Journal of Photochemistry & Photobiology, B: Biology 163 (2016) 277–283

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Caffeine metabolites not caffeine protect against riboflavin photosensitized oxidative damage related to skin and eye health

R.S. Scurachio [a], F. Mattiucci [a], W.G. Santos [a], L.H. Skibsted [b], D.R. Cardoso [a,*]

[a] Instituto de Químca de São Carlos, Universidade de São Paulo, Av. Trabalhador São Carlense 400, CP 780, CEP 13560-970, São Carlos, SP, Brazil
[b] Food Chemistry, Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 23 August 2016
Accepted 25 August 2016
Available online 27 August 2016

Keywords:
Caffeine metabolites
Riboflavin
Photosensitization
Skin and eye health

ABSTRACT

Caffeine metabolites were found to bind riboflavin with dissociation constant in the millimolar region by an exothermic process with positive entropy of reaction, which was found by ^{1}H NMR and fluorescence spectroscopy to occur predominantly by hydrogen bonding with water being released from riboflavin solvation shell upon caffeine metabolite binding to riboflavin. The caffeine metabolites 1-methyl uric acid and 1,7-dimethyl uric acid were shown by transient absorption laser flash photolysis to be efficient as quenchers of triplet riboflavin with second-order rate constant of 1.4 10^8 L mol^{-1} s^{-1} and 1.0 10^8 L mol^{-1} s^{-1}, respectively, in aqueous solution of pH 6.4 at 25 °C and more efficient than the other caffeine metabolite 1,7-dimethyl xanthine with second-order rate constant of 4.2 10^7 L mol^{-1} s^{-1}. Caffeine was in contrast found to be non-reactive towards triplet riboflavin. Caffeine metabolites rather than caffeine seem accordingly important for the observed protective effect against cutaneous melanoma identified for drinkers of regular but not of decaffeinated coffee. The caffeine metabolites, but not caffeine, were by time resolved single photon counting found to quench singlet excited riboflavin through exothermic formation of ground-state precursor complexes indicating importance of hydrogen bounding through keto-enol tautomer's for protection of oxidizable substrates and sensitive structures against riboflavin photosensitization.

1. Introduction

Recently epidemiological evidence has been presented indicating that higher coffee intake is associated with a decrease in risk of cutaneous melanoma [8]. Melanoma is the leading cause of skin cancer with exposure to UV components of sunlight as the important exogenous risk factor [3]. Accordingly, constituents in coffee seems to protect the skin against UV light, and since decaffeinated coffee was not found to be protective, caffeine seems to have been identified as the protective constituent in coffee [8].

Both UV-A (315–400 nm) and UV-B (290–350 nm) are part of the radiation that reaches the earth from the sun and are both associated with sunlight damage to skin and eye [8,12]. UV-B is considered a main cause of sunburn, photo-ageing and skin cancer as UV-B is absorbed directly by proteins and DNA, but UV-A is of higher intensity and may penetrate deeper into tissue [1]. UV-A is absorbed by other compounds in skin and eye such as melanin, porphyrins and flavins, which may act as photosensitizers [1,11]. Riboflavin, vitamin B2, is an effective photosensitizer widely found in tissue and food leading to oxidative damage of proteins, other vitamins, sterols and lipids upon absorption of UV-A and visible light [7].

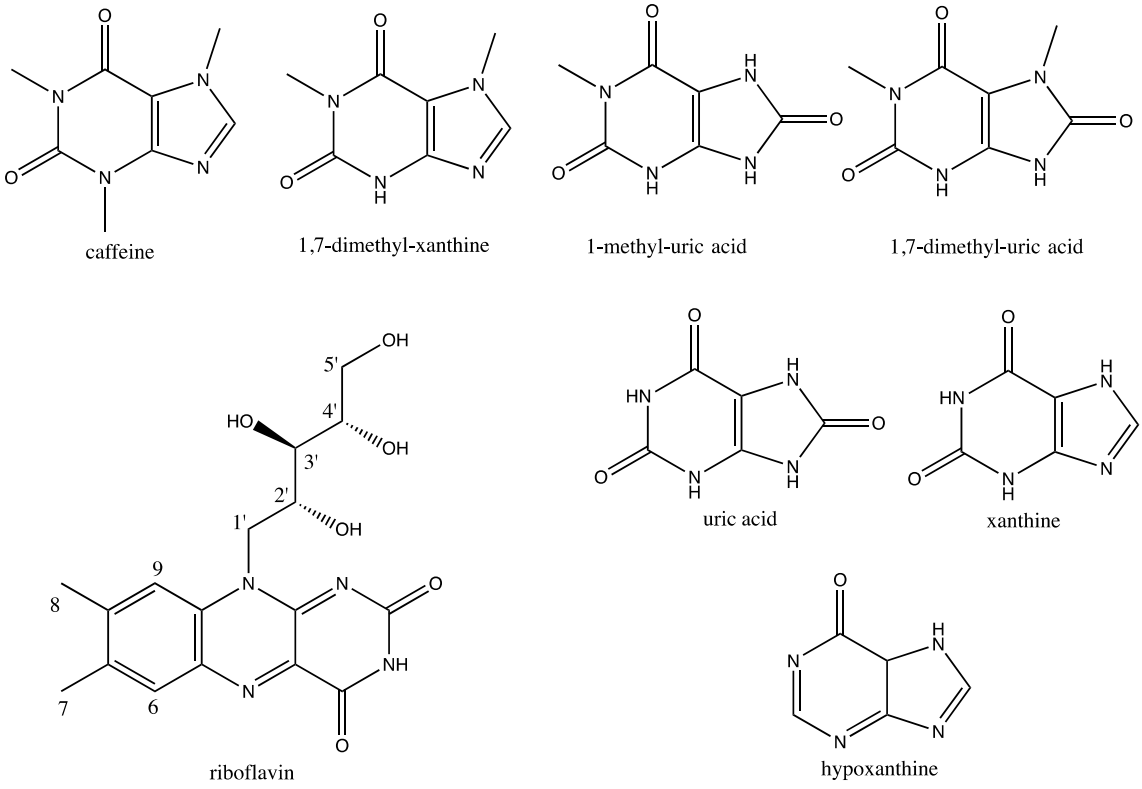
Caffeine is only absorbing a minor fraction of the UV-A component of daylight and a simple filter effect of caffeine, as present in tissue does not explain the protective affect seen for coffee against the development of cutaneous melanoma. Caffeine has also been found, when topically applied as eyes drops, to protect against cataractogenesis that is known at least partially to be induced by light exposure [8,17].

