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Studies on the Interaction between a Co(II) Complex with Salicylaldehyde-Aminoacetic Acid Schiff Base and Bovine Serum Albumin[1]

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Abstract—A Co(II) complex $[Co_3(L)_4(H_2O)_6] \cdot 2Cl$ (**I**), where L is salicylaldehyde-aminoacetic acid Schiff base, was synthesized and characterized via elemental analysis, UV, and single crystal X-ray crystallography. Complex **I** crystallizes in the orthorhombic system, space group *Pbcn* with lattice parameters $a = 9.569(4)$, $b = 12.301(5)$, $c = 36.931(14)$ Å, $V = 4347(3)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.608$ mg m^{-3}. At the same time, the binding reaction between complex **I** and bovine serum albumin (BSA) was studied by fluorescence spectroscopy combined with UV-Vis absorption measurements under simulative physiological conditions. The results indicated that its combination reaction is mainly a static quenching process. Complex **I** bound BSA with a molar ratio of 1 : 1 and the binding constant K_A values are 3.86×10^5 L mol^{-1} (25°C) and 1.17×10^5 L mol^{-1} (36°C). The shortest binding distance *r* between the donor BSA and acceptor (complex **I**) is 2.49 nm, which affirms that complex **I** has partly inserted into the hydrophobic pocket of BSA.

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INTRODUCTION

Serum albumin is an abundant protein in the circulatory system. The most important physiological function of serum albumin is to maintain the osmotic pressure and pH of blood and transport a wide variety of endogenous and exogenous compounds including fatty acids, amino acids, steroids, and drugs. Distribution and metabolism of biologically active compounds, such as drugs and other organic compounds in the body, are correlated with their affinities to serum albumin. Strong binding can reduce the concentrations of free drugs in plasma, whereas weak binding can lead to a short lifetime or poor distribution. Consequently, the study of the interaction between drugs and serum albumin is of great importance in pharmacology and pharmacodynamics. In this paper, bovine serum albumin (BSA) was selected as the protein model for its availability, low cost, stability, unusual ligand binding properties, and the high homology with human serum albumin (HSA) [1]. On the basis of the distribution of the disulfide bridges and of the amino acid sequence, it seems that BSA is composed of three homologous domains linked together (I–III), and each domain is subdivided into two subdomains (A, B). It has two tryptophan residues that possess intrinsic fluorescence: Trp-134, which is located on the surface of subdomain IB and Trp-212 locating within the hydrophobic binding pocket of sub-domain **IIA** [2]. By monitoring the intrinsic fluorescence transformation of BSA, information about the distribution, metabolism, and elimination of drugs in vivo and its pharmacological effect on SA can be obtained [3–5]. Schiff bases complexes have been widely investigated owing to their important biological activities, such as antibacterial, antitumor activities. Our present work was aimed at synthesizing a Co(II) complex with salicylaldehyde-aminoacetic acid Schiff, reporting its crystal structure and investigating the interaction mechanism between complex **I** and BSA regarding the quenching mechanism, the specific binding site, binding distances between BSA and complex **I**.

EXPERIMENTAL

Materials and methods. BSA (free of fatty acid, purity >99.0%) was purchased from biotechnology Co., Ltd. (Beijing, China), stored in refrigerator at 4.0°C and used without further purification. The BSA working solutions with a concentration of 2.0×10^{-6} mol L^{-1} were prepared by dissolving BSA in the Tris-HCl buffer solution (0.10 mol L^{-1} Tris base, 0.10 mol L^{-1} HCl and 0.10 mol L^{-1} NaCl, pH 7.40) and stored in dark prior to use. All other reagents and solvents were of analytical purity and doubly distilled water was used throughout the experiment. The weight measurements were performed on an AB204-N electronic analytic weight-

[1] The article is published in the original.

