Journal of Luminescence 157 (2015) 243–248

Contents lists available at ScienceDirect

Journal of Luminescence

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

Insights into the antioxidant activity of some flavones on silver nanoparticles using the chemiluminescence method

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ARTICLE INFO

Article history:
Received 27 March 2014
Received in revised form
20 August 2014
Accepted 27 August 2014
Available online 10 September 2014

Keywords:
Flavones
Proteins
Chemiluminescence
Luminol
Antioxidant activity

ABSTRACT

The work aims to simulate *in vitro* the effects caused by oxidation of five hydroxyflavones (HF) (some typical models of flavonols), (3-HF, 6-HF, 7-HF, 3,6-diHF and 3,7-diHF) on silver nanoparticles (SNPs) using the chemiluminescent system luminol–hydrogen peroxide, in phosphate buffer, pH 7.4. The contribution of bovine and human serum albumins to the antioxidant activity of the mentioned flavones, and the effect on the SNPs support, in the chemiluminescent system luminol–hydrogen peroxide, has been also investigated. The results are discussed with relevance to the oxidative stress process.

1. Introduction

Flavonoids are ubiquitous plant secondary products that contain 15 carbon atoms in their basic nucleus, arranged in a C_6–C_3–C_6 configuration: three aromatic rings labeled as A–C. It was reported that they have antioxidant activity, interact with protein phosphorylation, iron-chelating, and scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl. This fact was shown to be linked to an inhibition of the oxidative mechanisms that lead to degenerative diseases (heart disease, skin damage, cancer) [1–5]. Also, it was reported that flavonoid compounds exert antiviral actions due to their favorable oxidation potentials [6,7].

Using several evaluation methods, the antioxidant activity of flavonoids has been extensively reported [8–15] and there are some reports on the mechanism of oxidation or the structure–activity relationship. Considering these facts, spectrophotometric characterization of the behavior and of the predominant species of flavonoids in physiological buffer, such as the determination of solubility, lipophilicity and anticancer efficacy, has been recently investigated [16]. It was pointed out that the neutral forms of flavonoids are predominantly active molecules and the active sites responsible for anticancer activity are found in rings A and C, especially C4=O, C5–OH and C2=C3 [16].

The influence of polyphenol–plasma protein interaction on the antioxidant properties of polyphenols has been recently reported [17]. It was found that the effect of polyphenol–plasma protein interaction on the bioavailability of polyphenols is not equivocal, of importance being the structure characteristics of polyphenols, as well as the proteins [17]. The evaluation of the antioxidant activity of flavonoids (quercetin, glabridin, red clover and isoflavin Beta – an isoflavone mixture) by chemiluminescence method has been undertaken [18]. It was found that these flavonoids have a marked inhibition of oxidative stress, with a concentration-dependent action for quercetin and isoflavin Beta [18].

Nowadays the use of metal nanoparticles (NPs) represents a tool in biotechnology and clinical medicine, especially as carriers of biomolecules (proteins, enzymes, drugs) [19–23]. Metal NPs display interesting optical properties based on localized surface plasmons [23]. Among metal NPs, silver nanoparticles (SNPs) are the most extensively used, being employed as antibacterial agents; the chemical activity is due to their large surface to volume ratio [24]. Due to these facts, the influence of SNPs–Bovine Serum Albumin (BSA) and SNPs–BSA–antitumoral drug interaction with the protein structure as well as with the protein–drug binding, has been studied [23]. In recent works, photophysical properties of the serum albumins, BSA and HSA (Human Serum Albumin) proteins, as well as their interaction with Riboflavin on SNPs, have been investigated by spectroscopic techniques [25,26].

This work follows the previous paper [27] and aims to simulate *in vitro* the effects caused by the oxidation of five hydroxiflavones (HF)

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http://dx.doi.org/10.1016/j.jlumin.2014.08.058
0022-2313/

(some typical models of flavonols), (3-HF, 6-HF, 7-HF, 3,6-diHF and 3,7-diHF) on silver nanoparticles (SNPs) using the chemiluminescent system luminol–hydrogen peroxide, in phosphate buffer, pH 7.4. The contribution of bovine and human serum albumins to the antioxidant activity of the mentioned flavones, on SNPs support, in the luminol–hydrogen peroxide chemiluminescent system, has been also investigated. The results are discussed with relevance to the oxidative stress process.

2. Experimental

2.1. Materials.

The luminol (LH_2) (purchased from Fluka) – hydrogen peroxide (H_2O_2) (purchased from Sigma) system with concentration of $LH_2 = 2.5 \times 10^{-5}$ M and $H_2O_2 = 30$ mM in phosphate buffer (purchased from Sigma-Aldrich), 100 mM, pH = 7.4, was considered as reference system. 3-Hydroxyflavone (3-HF) and 7-HF were purchased from Sigma. 6-HF, 98%, 3,6-diHF, 98% and 3,7-diHF hydrate, 97% were purchased from Aldrich. All these HFs were used without further purification. The structure of the studied flavones is shown in Scheme 1. The stock solutions were prepared in methanol (of spectrophotometric grade, purchased from Sigma). Aliquots from stock solutions were used to a final working concentration found in the range of 86–180 μM.

