ARTICLE IN PRESS
EJBT-00186; No of Pages 6
Electronic Journal of Biotechnology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect
Electronic Journal of Biotechnology

Research article

Antioxidant activity and protective role on protein glycation of synthetic aminocoumarins

Akram Amin Jafari [a], Mehran Miroliaei [a,b,*], Violina T. Angelova [c], Rahman Emamzadeh [a], Mirjana M. Djukic [d], Ana Djuric [d], Luciano Saso [e]

[a] Biochemistry and Molecular Biology Division, Department of Biology, University of Isfahan, Iran
[b] Biomimetic Materials and Tissue Engineering Laboratories, Department of Chemical Engineering, University of South Carolina, Columbia, SC 29208, USA
[c] Faculty of Pharmacy, Medical University of Sofia, Bulgaria
[d] Department of Toxicology, Faculty of Pharmacy, University of Belgrade, Serbia
[e] Department of Physiology and Pharmacology "Vittorio Erspamer", Sapienza University of Rome, Italy

A R T I C L E I N F O

Article history:
Received 27 December 2015
Accepted 12 August 2016
Available online xxxx

Keywords:
Anti-glycation agents
Diabetes
4-Aminocoumarin derivatives
Congo red
Fluorescence
Spectropolarimetry
Spectrofluorimetry

A B S T R A C T

Background: Synthesized aminocoumarins are heterocyclic compounds possessing potential for the treatment of insulin-dependent diabetes mellitus with unexplored anti-glycative action.

Results: In this study 4-aminocoumarin derivatives (4-ACDs) were evaluated in vitro for antiglycation (AG) activities by using the human serum albumin (HSA)/glucose system, for 8 weeks of incubation. The glycation and conformational alteration of HSA in the presence of the tested compounds were evaluated by Congo red assay, fluorescence and circular dichroism spectroscopy. The antioxidant (AO) capacity were also tested by four different assays including: DPPH (2,2′-diphenyl-1-picrylhydrazyl radical), ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulphonate) diammonium salt), FRAP (ferric reducing antioxidant power) and β-carotene-linoleic acid assay. The tested compounds showed AG and AO effects. The intensity of the accomplished AO potential is related to the type of the used assay. Significant alterations in the secondary (monitored by CD spectropolarimetry) and tertiary structure (assessed by spectrofluorimetry) of HSA upon glycation were mitigated by the 4-ACDs, suggesting their suppressive role in the late stage (post-Amadori) of the HSA glycation.

Conclusions: By the analogues, in vitro ascertained AO and AG properties of 4-ACD may be recognized as rationale for their protective role against oxidative changes of proteins, thereby precluding diabetic complications in humans.

© 2016 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Coumarins are heterocyclic compounds, naturally occurring in green plants, fungi, bacteria and some fruits. An antioxidant and anti-inflammatory properties of coumarins and their derivatives have recognized to reduce the risk of cancer, diabetes, cardiovascular and brain diseases [1]. Moreover, *in vitro* inhibitory properties of 4-aminocoumarin derivatives (4-ACD) against human platelet aggregation, antioxidant, anticancer, antimicrobial and anti-mycobacterial activity have been described [2,3]. Likewise, cyclic 4-aminocoumarin derivatives have been reported to act on the viability of HepG2 cells through antioxidant activity [3]. Recently, antioxidant effect of coumarins was recognized as their novel mechanisms of action [4].

