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Critical insight into the interaction of naringenin with human haemoglobin: A combined spectroscopic and computational modeling approaches

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ABSTRACT

The present study demonstrates critical insight into the binding of a bioactive flavanone naringenin with normal human haemoglobin (NHb). Both spectrophotometric and spectrofluorimetric studies reveal that naringenin interacts with NHb. The binding affinity constant and number of binding sites appear to be approximately $(1.5 \pm 0.2) \times 10^4\ M^{-1}$ and 1, respectively. Static quenching seems to be an important factor in binding process, as evident from steady-state and time-resolved fluorescence spectroscopic studies. Far UV circular dichroism spectroscopy depicts that binding of naringenin to NHb causes no change in the secondary structure of the protein, which is also evident from Fourier transform infrared spectroscopic study. Free energy change (ΔG^0) for naringenin-NHb interaction, determined by spectroscopic and isothermal calorimetric method, appears to be −5.67 kcal/mol and −6.90 kcal/mol, respectively, and is close to the docking energy −6.84 kcal/mol. Molecular docking suggests that naringenin binds near the cavity of the tetrameric heme protein, forming hydrogen bonds with surrounding amino acid residues. The binding site is away from the heme moieties, implicating naringenin binding does not affect the oxygen binding capacity of NHb, which makes the protein a suitable carrier of the flavonoid.

1. Introduction

Bioflavonoids are widely found in various fruits, vegetables and beverages. Naringenin (Fig. 1a) is an important flavanone present in grapes and citrus fruits [1]. It possesses antioxidant, anti-inflammatory, anti-atherogenic, anti-hyperglycemic and immune system modulating properties [2–6]. Hence it is used against reactive oxygen species mediated diseases and also effective against cancers, cardiac and neuronal disorders [7–9]. Naringenin inhibits hepatitis C virus (HCV) generation by infected liver cells in culture medium [10]. It prevents metabolic syndrome-induced inflammation in low density lipoprotein receptor-deficient mice [11]. Naringenin also lowers the plasma cholesterol concentrations by down regulating hydroxy methyl glutaryl CoA reductase in cholesterol-fed rodents [12]. It appears to be a regulator of cytochrome P450 group of enzymes [13]. Although bioflavonoids including naringenin have several beneficial effects, poor solubility of the compounds in water limits their pharmacological applications. The hydrophobic ring structure is primarily responsible for the low bioavailability of naringenin within the animal system.

Normal adult haemoglobin (NHb) is an oligomeric conjugated protein with four peptide chains joined by non-covalent bonds. Besides transport of oxygen, which is a major function of NHb, the heme protein plays an important role in transporting drugs including antioxidants and bioflavonoids [14,15]. NHb thus facilitates bioavailability of drugs, and may be used for red blood cell (RBC) based drug transport [16,17]. Drug-NHb interaction needs to be explored to understand the heme protein-mediated drug delivery or designing RBC-based drug carrier. Interaction of NHb with dietary polyphenols namely, resveratrol, robinetin, apigenin, etc have been reported [18–20]. In recent years, spectroscopic and molecular modeling studies are widely used in understanding drug-protein interactions [14,19–23]. In the present study, the binding of naringenin with NHb has been characterised by employing both spectroscopic and molecular modeling techniques to understand the structure-affinity relationship, which is needed for designing a RBC-based carrier of a drug.

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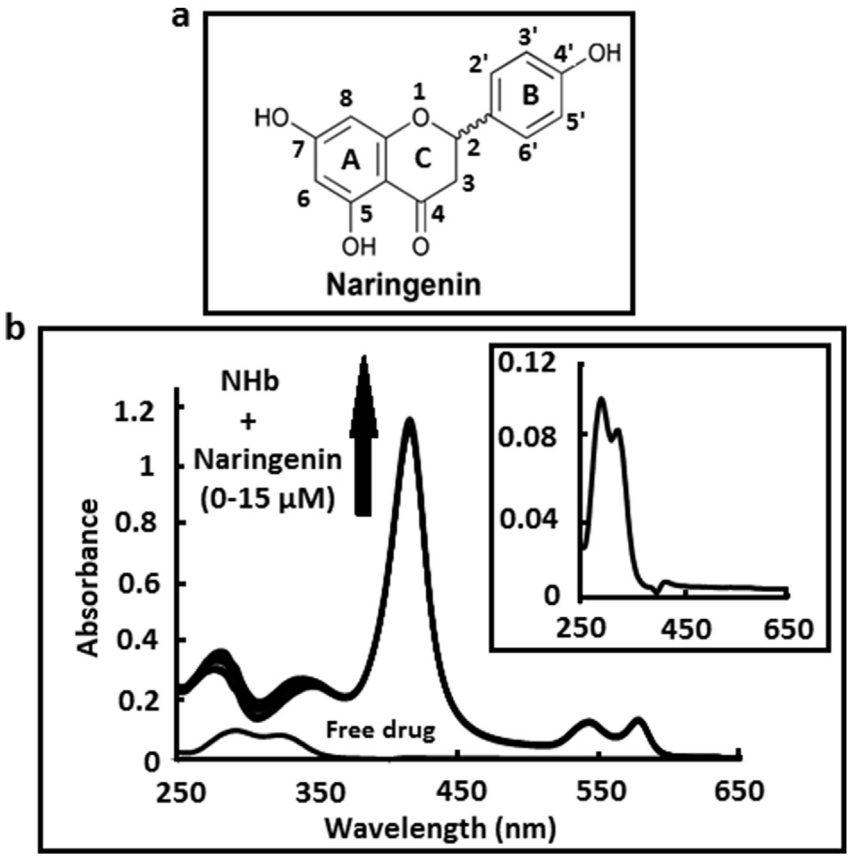

