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Spectroscopic study of 3-Hydroxyflavone - protein interaction in lipidic bi-layers immobilized on silver nanoparticles

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ABSTRACT

The interaction of 3-Hydroxyflavone with serum proteins (BSA and HSA) in lecithin lipidic bi-layers (PC) immobilized on silver nanoparticles (SNPs), was studied by fluorescence and Raman spectroscopy. BSA secondary structure was quantified with a deconvolution algorithm, showing a decrease in α-helix structure when lipids were added to the solution. The effect of temperature on the rate of the excited-state intra-molecular proton transfer and on the dual fluorescence emission of 3-HF in the HSA/PC/SNPs systems was discussed. Evaluation of the antioxidant activity of 3-HF in HSA/PC/SNPs systems was also studied. The antioxidant activity of 3-HF decreased in the presence of SNPs. The results are discussed with relevance to the secondary structure of proteins and of the 3-HF based nano-systems to a topical formulation useful in the oxidative stress process.

1. Introduction

3-Hydroxyflavone (3-HF) belongs to flavonols, the most widespread biologically active plant flavonoids, being one of the well-known model flavonol molecule which exhibits intra-molecular excited-state proton transfer (ESIPT) via intra-molecular H-bonding between the C═O and 3-OH group which results in the transformation of the initially excited state (N^* form) to the tautomer (T^*) form, the dual fluorescence emission [1–3]. The therapeutically beneficial role of flavonoids was first reported by Rusznyak and Szent-Gyorgyi [4]. The physiological potentials of flavonoids have attracted attention in relation to their role in the cellular and extracellular antioxidant defence against reactive oxygen species (ROS) with beneficial properties in human health including: cancers, tumors, allergies, AIDS, atherosclerosis, ischemia, neuronal degeneration, cardiovascular diseases [5–12].

Bovine serum albumin (BSA) and Human Serum albumin (HSA), transport proteins circulated in the body, have a conformational adaptability and act as carriers for several ligands [13–16]. BSA and HSA are characterized by low tryptophan (Trp) and high cystine contents, and except Trp localization (Trp^{214} in HSA structure and Trp^{134}/Trp^{212} in BSA structure), the amino acid compositions of these proteins are almost the same [13–16].

Interactions of flavonoid compounds with serum proteins and biological targets have been recently reported [17–22]. Studies on the microenvironments of 3-HF in model biomembranes, liposomes as well as in erythrocytes ghost were performed [23–25]. The influence of the bi-layer hydration on the emission ratiometric response of 3-HF showed the potency of 3-HF as excellent emission ratiometric probe to measure dipole potential in lipid membranes [26]. Also, it was found that lipid peroxidation of egg yolk phosphatidylcholine liposomes is significantly inhibited upon 3-HF binding, suggesting that 3-HF can be potentially useful as an inhibitor of peroxidative damage of cell membrane [27].

Recently, studies concerning the antimicrobial and antiviral effects as well as on the interaction of silver nanoparticles (SNPs) with biological molecules, especially proteins and nucleic acids, have been reported [28–36].

In this work, the interaction of 3-Hydroxyflavone with serum proteins (BSA and HSA) in lecithin lipidic bi-layers (PC) immobilized on silver nanoparticles (SNPs), was studied by fluorescence and Raman spectroscopy. BSA secondary structure was quantified with a deconvolution algorithm, showing a decrease in α-helix structure when lipids were added to the solution. The effect of temperature on the rate of the excited-state intra-molecular proton transfer and on the dual fluorescence emission of 3-HF in the HSA/PC/SNPs systems was discussed. Evaluation of the antioxidant activity of 3-HF in HSA/PC/SNPs systems was also studied. The antioxidant activity of 3-HF decreased in the presence of SNPs. The results are important in the secondary structure analysis of proteins as well as of the 3-HF concerning its

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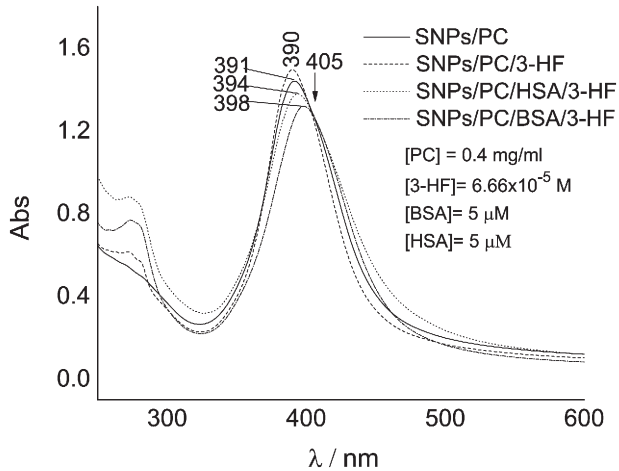

Fig. 1. Absorption spectra of SNPs/PC/protein/3-HF systems.

therapeutic properties into the proteins based nano-systems, to a topical formulation useful in the oxidative stress process.

2. Experimental

2.1. Materials

3-Hydroxyflavone (3-HF) was purchased from Sigma and used without further purification. Stock solution of 3.6 mM was prepared in methanol (of spectrophotometric grade, purchased from Sigma, 99%). Depending on the experimental method, aliquots from the stock solution were added to the working sample to reach the final concentration in the range of 6×10^{-5} M to 1.8×10^{-4} M.

Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) were purchased from Merck and Sigma and in a typical experiment, the final working concentration was in the range of 0.05 μM to 10^{-4} M.

L-α-phosphatidylcholine (PC) from egg yolk, ~60%, was purchased from Sigma and the stock solution, 8 mg/ml, was prepared in ethanol (from Merck).

Silver nanoparticles (SNPs) were synthesized according to ref. [37] by adding under vigorous stirring appropriate aliquots of 1 mM $AgNO_3$ (purity 99.99%, purchased from Sigma-Aldrich) solution to a solution containing $NaBH_4$ (purity 99.8%, purchased from Sigma-Aldrich), to final working concentrations of 0.1 mM $AgNO_3$ and 7 mM $NaBH_4$. SNPs were electrostatically stabilized and no capping agent was needed [37]. The formed SNPs aqueous solution was first treated with PC (0.4 to 0.5 mg/ml) and then BSA/HSA protein (5 μM) and 3-HF (6.66×10^{-5} M) were added.

Luminol (LH_2) was purchased from Fluka, the hydrogen peroxide (H_2O_2) was purchased from Sigma, with their working concentrations of $LH_2 = 2.5 \times 10^{-5}$ M and $H_2O_2 = 30$ mM in phosphate buffer (purchased from Sigma-Aldrich) 0.1 M, pH = 7.4, used as reference system.

Table 1
DLS data (the hydrodynamic diameter, $Z_{average}$; the polydispersity index, *PDI* and the zeta potential, ξ) of SNPs/PC/protein/3-HF systems.

System	$Z_{average}$, nm	PDI	ξ–potential, mV ± SD
SNPs [48]	33	0.50	−50 ± 1.35
SNPs/3-HF [48]	110	0.37	−26 ± 1.07
SNPs/PC	78	0.25	−37 ± 1.71
SNPs/PC/3-HF	75	0.30	−27 ± 1.38
SNPs/PC/HSA/3-HF	74	0.33	−30 ± 1.40
SNPs/PC/BSA/3-HF	74	0.21	−33 ± 0.90

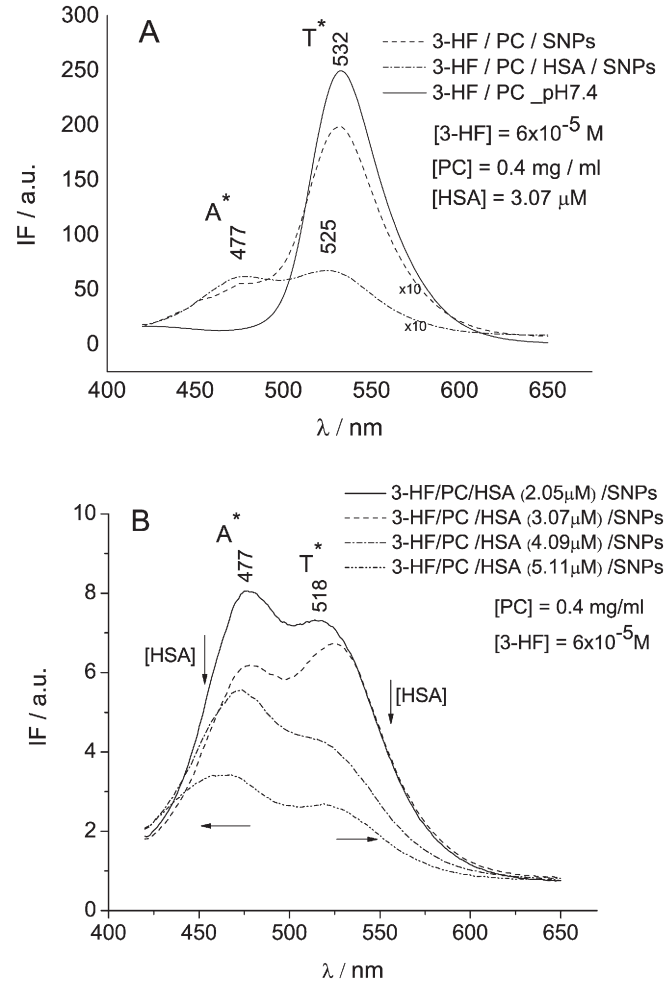

Fig. 2. Fluorescence emission spectra of 3-HF in PC/HSA/SNPs systems; $\lambda_{ex} = 365$ nm.

2.2. Methods

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 ($Z_{average}$), *zeta potential* (ξ) and the *polydispersity index* (*PDI*) were measured by Dynamic Light Scattering (DLS) technique, 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 fluorescence emission and excitation spectra were recorded with a Jasco FP-6500 Spectrofluorometer, using 3 nm bandpass for the excitation and the emission monochromators, the detector response of 1 s, data pitch of 1 nm, the scanning speed of 100 nm/min. The excitation wavelength was 365 nm.

The Raman spectra were measured on a JASCO NRS-3100 with a 785 nm laser of 160 mW of power with a focusing beam diameter of 1 μm.

Deconvolution of spectra was made for amide I, amide III and the disulphide bridges bands, considering the spectral ranges of 1500–1750, 1140–1500 and 450–600 cm^{-1}, respectively. These ranges contain some bands overlapped with the ones of interest. The number of bands taken into account was equal to the number of negative maxima of the fourth derivative of the spectrum and the respective positions were taken as an initial guess. The use of the fourth derivative revealed some strongly overlapped bands which could not be detected in the second derivative profile. Nonetheless, this method cannot discern