Inhibitory effect of condensed tannins from banana pulp on cholesterol esterase and mechanisms of interaction

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SUPPORTING INFORMATION

Fluorescence resonance energy transfer phenomena (FRET)

Energy transfer between small molecules and proteins is divided into radioactive energy transfer and nonradioactive energy transfer. If the emission spectrum of the donor (tryptophan) significantly overlaps with the absorption spectrum of the acceptoe (CTs), these donor-acceptor pairs will consider in Förster distance and then we could ascertain the possibility of energy transfer phenomenon. Therefore, Förster energy transfer theory depends upon these conditions: (a) the donor can produce fluorescence light; (b) the donor's fluorescence spectrum overlaps the acceptor's UV-vis spectrum; (c) the distance between donor and acceptor is in the range of 1 to 10 nm.

1. Methods

1.1 The measurement of fluorescence quantum yield of CEase

The fluorescence quantum yield of CEase was measured according to the previous reference with some modifications. [1, 2] The fluorescence spectrum of standard and sample was collected in the condition of the excitation wavelength 295 nm and emission wavelength 300-500 nm. And the fluorescence quantum yield of CEase was calculated as follow.

$$\varphi_x=\varphi_s*(F_x*A_s)/(F_S*A_x)*(n_x^2/n_s^2) \qquad (1)$$

Where φ_x and φ_s are the fluorescence quantum yield of the sample and standard, F_x and F_s are the integral intensities (squares under the curves of fluorescence) of sample and standard, A_x and A_s are the absorbance of the sample and standard

measured at the wavelength of excitation, n_x and n_s are the refractive indexes of media in which sample and standard dissolved. In this assay, tryptophan was used as a standard and its fluorescence quantum yield in water is 0.14. [3] And the absorbance of sample and standard need below 0.08 at the wavelength of excitation.

1.2 Fluorescence resonance energy transfer phenomena

The fluorescence resonance energy transfer phenomena between CEase and CTs was measured according to the previous reference with minor modifications. [2] The fluorescence spectra of CEase (0.083 mg/mL) and absorption spectra of CTs (0.0167 mg/mL) were collected in a similar way as given in method section 2.7 'Fluorescence and UV-vis adsorption spectroscopy measurement'.

The efficiency of energy transfer (E) is calculated through following equation:

$$E_{FRET}=1-F/F_0=R_0^6/(R_0^6+R^6) \qquad (2)$$

Where F_0 and F were the fluorescence intensities of CEase in the absence and presence CTs; R is the distance between donor and acceptor; R_0 is the critical distance at which transfer effificiency equals to 50% and can be calculated by following equation:

$$R = 8.79 * 10^{-25} k^2 n^{-4} \phi J \qquad (3)$$

where, k^2 is the orientation factor related to the geometry of the donor and acceptor of dipoles, n is the refractive index of the medium, φ is the fluorescence quantum yield of the donor in the absence of acceptor; and J expresses the degree of spectral overlap between the donor emission and the acceptor absorption which can be evaluated by integrating the overlap spectral area in between 300-500 nm from

following equation:

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda}{\int_0^\infty F(\lambda)d\lambda} \qquad (4)$$

where, F(λ) is the fluorescence intensity of the donor at wavelength λ, ε(λ) is the molar absorption coeffificient of acceptor at wavelength λ.

2. Result and discussion

According to equation (1) and using φ_s=0.15, n_x=n_s=1.333, the fluorescence quantum yield of CEase is 0.143. The overlap of the absorption spectrum of CTs and fluorescence emission spectrum of CEase was shown in Fig.S1. The value of J could be evaluated by integrating the overlap spectra at λ=300-500 nm. According to Eqs. (2)-(4) and using k^2=2/3, n=1.333, φ=0.143 for CEase, the following data were obtained: J=2.95*10^{-14} cm^3 L mol^{-1}, R_0=4.44 nm and R=5.56 nm for the CTs – CEase system. Obviously, the donor-acceptor distances was less than 8 nm, which accorded with conditions of Foster's non-radiative energy transfer theory. The result confirmed that the interactions of CTs with CEase was initiated by the static quenching and the energy transfer from CEase to CTs occurred efficiently. The result was consistent with fluorescence spectra results obtained.

3. Reference

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