Determination of Serum Albumin by its quenching effect on the fluorescence of Zn^{2+}-Morin complex

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Keywords: Morin, BSA,Zn^{2+},fluorescence spectrum,UV absorption spectra.

Abstract. In this paper, the mode of action of Morin and protein has been studied through UV and fluorescence spectrum. This paper found that in the pH = 8.50 Tris-HCl buffer solution,at the presence of Zn^{2+}, the protein can cause Morin fluorescence quenching and the quenching degree of fluorescence is in proportional to the amount of protein to a certain extent. At the best condition, the detection limit of BSA and HSA is 0.22μg·mL^{-1}、 0.18μg·mL^{-1} respectively. Compared with other analysis methods for proteins that have been reported,this method has a relatively wide linear range and high sensitivity.

Introduction

Morin (3, 5, 7, 2′, 4′-pentahydroxyflavone) is the effective component of a common Chinese medicinal herb. Morin belongs to a kind of hydroxyl flavonoids and its structure as shown in Fig.1. Morin consists of two aromatic rings (A and B) linked by an oxygen-containing heterocycle (ring C).

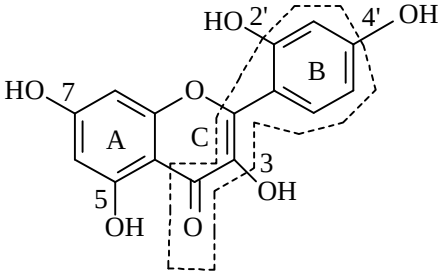

Fig.1. The structure of Morin

Recently, Morin has attracted more and more interest because it exhibits a broad spectrum of biological and important therapeutic applications (eg. antibiosis, antiphlogosis, antioxidation, antiviral, antitumor activities [1-3]). Morin exerts its functions on various proteins [4–8], so it is necessary to study the interaction between Morin and protein. Bovine serum albumin (BSA), a model protein that is frequently used as a testing ground for initial characterization of novel optical probes for proteins was chosen for the present investigation.

Previous work has primarily been about the usage of the intrinsic fluorescence emission properties of proteins [9-10]. In this paper, the intrinsic fluorescence of Morin is employed as a probe for understanding protein–natural flavonoid interaction from both qualitative as well as quantitative perspectives.

Experimental

Apparatus. Fluorescence spectra were recorded with an F-2500 spectrofluorometer (Hitachi, Japan). Absorption spectra were taken with an UV-2401PC spectrophotometer (Shimadzu, Japan). All pH measurements were made with a pHS-2F digital acidity meter (Leici, Shanghai, China). Three times distilled water was made by SZ-93A automatic dual water distiller (Yarong biochemical instrument factory, Shanghai). Analytical balance is CP225D electronic balance (sartorius AG).

Reagents. Protein bovine serum albumin (BSA) and human serum albumin (HSA) were purchased from Shanghai Boao Biochemical Technology Co. and Sigma, respectively. Morin was purchased from

Sigma-Aldrich. All the chemicals used were of analytical reagents grade, and deionized water was used for all experiments.

Stock solutions of BSA and HSA at $1.00 g \cdot L^{-1}$ were prepared by dissolving them in water. The Morin solution at $4.00\times10^{-3} mol \cdot L^{-1}$ was prepared by dissolving Morin in ethanol. The Zn^{2+} solution at $1.00\times10^{-3} mol \cdot L^{-1}$ was prepared by dissolving $ZnCl_2$ in water. A series of Tris -HCl buffer solutions ($0.2 mol \cdot L^{-1}$) were used for the pH adjustment. The above solutions were stored at 0-4ºC.

Procedures. For spectral measurement, all samples were prepared according to the following procedure: 1.00mL Tris -HCl (pH8.50), 0.80mL of Morin (4.00×10^{-4} $mol \cdot L^{-1}$), 1.00mL of Zn^{2+} (1.00×10^{-3} $mol \cdot L^{-1}$)and certain standard BSA (or sample solution) in turn, and then the mixture was diluted to 10mL with water and allowed to stand for 20 minutes. The fluorescence intensity was measured in a 1 cm quartz cell at λex/λem = 320nm/520nm with a slit of 10.0nm for the excitation and emission. The quenched fluorescence intensity of Morin by BSA was represented as $\Delta I_f(\%)=(I_0 - I_f)/ I_0\times100\%$. Here, I_f and I_0 were the intensities of the systems with and without BSA, respectively.

Results and discussion.