Table 1. Crystal data and structure refinement parameters for **I**

Parameter	Value
Empirical formula	$C_{36}H_{28}N_4O_{18}Cl_2Co_3$
Formula weight	1052.31
Crystal system	Orthorhombic
Space group	*Pbcn*
Crystal size, mm	0.50 × 0.08 × 0.04
Diffractometer, scan mode	CCD area detector, φ/ω
θ Range for data collect, deg	2.21–26.01
a, Å	9.569(4)
b, Å	12.301(5)
c, Å	36.931(14)
V, Å^3	4347(3)
Z	4
ρ_{calcd}, mg/m^3	1.608
F(000)	2124
Temperature, K	296(2)
Index range *h*, *k*, *l*	$-11 \le h \le 11$, $-9 \le k \le 15$, $-45 \le l \le 44$
Completeness, %	99.0
Reflections collected	15486
Independent reflections (R_{int})	4244
Reflections with $I > 2\sigma(I)$	2138 (0.0936)
Numbers of parameters	286
Final *R* indices ($I > 2\sigma(I)$)	$R_1 = 0.0954$, $wR_2 = 0.2586$
R indices (all data)	$R_1 = 0.1938$, $wR_2 = 0.3478$
Goodness-of-fit on F^2	1.088
$\Delta\rho_{max}/\Delta\rho_{min}$, *e* Å^{-3}	1.156 and −2.337

ing scale (Mettler-Toledo Co., Ltd.) with a resolution of 0.1 mg. All fluorescence measurements were performed on an F-4500 FL mode spectrophotometer equipped with a quartz cell and a thermostat bath. Fluorescence emission spectra were recorded at two different temperatures (298, 309 K) in the range of 303–500 nm. The width of the excitation and emission slits was set to 5 nm. An excitation wavelength of 283 nm was chosen and the temperature of sample was kept by recycle water during the experiment. UV-Vis absorption spectrum was recorded on a Lambda-35 spectrophotometer (PerkinElmer corporate, America), equipped with 1.0 cm quartz cells. The recorded wavelength was ranging from 500 to 200 nm. The binding constant K_A and binding site *n* between complex **I** and BSA were measured using the fluorescence titration method, in which by fixing the concentration of BSA (2.0×10^{-6} mol L^{-1}) and complex **I** (1.0×10^{-3} mol L^{-1}), then gradually added to the BSA ($V_{BSA} : V_{complex\ I} = 1 : 1$). An excitation wavelength of 283 nm was selected, and the fluorescence spectra were recorded in the range of 303–500 nm.

Synthesis of complex I. An ethanol solution (10 mL) of aminoacetic acid (1 mmol) was dropwise added into an ethanol solution (15 mL) of salicylaldehyde (1 mmol) with stirring, the pH value of the mixture solution was adjusted to 6.5. Then an aqueous solution (5 mL) of $CoCl_2 \cdot 6H_2O$ (1 mmol) was added to the above solution. The mixture was refluxed for 60 min. After cooling to room temperature, the solution was filtered. Brown single crystals of the title compound were obtained from the filtrate after 4 days.

X-ray crystal determination. Data collection for complex **I** was performed on a Bruker SMART APEX II CCD area detector equipped with a graphite monochromated MoK_α radiation (λ = 0.71073 Å). Multi-scan absorption corrections were applied using the SADABS program [6]. The structure was solved by a direct method using the SHELXS-97 program [7]. Refinements on F^2 were performed using SHELXL-97 [8] by a full-matrix least-squares method with anisotropic parameters for all non-hydrogen atoms. The hydrogen atoms of the ligand were generated geometrically. The H atoms of the coordination water molecules were not located due to disorder.

