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Study On The Interaction Of Bovine Serum Albumin And Diethyl Flavone-7-yl phosphate By Fluorescence Method

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ABSTRACT

Fluorescence method was used to study the interactions between BSA and diethyl flavone-7-yl phosphate and 7-hydroxyflavone. The results showed that the phosphorylated flavonoid can form non-covalent complexes BSA and showed higher binding affinity with the protein than 7-hydroxyflavone did. The association constants of BSA and diethyl flavone-7-yl phosphate were determined from a Line weaver-Burk plot. Experiments demonstrated that the higher the temperature was, the lower the slops of quenching curve of BSA was in presence of different amounts of diethyl flavone-7-yl phosphate. It was confirmed that the combination for diethyl flavone-7-yl phosphate with BSA was a single static quenching process. According to the nonradiative transfer of energy, the distance was measured between the diethyl flavone-7-yl phosphate and tryptophane. From thermo dynamical coordination it could be judged that the binding power between diethyl flavone-7-yl phosphate and BSA was static electric power and. hydrophobic force.

KEYWORDS

Fluorescence quenching;
BSA;
Diethyl flavone-7-yl phosphate;
Energy transition.

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INTRODUCTION

The binding study of drugs with proteins is of great important in pharmacology, Several well established spectroscopic techniques are used to study biomolecular interactions including circular dichroism CD, Infra-Red IR and ultraviolet UV spectroscopy, nuclear magnetic resonance, ESI-MS and X-ray crystallography. There are three amino acid residues in the protein can emit fluorescence, so the fluorescence is a efficient method by which to study biomolecular nocovalent interactions with small molecules. The basic information contained in fluorescence measurement relates to the molecular environment of the chromophore, fluorescence of tryptophan residues is very sensitive to the changes in their vicinity, thus it is widely used to study variations of the molecular conformations of proteins.

Serum albumins are the most abundant proteins in plasma. As the major soluble protein constituents of the circulatory system, they have many physiological functions. They contribute to colloid osmotic blood pressure and are chiefly responsible for the maintenance of blood pH[1]. Since albumin serves as a transport carrier for drugs, it is important to study the interactions of small molecules with this protein. The effectiveness of these compounds as pharmaceutical agents depends on their binding ability.

It is known that esters of phosphoric acid have wide bioactivities and play a vital role in many biological processes. They appear to be synthesized and to undergo interconversion with great ease in living organisms[2-5]; in recent years, flavonoids have attracted increasing interest due to their various beneficial pharmacological effects. In this paper we selected 7-hydroxyflavone, a representative flavone, to synthesize its phosphate ester through simplified Atherton-Todd reaction. Then the binding affinity of the flavonoid and its phosphate ester with serum albumins was studied and compared using fluorescence quenching.(SCHEME 1)

EXPERIMENTAL

Materials

Bovine serum was purchased from Sigma chemicals and was used without further purification. BSA was dissolved in Tris-HCl buffer (pH7.4 including 0.1mol/LNaCl) and the concentrations was 1.74×10^{-6}mol/L. Flavonoid and diethyl flavone-7-yl phosphate were obtained from our laboratory and were dissolved in water-methanol. All other chemicals were of analytical grade.

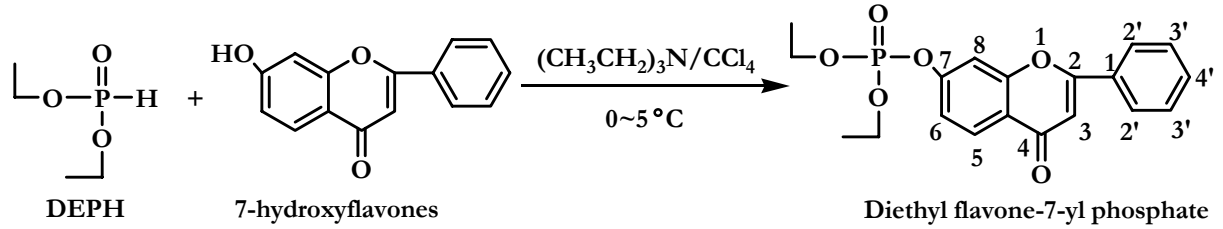
Fluorescence measurements

Fluorescence spectra were taken with a F4500 spectrofluorometer using excitation wavelength of 280nm and the emission range set between 290nm and 450nm. The slit was 10nm. The quenching experiment was performed for the same BSA concentration with different quantity flavonoid and its phosphate ester at the temperature 20°C and 35°C. We only found the quenching in the BSA-diethyl flavone-7-yl phosphate system but not found in the BSA-flavonoid system (Figure 1).