Fluorescence Spectra. The fluorescence spectra of the (1) Morin+Zn^{2+}; (2) Morin+Zn^{2+}+BSA; (3) Morin; (4) Morin+BSA ;(5) Zn^{2+}+BSA ;(6) Zn^{2+};(7) BSA systems are shown in Fig.2. From this figure, It can be seen that in a Tris -HCl buffer at pH=8.50, after the excitation of 320 nm, the intensity of characteristic fluorescence of BSA at the emission peak of 520nm can be quenched by the addition of Morin+Zn^{2+}. This indicates that there is interaction among Morin+Zn^{2+} and BSA.

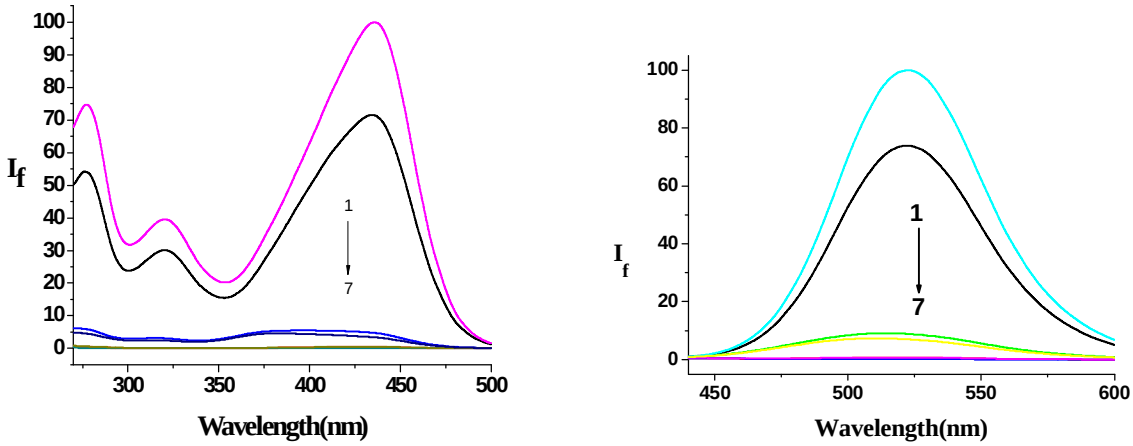

Fig. 2 Excitation spectra (a) (λem=520nm) and emissionspectra (b) (λex=320nm)

1.buffer+Morin+Zn^{2+} ; 2.buffer+Morin+Zn^{2+}+BSA ; 3.buffer+Morin ; 4.buffer+Morin+BSA ; 5.buffer+Zn^{2+}+BSA ; 6.buffer+Zn^{2+} ; 7.buffer+BSA

Conditions: pH=8.50; Morin:$3.20\times10^{-5} mol \cdot L^{-1}$; Zn^{2+}:$1.00\times10^{-4} mol \cdot L^{-1}$; BSA:$0.050 g \cdot L^{-1}$;

Effects of pH and Buffers. The effect of pH on the quenched fluorescence intensity $\Delta I_f(\%)$ of the system is shown in Fig. 3. The experimental results indicate that the $\Delta I_f(\%)$ reaches a maximum around pH =8.50. The effects of different buffers on the $\Delta I_f(\%)$ of this system are also tested at the same pH (pH=8.50). The $\Delta I_f(\%)$ for Tris-HC;Borax; KH_2PO_4-NaOH; BR-; citric acid - NaOH are 100, 35.89; -1.16; -1.62;-5.17 respectively. So Tris-HC is the most suitable buffer. Tris-HC buffer solution

(pH=8.50) was selected for the assay and the optimum volume is 1.00 mL.

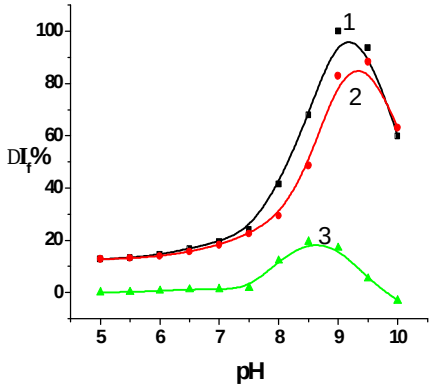

Fig. 3 The effect of pH on the fluorescence intensity
1:I_f^0; 2: I_f; 3 I_f^0 -I_f
Conditions: pH=8.50; Morin: 3.20×10^{-5}mol·L^{-1};
Zn^{2+}:1.00×10^{-4}mol·L^{-1}; BSA:0.050g·L^{-1} ;