A summary of crystallographic data and refinement parameters is given in Table 1. The atomic coordinates and other parameters of structure **I** have been deposited with the Cambridge Crystallographic Centre (no. 827143; deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

RESULTS AND DISCUSSION

As illustrated in Fig.1, the asymmetric unit of **I** consists of two $[Co(1)(HL)_2]$, $[Co(2)(H_2O)_6]^{2+}$ and two Cl^- anions. The $Co^{2+}(1)$ in **I** is linked with two Schiff bases with the –COOH group of aminoacetic acid deprotonated. The $Co^{2+}(1)$ possesses distorted octahedral coordination environment and is surrounded by four O atoms (O(1), O(3), O(4), O(6)) and two N atoms (N(1), N(2)) from two deprotonated Schiff bases. Two O atoms (O(3) and O(6)) are from –OH group of salicylaldehyde, while another two O atoms (O(1) and O(4)) are from $-COO^-$ group of aminoacetic acid deprotonated. The Co(1)–O(OH) bond distances Co(1)–O(3) and Co(1)–O(6) are 1.865(6) and 1.877(7) Å, correspondingly; the Co(1)–O(COO) bond distances Co(1)–O(1) and Co(1)–O(4) are 1.920(7) and 1.925(6) Å, correspondingly. Two N atoms (N(1) and N(2)) are from two different Schiff bases with bond distances of 1.910(8) and 1.902(8) Å for Co(1)–N(1) and Co(1)–N(2), respectively. The OCo(1)O bond angles are in the range 88.3(3)°–90.6(3)°, while the OCo(1)N bond angles are in the

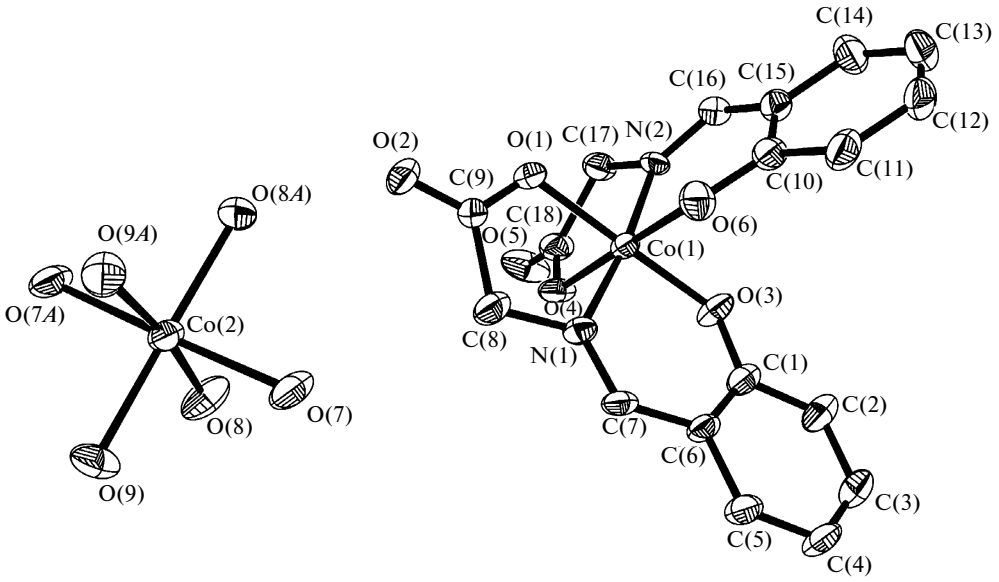

Fig. 1. Crystallographically independent structure fragment in complex **I**. Hydrogen atoms are omitted for clarity. Atomic displacement ellipsoids are shown at the 30% probability level.

range of 84.7(3)°–97.1(3)°. The $Co^{2+}(2)$ possesses a distorted octahedral coordination environment and is coordinated by six O atoms (O(7), O(8), O(9), O(7*A*), O(8*A*) and O(9*A*)) from six water molecules with bond distances 2.141(6), 2.050(7) and 2.073(7) Å for Co(2)–O(7), Co(2)–O(8) and Co(2)–O(9), respectively, Selected bond distances and angles are presented in Table 2. Rich hydrogen-bonds link the molecules to form 3D networks, as shown in Fig. 2.