RESULTS AND DISCUSSION

Calculation of binding constants

From figure 1, we can see that with the diethyl flavone-7-yl phosphate increasing concentrations



DEPH + 7-hydroxyflavones (1) → Diethyl flavone-7-yl phosphate (2)

SCHEME 1

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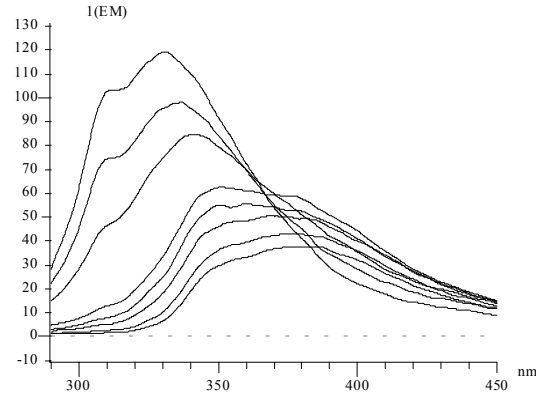

Figure 1: The effect of diethyl flavone-7-yl phosphate on fluorescence spectra of bovine serum albumin

caused a linear reduction in the fluorescence of BSA solution. In order to make clear the style of the quenching process, a first approach to describe the fluorescence behavior is Stern-Volmer[6] equation, given by

$$F_0 / F = 1 + K_{sv}[Q]$$

Where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively. K_{sv} is the Stern-Volmer constant and [Q] is the molar concentration of quencher. Draw F_0 / F - [Q] lines at temperature 20°C and 35°C respectively. (Figure 2 Line 1-20°C; line 2-35°C). The experiments demonstrated that the higher the temperature was, the lower the slops of quenching curve of BSA was in presence of different amounts of diethyl flavone-7-yl phosphate. It was confirmed that the combination for diethyl flavone-7-yl phosphate with BSA is a single static quenching process[7]. So the quenching data were analyzed according to other equation, given by

$$(F_0-F)^{-1} = F_0^{-1} + K^{-1}F_0^{-1}[Q]^{-1}$$

Draw Line 3weaver-Burk plots (Figure 3 line 1-20°C; line 2-35°C). The linearly dependent coefficients are 0.99 and 0.998. According to this equation the binding constants at different temperature could be calculated to be 3.3×10^4(20°C) and 2.9×10^4(35°C) respectively. These results showed that the phosphorylated flavonoid formed non-covalent complexes BSA and showed high binding affinity with the protein.

The sorts of binding force determination

The sorts of binding force between small molecules and protein are mainly electrostatic force, hydrophobic force and van der waals force. We regarded the enthalpy ΔH as a constant when the temperature shifted a little. Then the thermodynamics functions of BSA binding with diethyl flavone-7-yl phosphate could be calculated by these formulas[8].

$$\ln(k_2 / k_1) = \Delta H/R(1/T1 - 1/T2);$$
$$\Delta G = \Delta H - T\Delta S;\ \Delta G = -RT\ln k$$

At the temperature 20°C, the enthalpy ΔH = -6.45kJ·mol^{-1}; the entropy ΔS = 64.3 J·mol^{-1}·K^{-1}· ΔS>0

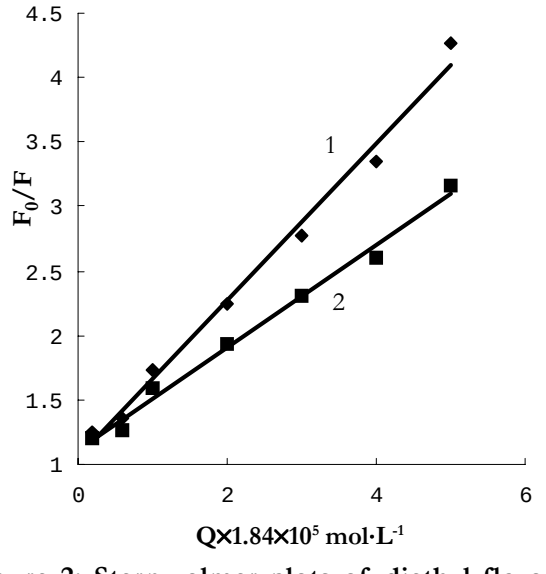

Figure 2: Stern-volmer plots of diethyl flavone-7-yl phosphate and bovine serum albumin