Increased content of free radicals (FRs) in living organisms occurs due to their increased production or insufficient sequestration by the innate antioxidative defense system (AODS). Free radicals initiate oxidative stress (OS), *i.e.* oxidative injury of all classes of biomolecules (proteins, lipids, DNA). This pathophysiological mechanism has been documented in major ailments such as diabetes, carcinogenesis, atherogenesis, aging, etc. [5,6]. Protein glycation occurs spontaneously, but increasingly in the presence of oxidizing agents such as FRs. Glycated proteins are involved in long term complications of diabetes [7,8]. Reduced antioxidant status coexistence with hyperglycemia results in formation of heterogeneous molecules complexes known as advanced glycation end products (AGEs) [9]. Cytotoxicity of AGE adducts have been hypothesized to be tightly intertwined with OS [10,11]. Metal-catalyzed oxidation reactions were also found to increase the rate of AGEs production. Accordingly, compounds with

* Corresponding author at: Biochemistry and Molecular Biology Division, Department of Biology, University of Isfahan, Iran.
E-mail addresses: m.miroliaei@sci.ui.ac.ir, mmiroliaei@yahoo.com (M. Miroliaei).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2016.08.004
0717-3458/© 2016 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. All rights reserved. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article as: Jafari AA, et al, Antioxidant activity and protective role on protein glycation of synthetic aminocoumarins, (2016), http://dx.doi.org/10.1016/j.ejbt.2016.08.004

both antioxidant and antiglycation (anti-glyoxidants) properties would be ideal candidates to suppress harmful effects caused by FRs in biological systems, as well as to obstruct the AGE-formation-based mechanism — pathways in diabetic patients. Recently, we reported that anti-AGE activity of balm extract was associated with its antioxidant properties [12]. Madhu and Devi demonstrated that OS was diminished by vitamins C and E intake in diabetic rats [13]. Moreover, it has been found that the hemoglobin glycation decreases with the supplement of vitamin C in the diabetic patient [14].

Current efforts have being made to synthetize coumarin modified analogs with better antioxidant properties and reduced adverse effects. In a previous paper, 4-hydroxycoumarin-3-carboxamide derivatives have been synthesized as potential drugs for the treatment of insulin-dependent diabetes mellitus [6]. The objective of this study was to evaluate if synthesized 4-ACDs exhibit antioxidant properties and if it is associated with antiglycemic activity.

2. Materials and methods

2.1. Chemicals

2,2-Azinobis(3-ethylbenzothiazoline-6-sulphonate) diammonium salt (ABTS), ascorbic acid, b-carotene, 2-deoxy-D-ribose, 2,2′-diphenyl-1-picrylhydrazyl (DPPH), disodium salt of ethylenediamine tetraacetic acid (EDTA), ferrozine, Folin–Ciocalteu reagent, gallic acid, hypoxanthine, iron(III) chloride, iron(II) chloride, linoleic acid, potassium hexacyanoferrate, trichloroacetic acid (TCA), Trolox, human serum albumin (HSA) and glucose were from Sigma Chemical Company (Germany).

2.2. Synthesized/tested compounds: analogues of 4-aminocoumarin

Three 4-ACDs were synthesized and diluted in relation to their water solubility [3 mg/mL], according to Ivanov et al. [15]: N-{2-[(2-oxo-2H-chromen-4-yl)amino]ethyl}acetamide or aminoethylacetamide (1), 4-[2-hydroxypropyl)amino]-2H-chromen-2-one or aminoalcohol (2) and N-{2-chromen-4-yl)ami-no]propyl}acetamide or aminopropylacetamide (3). (See Scheme 1.)

2.3. In vitro antioxidant potential measurements

2.3.1. DPPH free-radical scavenging activity

The DPPH assay is based on the ability of an antioxidant [16] to donate hydrogen to DPPH radical (DPPH•). The change in color of DPPH• (from purple to yellow) is the measure of free radical scavenging activity. The hydrogen-donating activity of the 4-ACDs was measured according to the method by Gyamfi et al. [17]. By accepting hydrogen (H+ and e-), purple-colored DPPH• is being converted into the non-radical form (DPPH-H), yellow-colored diphenylpicrylhydrazine. Briefly, 50 mL of dissolved 4-ACDs was mixed with 450 mL of Tris–HCl buffer (50 mmol/L, pH 7.4) and 1 mL of (0.1 mmol/L) DPPH• (dissolved in methanol). After 30 min, the absorbance was recorded at 517 nm (absorption max for DPPH•). The percentage of inhibition was calculated using [Equation 1] and the concentration of the compound at which it exhibits 50% inhibition (IC_{50}) value was estimated using a non-linear regression algorithm.