In the present work, the fluorescence quenching spectra of BSA in the presence of different concentrations of complex **I** at 298 and 309 K were measured to elucidate the quenching mechanism. Effects of complex **I** on the fluorescence spectra of BSA at corresponding temperatures are shown in Fig. 3. Obviously, BSA has a strong fluorescence emission band at 340 nm when excited at 283 nm, which should mainly due to the fluorescence emission of tryptophan residues; while complex **I** has no fluorescence emission under the same condition. In order to study if the $[Co(H_2O)_6]^{2+}$ ions have some effect, different concentrations of $CoCl_2$ solutions have been used to test fluorescence intensity of BSA, and the experiment indicated that the fluorescence intensity of BSA were not changed. So we can conclude that $[Co(H_2O)_6]^{2+}$ has no effect on BSA, the interaction is cased by the interaction between complex **I** and BSA. When different amount of complex **I** was added to a fixed concentration of BSA, a gradually

Table 2. Selected bond distances (Å) and bond angles (deg) for **I**

Bond	*d*, Å	Bond	*d*, Å	Bond	*d*, Å
Co(1)–O(1)	1.920(7)	Co(1)–O(3)	1.865(6)	Co(1)–O(4)	1.925(6)
Co(1)–O(6)	1.877(7)	Co(1)–N(1)	1.910(8)	Co(1)–N(2)	1.902(8)
Co(2)–O(7)	2.141(6)	Co(2)–O(8)	2.050(7)	Co(2)–O(9)	2.073(7)
Angle	ω, deg	Angle	ω, deg	Angle	ω, deg
O(6)Co(1)O(3)	90.6(3)	O(6)Co(1)N(2)	95.1(3)	O(3)Co(1)N(2)	87.8(3)
O(6)Co(1)N(1)	89.1(3)	O(3)Co(1)O(4)	88.3(3)	N(2)Co(1)O(4)	85.4(3)
O(6)Co(1)O(1)	90.5(3)	N(2)Co(1)O(1)	90.3(3)	N(1)Co(1)O(1)	84.7(3)
N(1)Co(1)O(4)	90.4(3)	O(1)Co(1)O(4)	90.6(3)	O(3)Co(1)N(1)	97.1(3)
O(8)Co(2)O(8*A*)	98.5(5)	O(9*A*)Co(2)O(7*A*)	87.4(3)	O(8*A*)Co(2)O(9*A*)	87.7(3)
O(8)Co(2)O(9)	87.7(3)	O(9)Co(2)O(7*A*)	94.5(3)	O(9*A*)Co(2)O(9)	86.6(4)
O(8)Co(2)O(7)	90.2(3)	O(8*A*)Co(2)O(7)	88.2(3)	$O(9)^{\#1}$Co(2)O(7)	94.5(3)
O(9)Co(2)O(7)	87.4(3)	O(8)Co(2)O(7*A*)	88.2(3)	O(8*A*)Co(2)O(7*A*)	90.2(3)

