), 6.29 (1H, d, $J = 15.9$, H (β)), 6.92 (1H, d, $J = 8.1$, H(5)), 7.03 (1H, d, $J = 1.8$, H(2)), 7.07 (1H, dd, $J = 1.8$,8.1, H(6)), 7.61 (1H, d, $J = 15.9$, H(α)); ^{13}C NMR δ(DMSO): 14.7 (CH_3), 20.1 (CH2), 31.7 (CH_2), 56.8 (CH_3), 65.2 (CH_2), 110.2 (C5), 115.6 (C2), 116.5 (Cα), 123.9 (C6), 127.9 (C1), 145.6 (Cβ), 147.7 (C–OH), 158.1 (C–OCH_3), 168.3 (COOH).

2.4. Measurement of antioxidant activity

2.4.1. DPPH radical scavenging activity

DPPH radical scavenging activity of caffeic acid and ferulic acid derivatives (Scheme 1) was assessed as previously described with some modifications [26,27]. Briefly, the test compound was dissolved in dimethyl sulfoxide and 4 different concentrations were mixed with a methanolic solution of DPPH 100 μM in duplicate. After 30 min of incubation at room temperature in the dark, the absorbance at 517 nm was measured by a spectrophotometer (Bio-Tek, Model Uvikon XL). The concentrations (in the range 1–100 μM) were carefully chosen for each compound in order to produce a suitable dose–response curve. The percent inhibition of the radical was calculated based on the absorbance of the mixture compared to the absorbance of DPPH solution alone. IC_{50} values were calculated by the software Curve-Expert (for Windows, version 1.34).

2.4.2. Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed as described previously [28,29]. Briefly, the FRAP solution was freshly prepared by mixing 10 ml of acetate buffer 300 mM at pH 3.6, 1 ml of ferric chloride hexahydrate 20 mM dissolved in distilled water and 1 ml of 2,4,6-*tris*(2-pyridyl)-*s*-triazine 10 mM dissolved in HCl 40 mM. Ten μl of caffeate and ferulate derivatives and quercetin dissolved in dimethyl sulfoxide were mixed with 190 μl of the FRAP solution in 96-well microplates in triplicate at the final concentrations of 10, 40 and 20 μM, respectively. After 30 min of incubation at room temperature, absorbance at 595 nm was measured by a microplate reader (Bio-Rad, model 680). Light absorbance of the FRAP solution in the presence of test compounds and quercetin were measured and FRAP values for hydroxycinnamic acids (HCAs) were expressed as mM equivalent of quercetin per mole of compound.

2.5. Electrochemical measurements

Stock solutions of caffeic acid and ferulic acid and their ester derivatives (10 mM) were prepared by dissolving an appropriate

Download English Version:

https://daneshyari.com/en/article/10803753

Download Persian Version:

https://daneshyari.com/article/10803753

Daneshyari.com