Caffeine, not being protective simply by absorbing damaging light shielding sensitive tissue components, may accordingly be protective against cataract and cutaneous melanoma through other mechanisms. Caffeine or caffeine metabolites may, as it is known for uric acid, be quenchers of photosensitizers like the bioactive forms of riboflavin present in skin tissue and eye liquids. Riboflavin becomes strongly oxidizing upon light absorption in a relatively long-lived triplet state, and efficient quenchers of this state may be strongly protective against photosensitized oxidative damage of tissues [7].

Aiming to provide a better understand on the mechanism behind the photoprotection of sensitive structures in eyes and skin against UV-A photoinduced damage sensitized by flavins, herein, we report result of kinetic studies of the deactivation of triplet-excited riboflavin by caffeine and its biological relevant metabolites, Scheme 1.

* Corresponding author.
E-mail address: drcardoso@iqsc.usp.br (D.R. Cardoso).

http://dx.doi.org/10.1016/j.jphotobiol.2016.08.042
1011-1344/



Scheme 1. Chemical structure of riboflavin and purine derivatives.

2. Materials and Methods

2.1. Chemicals

Caffeine (1,3,7-trimethyl xanthine), 1,7-dimethyl uric acid, 1,7-dimethyl xanthine, 1-methyl uric acid, and riboflavin, and tetrabutylammonium hexafluorphosphate salt were purchased from Sigma-Aldrich (Steinheim, Germany). Water was purified (18 MΩ·cm^{-1}) by means of a Milli-Q purification system from Millipore (Billerica, MA, USA).

2.2. Transient Absorption Laser Flash Photolysis

Laser flash photolysis (LFP) experiments were carried out with LFP-112 ns laser flash photolysis spectrometer from Luzchem (Ottawa, Canada) using the third harmonic (355 nm) of a pulsed Q-switched Nd:YAG laser (Brilliant-B, LesUlis, France) attenuated to 10 mJ·cm^{-2} as the excitation source with 8 ns of pulse duration. The signal from the photomultiplier detection system was captured by a Tektronix TDS 2012 digitizer (Beaverton, OR, USA). The FFP-112ns and the digitizer were connected to a personal computer via General Purpose Instrumentation Bus (GPIB) and serial interfaces controlling all the experimental parameters and providing suitable processing and data storage capabilities using a proprietary software package developed in LabView environment and compiled as a stand-alone application (Luzchem, Ottawa, Canada). Each kinetic trace was averaged 16 times, and observed rate constants were determined by parameter fitting to exponential decay functions. All measurements were made with aqueous phosphate buffer solutions pH 6.4 (ionic strength = 0.2 mol L^{-1}), thermostatted at 25.0 ± 0.5 °C, and purged with high-purity N_2 for at least 30 min before the experiment.

2.3. Time Resolved Single Photon Counting

Time-resolved fluorescence were measured by time-correlated single-photon counting using an picosecond spectrometer equipped with Glan-Laser polarizers (Newport, Irvine, CA), a Peltier-cooled PMTMCP from Hamamatsu model R3809U-50 (Hamamatsu, Japan) as the photon detector, and Tennelec-Oxford (Oxford, Abingdon, UK) counting electronics. The light pulse was provided by frequency doubling the 200 fs laser pulse of a Mira 900 Ti-Sapphire laser pumped by a Verdi 5 W coherent laser (Santa Clara, CA), and the pulse frequency was reduced to 800 kHz using a Conoptics pulse picker. The fluorescence decays were taken at the magic angle (λ_{exc} = 400 nm) and analyzed by a re-convolution procedure with instrument response function with exponential decay models, and the goodness of the fit was evaluated by the statistical parameters $\chi 2$. The fluorescence was measured at 25.0 ± 0.1 °C in phosphate buffer solutions with pH = 6.4 and 0.2 mol L^{-1} of ionic strength.

2.4. Steady-state Fluorescence Spectroscopy

Fluorescence measurements were carried out using a Hitachi F-7000 Fluorescence Spectrometer (Hitachi High-Tech, Tokyo, Japan). The samples were excited in 1.0 cm × 1.0 cm fluorescence cuvettes from Hellma (Mulheim, Germany) at 440 nm. Emission spectra from 480 nm to 600 nm were recorded using a 2.0 nm band-pass for the excitation monochromator and a 4.0 nm band-pass for the emission monochromator. The recorded spectra were corrected for instrument response. The fluorescence was measured at 25.0 ± 0.2 °C in phosphate buffer solutions with pH = 6.4 and 0.2 mol L^{-1} of ionic strength.

2.5. Cyclic Voltammetry

Cyclic voltammetry of caffeine and its metabolites (1 × 10^{-3} mol L^{-1}) dissolved in nitrogen-purged phosphate buffer