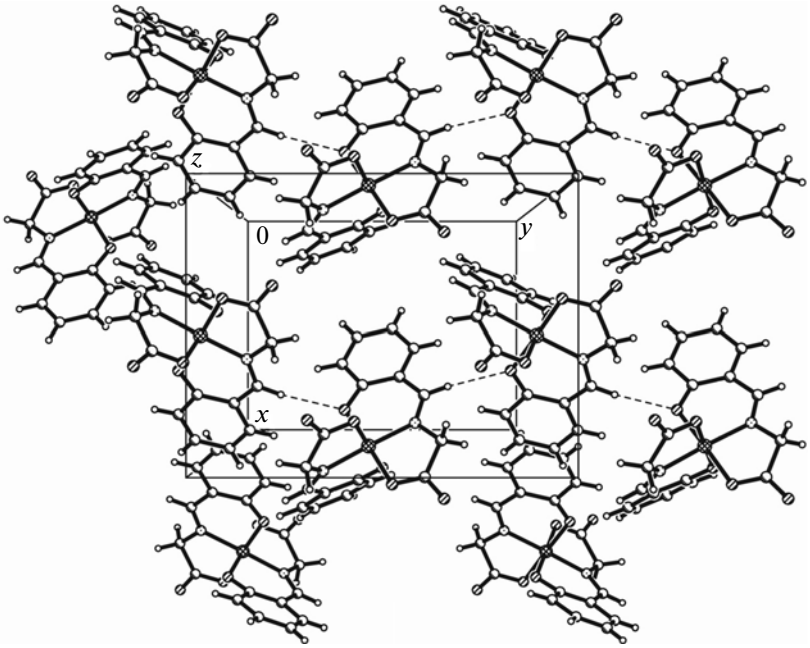

Fig. 2. Crystal packing perspective view of **I**.

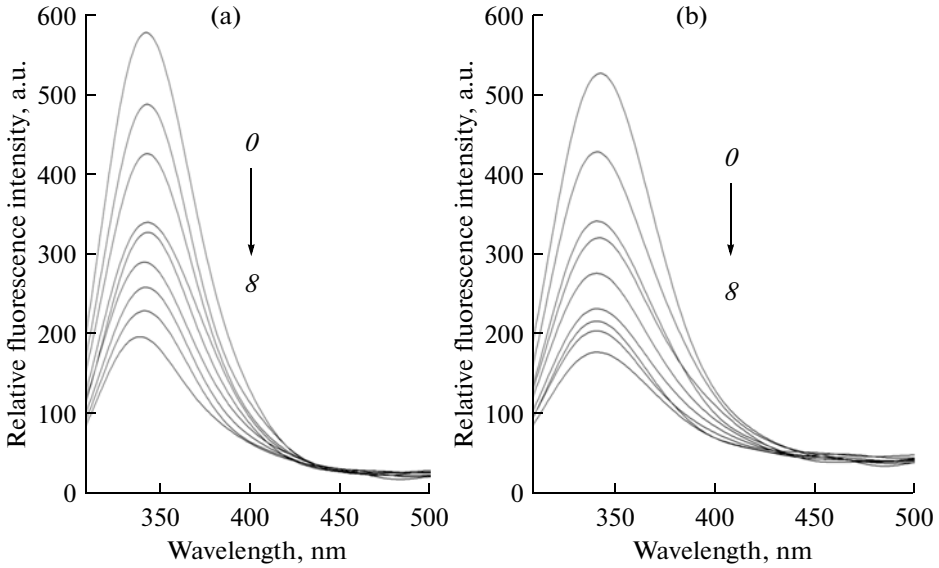

Fig. 3. Influence of complex **I** on fluorescence spectra of BSA at 298 (a) and 309 K (b). $c_{BSA} = 2.0 \times 10^{-6}$ mol L^{-1} in all cases, lines from up to down, *0*–*8*: $V_M = 0$, 5, 10, 15, 20, 25, 30, 35, 40 μL, $c_{complex\ I} = 1 \times 10^{-3}$ mol L^{-1}.

decrease in the fluorescence intensity of BSA was observed, indicating that complex **I** could interact with BSA and quench its intrinsic fluorescence. The fluorescence quenching was usually analyzed using the well-known Stern–Volmer equation [9]:

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q],$$

where F_0 and F denote the steady-state fluorescence intensities in the absence and presence of quencher (complex **I**), respectively. K_{SV} is the Stern–Volmer quenching constant and [Q] is the concentration of quencher. K_q is the quenching rate constant of the biological macromolecule and K_q is equal to K_{SV}/τ_0, τ_0 is the average lifetime of the molecule without any quencher and the fluorescence lifetime of the biopoly-

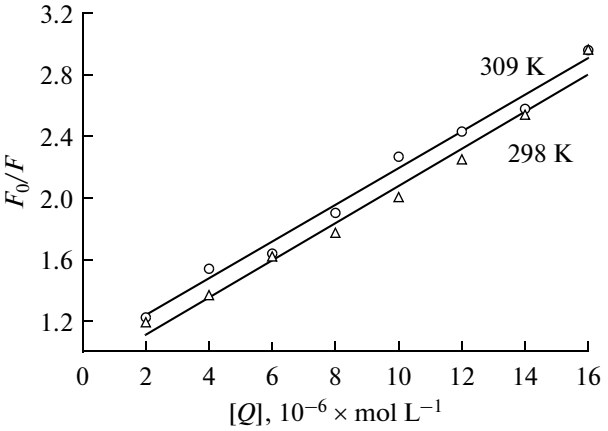

Fig. 4. Stern–Volmer plots of complex **I**–BSA systems.