Fig. 1. a) Structure of naringenin. **b)** Absorption spectra of NHb (10 μM) with increasing concentrations of naringenin (0, 2.5, 5, 10, 12.5 and 15 μM) in phosphate buffer. Inset: Enlarged absorption spectrum of naringenin (15 μM).

2. Materials and methods

2.1. Materials

Naringenin and Sephadex G-100 beads were purchased from Sigma-Aldrich, USA. All other chemicals of purified grade were obtained from Sisco Research Laboratories, India.

2.2. Steady-state spectroscopic analysis of naringenin and NHb binding

Blood was collected from healthy volunteers of 25–28 years age and NHb was purified by Sephadex size exclusion column chromatography [24]. The concentration of NHb in 10 mM phosphate buffer, pH 7.4 was determined from the absorbance at 415 nm ($\varepsilon_{415\ nm}$ = 125 $mM^{-1}cm^{-1}$). For absorption and fluorescence spectroscopic studies, methanolic naringenin solution (final concentration 0, 2.5, 5, 10, 12.5, 15, 17.5 and 20 μM) was mixed to 10 μM NHb solution in 1 ml cuvette, and the spectra were recorded in V-630 Jasco spectrophotometer and Hitachi F-3010 spectrofluorimeter, respectively. Final concentration of methanol in the mixture was kept <1% (v/v). Steady state absorption spectra were recorded in 250–650 nm range. Steady state fluorescence spectra were recorded in 300–400 nm range with excitation at 280 nm (excitation band pass = 5 nm, emission band pass = 10 nm). Solvent correction was done in all the studies.

2.3. Time-resolved fluorescence decay measurements

Tryptophan fluorescence decay parameters for the interaction of naringenin with NHb were measured using Jobin-Yvon nanosecond setup (nanoLED-295) at 295 nm diode excitation source. The fluorescence decay profiles of the samples were monitored at 340 nm. Other parameters were as follows: counts = 5500, band slit = 12 nm and FWHM ~ 760 ps. Intensity decay outputs were deconvoluted using DAS6 Software attached with the instrument and fitted in multi-exponential decay profile, $F(t) = \Sigma_i \alpha_i \exp(-t/\tau_i)$, where α_i and τ_i stand for amplitudes and decay component times, such that $\Sigma_i \alpha_i = 1$. Goodness of fit was analyzed by using χ^2 values. Mean lifetimes of tryptophan $<\tau>$ were derived from the expression [25]:

$$<\tau> = (\alpha_1\tau_1^2 + \alpha_2\tau_2^2)/(\alpha_1\tau_1 + \alpha_2\tau_2)$$

2.4. Secondary structure analysis by circular dichroism (CD) and Fourier transform infrared spectroscopy (FTIR) experiments

CD spectra were recorded in a Biologic spectropolarimeter (MS-500) at room temperature. For each experiment, three different measurements were carried out and averaged after buffer correction. CD spectra were recorded in the far UV region (200–250 nm) using 5 μM NHb and 0–20 μM naringenin in a quartz cell of 1 mm path length. FTIR spectra of the liquid samples (protein and protein-naringenin mixture) were recorded in Perkin Elmer Spectrum Two spectrophotometer in the frequency range of 450–4000 cm^{-1} with resolution 4 cm^{-1}. The concentrations of NHb and naringenin used were 300 μM and 300 μM, respectively.