$$\text{Percentage inhibition} = \left[\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)/\text{Abs}_{\text{control}}\right] \times (1) \qquad \text{[Equation 1]}$$

2.3.2. $ABTS^{\bullet +}$ free-radical scavenging activity

The antioxidant capacity of the tested compounds was estimated by the method of Re et al. [18]. The blue/green colored $ABTS^{\bullet +}$ solution used for the measurement of 4-ACDs antioxidative activities was prepared by mixing ABTS (10 mL of 7 mmol/L) with oxidizing agent $K_2S_2O_8$ (5 mL of 2.45 mmol/L) for 12–16 h in the dark and subsequently diluted with ethanol (a dilution of between 1/50 and 1/400 was performed in order to obtain absorbance value of 0.700, at 734 nm). The reduction of the radical cation ($ABTS^{\bullet +}$) by 4-ACDs was determined as decolorization at 734 nm, *i.e.* the percentage inhibition of absorbance of the $ABTS^{\bullet +}$ solution (since 1 min upon mixing of 1.5 mL of the prepared $ABTS^{\bullet +}$ solution with 15 μL of 3 mg/mL4-ACDs samples, at 5 min intervals, for 40 min).

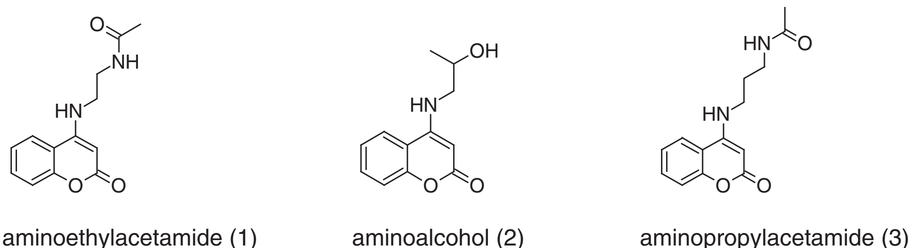
The results are expressed as the Trolox equivalent antioxidant capacity (TEAC, mmol/L Trolox) at different time intervals.

2.3.3. FRAP (ferric reducing antioxidant power) assay

The principle of this assay is based on one-electron reduction of Fe (III)/ferricyanide complex to the ferrous form Fe (II) [19]. In brief, 1 mL of 3 mg/mL compound was mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of a 10 g/L $K_3Fe(CN)_6$, and incubated at 50°C, for 30 min. After the incubation, 2.5 mL of a 100 g/L TCA solution was added to terminate the reaction and the mixture was centrifuged for 10 min (1800 rpm). Finally, 2.5 mL of supernatant was used to mix with 2.5 mL ultra-pure water and 0.5 mL of a 1 g/L $FeCl_3$. The absorbance was recorded at 700 nm and the data were presented as ascorbic acid equivalents (AscAE; mmol ascorbic acid/g sample).

2.3.4. β-Carotene-linoleic acid bleaching inhibition

The determination of antioxidant activity was evaluated by the ability of the compounds to inhibit the bleaching of the β-carotene by linoleic acid. Namely, during the incubation at 50°C linoleic acid produces peroxyl radical which becomes neutralized by the presence of antioxidants, at the same time the β-carotene oxidation is avoided (*i.e.* inhibition of β-carotene bleaching occurs, thus yellow color of b-carotene in the system persists in the presence of antioxidant) [20]. Briefly, 0.2 mg β-carotene dissolved in 1 mL chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were transferred into a round-bottom flask. Once the chloroform had been removed under the nitrogen stream, 50 mL distilled H_2O was added and the resulting



Scheme 1. Structures of tested compounds.

UNCORRECTED PROOF

Download English Version:

https://daneshyari.com/en/article/6618924

Download Persian Version:

https://daneshyari.com/article/6618924

Daneshyari.com