The silver source, silver nitrate ($AgNO_3$, purity 99.99%) and the reducing agent, sodium borohydride ($NaBH_4$, purity 99.8%) were purchased from Sigma-Aldrich. Ag (0) nanoparticles (SNPs) were prepared according to Ref. [28]. The stock solutions of HFs were prepared in methanol. Aliquots of stock solutions of HFs were previously dried at room temperature and then SNPs added, the concentration of HFs being in the range of $5.45–6 \times 10^{-5}$ M. Aliquots from this solution were used to a final working concentration of HFs which was in the range of 0.5–3 μM. Also, an 1:1 (v/v) of HFs: protein (Bovine Serum Albumin, BSA or Human Serum Albumin, HSA) have been used. Methanol of spectrophotometric grade was purchased from Sigma. BSA and HSA were purchased from Merck and used without further purification.

2.2. Methods and apparatus.

The absorption measurements were recorded using a Perkin Elmer, Lambda 35, UV–vis spectrometer at a scan rate of 480 nm/min and a spectral resolution of 1 nm.

Particles size ($Z_{average}$) and *zeta potential* (ξ) were measured by Dynamic Light Scattering (DLS) technique on the as-synthesized dispersions, using a Zetasizer Nano ZS instrument (Malvern International Ltd.). Zetasizer Nano ZS for the measurement of size and zeta potential of dispersed particles in solution includes a 4 mW He–Ne laser.

The chemiluminescence (CL) measurements have been performed with a TD 20/20 chemiluminometer Turner Design. The CL method, data acquisition and the used chemiluminescent system, luminol–hydrogen peroxide, were previously described [29–35].

In vitro antioxidant activity, *S* (%), of the studied HFs and HFs–protein systems, by means of the free radicals scavenging was calculated according to the equation below

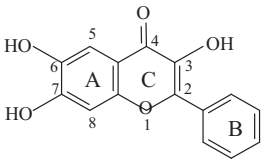
$$S(\%) = \frac{(I_0 - I)}{I_0} \times 100$$

with I_0 and I, the CL intensity (I_{CL}) measured for the reference system in absence and in the presence of HFs and HFs–BSA/HSA, respectively. Both values were measured 5 s after the beginning of the reaction.

The rate constant of the CL reaction was estimated from $I_{CL} = f(t)$, for the upward part of the plot, $-k$, (attributed to the consumption of free radicals), considering that the CL reaction is of the first order

$$k = 1/\Delta t \times \ln(I_i/I_0), \quad \Delta t = t_i - t_0$$

with t_i, the time at the i moment; t_0, the initial time; I_i is the intensity of CL signal at the moment i and I_0 is the intensity of the CL signal at the initial moment. The value of the rate constants were calculated in the 5–150 s time range, for the all investigated systems.



Scheme 1. Molecular structure of the studied hydroxyflavones (HFs).

3. Results and discussion

For the antioxidant activity of mentioned HFs, they were tested in various concentrations, as follows: 36–216 μM for 3-HF; 72–216 μM for 6-HF and 7-HF; 57.6–172.8 μM for 3,6-diHF and 3,7-diHF. Fig. 1A shows the effect of the HFs on the CL intensity of the LH_2–H_2O_2, in phosphate buffer, pH 7.4. As it can be observed, for the same concentration, 180 μM, 3-HF presents a strong CL intensity comparing with 6-HF and 7-HF. Also, having the same concentration (144 μM) of 3,6-diHF and 3,7-diHF, no significant differences in the CL intensity were observed.

The antioxidant activity of 7-HF (~10%) was found to be lower than that of 6-HF (~60%). In this case, the oxidation of 7-HF by hydrogen peroxide, with the formation of phenoxyl radical is considered. According to the literature [36] the B ring hydroxyl configuration of the flavones structure is involved in reactive oxygen scavenging, while substitution of the rings A and C has no significant impact on superoxide anion radical scavenging [37,38]. Also, it is well known that 7-HF represents a model of biologically active flavone which is oxygenated in the 7-position [39].

No significant differences between 3,6 and 3,7-diHF were observed, their antioxidant activity is ~88%. Also, as it can be noticed, Table 1, a significant enhancement of antioxidant activity (~94%) occurred in the case of 3-HF.

The behavior of the serum albumins (BSA and HSA proteins) in the CL system, luminol–hydrogen peroxide, pH 7.4 is presented in Fig. 1B. As it can be seen, BSA and HSA antioxidant activity is time depending: HSA protein scavenges the formation of the free radicals in the CL system 20 s after the beginning of the CL reaction while, BSA protein seems to scavenge the free radicals after 60 s. Therefore, there are more radicals in the early stages in the presence of serum albumins than in the reference system. This behavior may be due to oxidation of Cys34 residue (the only free Cys in the native HSA) to sulfenic acid (HSA–SOH) and then, as a function of the CL reaction time, it may be reduced leading to the initial HSA–SH reduced form [40] and in this way an enhancement of the antioxidant activity of the serum albumins is observed.

Fig. 1C presents the influence of the BSA and HSA proteins on the antioxidant properties of the studied HFs. It can be observed that even in the presence of HSA in low concentration, 0.64 μM, the CL intensity of the 7-HF is higher by comparison with the other HFs. This fact may be an indication that in the presence of HSA, the –OH group from 7-position of the HF is faster oxidized and leads to the formation of the phenoxyl radical, more rapidly. Hence,