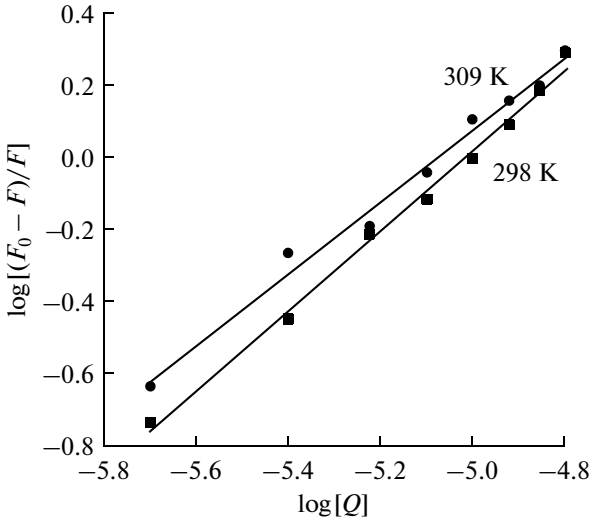

Fig. 5. Logarithmic plots of fluorescence quenching of BSA.

mer is 10^{-8} s [10]. K_{SV} and K_q obtained from fitting the curves in Fig. 4.

T, K	K_{SV}, L mol^{-1}	K_q, L mol^{-1} s^{-1}	R
298	1.2095×10^5	1.2095×10^{13}	0.9893
309	1.1938×10^5	1.1938×10^{13}	0.9929

K_q is far greater than the maximum quenching rate constant (2×10^{10} L mol^{-1} s^{-1}) of the biological macromolecule to BSA, which indicate that the fluorescence quenching of BSA was probably induced by the formation of a **I**–BSA complex rather than by dynamic collision.

The binding constants K_A and binding sites n between complex **I** and BSA were calculated according to the equation:

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_A + n\log[Q],$$

which can be obtained from [11]. Accoding to the data of Fig. 4, the logarithmic plots of fluorescence quenching of BSA was obtained in Fig. 5. K_A and n obtained from fitting the curves in Fig. 5:

T, K	K_A, L mol^{-1}	R	n
298	3.8628×10^5	0.9972	1.1137
309	1.1679×10^5	0.9943	0.9985

These parameters indicated that complex **I** strongly bound BSA with a molar ratio of 1 : 1.

Generally, the interaction forces between small organic molecules and biological macromolecules may include hydrophobic force, hydrogen bond, van der Waals force and electrostatic interactions, etc. [12]. The enthalpy (ΔH) of the reaction of the complex and BSA can be treated as a constant in case there is no remarkable change on temperature and the thermodynamic parameters can be calculated from the Van't Hoff equation:

$$\ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R},$$

where K corresponds to the effective binding constant K_a and R is the gas constant. The value of ΔH and ΔS can be calculated from the slope and intercept of the plot of $\ln K$ versus $1/T$. And the value of ΔG also can be calculated according to the equation:

$$\Delta G = \Delta H - T\Delta S = -RT\ln K.$$

Thermodynamic parameters of binding for BSA and complex **I** are the following:

T, K	ΔH, kJ mol^{-1}	ΔG, kJ mol^{-1}	ΔS, J mol^{-1} K^{-1}
298	−83.25	−31.87	−172.42
309	−83.25	−29.98	−172.42

According to Ross [13], the values of ΔH (−83.25 kJ mol^{-1}) and ΔS (−172.42 J mol^{-1} K^{-1}) indicate that the hydrogen bond and van der Waals force played a major role in the interaction of complex **I** with BSA.