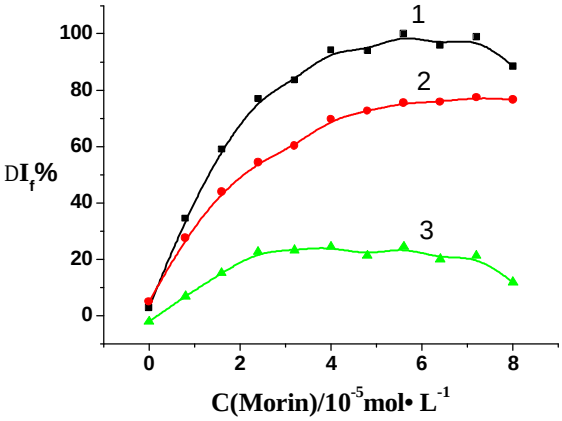

Fig. 4The effect of the concentration of Morin
1:I_f^0; 2: I_f; 3 I_f^0 -I_f
Conditions: pH=8.50; Zn^{2+}:1.00×10^{-4}mol·L^{-1};
BSA:0.050g·L^{-1} ;

Effect of Morin Concentration. The effect of Morin concentration on the fluorescence intensity of Morin-BSA-Zn^{2+} system is studied. From Fig. 4, it is found that the quenched fluorescence intensity of Morin system reaches a maximum when the concentration of Morin is 3.20×10^{-5}mol·L^{-1}. So 3.20×10^{-5}mol·L^{-1} of Morin is chosen in the research.

Effect of Zn^{2+} concentration. The effect of Zn^{2+} concentration on the fluorescence intensity of Morin-BSA-Zn^{2+} system is studied.It is found that the quenched fluorescence intensity of Zn^{2+} system reaches a maximum when the concentration of Zn^{2+} is 1.00×10^{-4}mol·L^{-1}. So 1.00×10^{-4}mol·L^{-1} of Zn^{2+} is chosen in the research.

Effect of reaction time Tests show that the ΔI_f reached a maximum within 20 minutes after reagents had been added, and the ΔI_f remained stable for at least 2 hours. Therefore the system exhibited good stability. In this research, 20 minutes of sample incubation time was set for all the fluorescence measurements.

Interfering substances. With the standard analytical procedure established at the above optimized condition, interferences of foreign substances including various ions and biomolecules on fluorescence of the Morin-BSA- Zn^{2+} system were evaluated. The results in Table 1 indicate that these foreign substances had little or no effect on the determination of proteins under the permission of ±5% relative errors, and thus the proposed method exhibits good selectivity in protein measurement.

Table1 Interference from foreign substances

Foreign substances	Concentration (10^{-6}mol·L^{-1})	ΔI_f (%)	Foreign substances	Concentration (10^{-6}mol·L^{-1})	ΔI_f (%)
Na^+, Cl^{-f}	2	-3.98	Mg^{2+} , SO_4^{2-}	2	-2.85
Ca^{2+},Cl^-	2	-3.18	Cu^{2+},Cl^-	2	-5.95
NH_4^+, Cl^-	2	-2.71	Zn^{2+},SO_4^{2-}	2	-2.92
Zn^{2+}, Cl^-	2	-3.84	L-Glu	2	-3.69
Mn^{2+}, Cl^{--}	2	-4.67	sucrose	2	-4.50
Na^+, SO_4^{2-}	2	4.55	L-Leu	2	-5.75
K^+ , Cl^-	4	-3.76	Glucose	2	-5.94

pH=8.50; Morin:3.20×10^{-5}mol·L^{-1}; BSA:5.0×10^{-4}g·L^{-1}; Zn^{2+}:0.80×10^{-4}mol·L^{-1}

Analytical parameters

Under the optimum conditions defined, the calibration graphs for BSA and HSA were obtained. They showed that there was a linear relationship between the ΔI_f and the concentration of proteins listed in Table 2. It shows that this method has high sensitivity and a wide linear range. It can be seen that this method has a higher sensitivity and a wider linear range for protein measurement.

Table2 Analytical parameters of this method

	Linear range ($g \cdot mL^{-1}$)	Linear regression equation	Correlation coefficient r	Limit of detection($\mu g \cdot mL^{-1}$)
BSA	4.00×10^{-7}–1.00×10^{-4}	ΔI_f=14.57+43.14C	0.9910	0.22
HSA	4.00×10^{-7}–1.00×10^{-4}	ΔI_f=14.65+52.21C	0.9922	0.18

Conclusion

In this work, the fluorescence property of the Morin-BSA--Zn^{2+}system has been systematically investigated by using UV absorbance spectroscopy and fluorescence spectroscopy techniques. Results show that the fluorescence of Morin or BSA can all be intensely quenched by the other. The quenching in Morin fluorescence intensity is quantitatively proportional to the concentration of proteins in a wide range.

Acknowledgements

The project of this paper are supported by Shandong Provincial Natural Science Foundation(ZR2012BL06) and Higher school teaching reform project in shandong province (2012496).

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