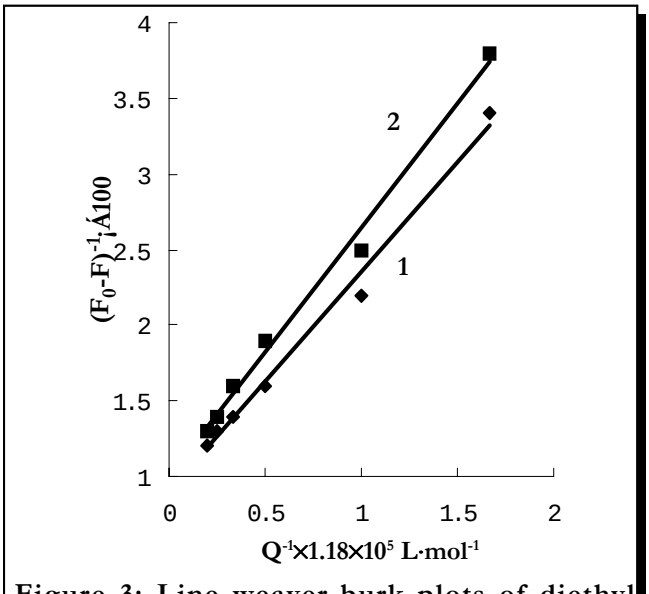

Figure 3: Line weaver-burk plots of diethyl flavone-7-yl phosphate vs bovine serum albumin^{-1}

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and $\Delta H^3 0$. So it was considered that the electrostatic force was main binding force, and the hydrophobic force had also influence the binding process[9].

The distance between BSA and diethyl flavone-7-yl phosphate

Although fluorescence-quenching studies have provided information on the interaction of small molecules and protein, fluorescence resonance energy transfer (FRET) has provided more quantitative information on distances between sites on proteins. Fluorescence resonance energy transfer (FRET) involves the nonradiative transfer of energy from an excited state donor fluorophore to a nearby acceptor[10-14]. The energy transfer efficiency E is related to the distance r separating a given donor and accepter pair by

$$E = R_0^6 / (R_0^6 + r^6)$$

The resolution of FRET is thus defined by R_0, which is typically <10-70 Å. R_0 for a given donor and acceptor pair is a function of the extent of overlap between the donor emission and acceptor absorption spectra (J Figure 4), the absorption coefficient for the acceptor(ε_A), the quantum yield of the donor(Φ), and the relative orientation of the donor and acceptor (k^2). Because FRET falls off as the sixth power of the separation between the donor and acceptor, when distances of separate the donor and acceptor >$2R_0$, no FRET occurs. The distance between BSA and small molecule r can be calculated by:

$$R_0^6 = 8.8\times10^{-25}(K^2\cdot F\cdot n^{-6}\cdot J)$$

$$J(\lambda)=\int_0^{\infty} F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda$$

Where F_D is the fluorescence intensity of BSA at the wavelength λ. Energy transfer efficiency can be quantitated simply as $E=1-F/F_0$, where F_0 and F are the intensity of fluorescence in the absence and presence of the accepter at the same concentration with protein. The energy transfer efficiency of BSA and diethyl flavone-7-yl phosphate was calculated to be E=0.055. Then r was calculated r=41.3Å (<$2R_0$). That's to say the nonradiative transfer of energy occurred between BSA and diethyl flavone-7-yl phosphate.

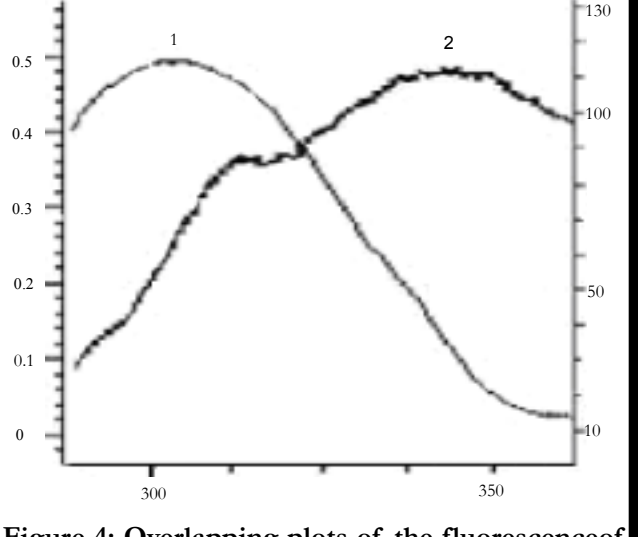

Figure 4: Overlapping plots of the fluorescenceof BSA 2 with absorption spectra of diethyl flavone-7-yl phosphate 1

CONCLUSION

Tryptophan present in the proteins can act as intrinsic fluorescence probes[15] and it is the typical site of coordination for several substances. It was clear that the binding site of phosphorylated flavonoids in BSA, which is very close, in contact with tryptophan residue. The maximum of emission wavelength of protein shift from 320 nm to 360 nm indicates that tryptophans are, on average, more exposed to the solvent, and the ligand binding changed the geometry of the structure of albumin when diethyl flavone-7-yl phosphate was added to the protein solution, due to electrostatic and hydrophobic contacts of the chromophore within the protein interior[16], while the influence of flavonoid on the BSA couldn't be found. This conclusion confirmed that phosphorylated flavonoids possess relatively stronger affinities for proteins than flavonoids.

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