There are different degrees of overlap between the absorption spectra and the fluorescence emission spectra with a moral ratio 1 : 1 (BSA : complex) (Fig. 6). The binding distance between BSA and complex **I** can be calculated based on Föster's non-radioactive energy transfer theory [14], the efficiency of energy transfer (E) between the BSA and complex **I** can be calculated by the equation:

$$E = 1 - F/F_0 = R_0^6/(R_0^6 + r_0^6),$$

where F is fluorescence intensity of BSA, F_0 is the fluorescence intensity in the absence of complex **I**, r_0 is the real distance between donor and acceptor; R_0 is the distance at 50% transfer efficiency (E), also called Föster distance. R_0 can be calculated by the equation:

$$R_0^6 = 8.8 \times 10^{-25} K^2 N^{-4} \Phi J(\lambda),$$

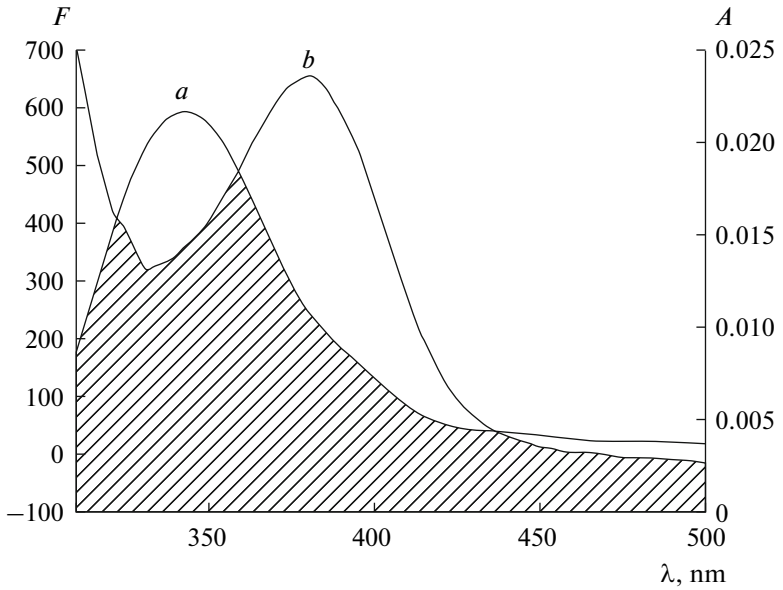

Fig. 6. The overlap of the fluorescence emission spectra (*a*) and the absorption spectra (*b*) when the molar ratio to complexes is 1 : 1.

where the $K^2(2/3)$ is the spatial orientation factor of the dipole; N is the refractive index of medium, usually used the average refractive index (1.336) of water and organic matter; Φ is the fluorescence quantum yield, usually taken quantum yield of tryptophan 0.118 [11]; J is the overlap integral of the fluorescence emission spectra and the absorption spectra. J is calculated by the equation:

$$J(\lambda) = \int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 d\lambda \Big/ \int_0^\infty F(\lambda)d\lambda,$$

where $F(\lambda)$ is the fluorescence intensity of fluorescence donor (BSA), $\varepsilon(\lambda)$ is the molar absorbance coefficient of the complexes. The overlap was divided into the smallest matrix area from these relationships, the overlap J can be calculated. R_0, r and E have been listed below:

Complex	J, cm^3 L mol^{-1}	E	R_0, nm	r, nm
I	1.28×10^{-14}	0.16	2.02	2.49

Non-radiation energy transfer includes the intramolecular and intermolecular energy transfer. If the donor combined with the receptor to form a chemical compound, it was the intramolecular energy transfer. The distance r between the various complex and BSA is less than 7 nm, which indicated that non-radiation energy transfer was occurred between the complex **I** and BSA.

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