

Study on the Interaction of Retinoic Acids to Human Serum Albumin by Fluorescence and Circular Dichroism Spectroscopy

Heyong Huang^{1,2}, Xiansheng Liu¹, Ruiming Han¹ and Guoxiang Wang^{1*}¹Jiangsu Key Laboratory of Environment Change and Ecological Construction, Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, College of Geographical Science, Nanjing Normal University, Nanjing 210023, China²Analysis and Testing Center, Nanjing Normal University, Nanjing 210023, China

Abstract

Retinoic acids (RAs) are considered to be endocrine disruptor chemicals and toxic environmental priority pollutants. In this paper, the interactions between RAs and human serum albumin (HSA) were examined by steady state fluorescence, time-resolved fluorescence and circular dichroism spectroscopy (CD). The RAs quenched the fluorescence of the protein remarkably and the mechanism of quenching was found to be static in nature. Synchronous fluorescence studies suggested that the polarity around the tryptophan(Trp) residues and tyrosine(Tyr) residues was not altered in the presence of RAs. The thermodynamic parameters of the binding reactions (ΔG^\ominus , ΔH^\ominus , ΔS^\ominus) were measured, and they indicated the presences of hydrophobic forces and hydrogen interactions in the RAs–HSA interactions. The alterations of HSA secondary structure in the presence of RAs were confirmed by CD and time resolved fluorescence spectroscopy.

Keywords: Retinoic acids; Human serum albumin; Fluorescence; Circular dichroism

Introduction

Retinoic acids (RAs) are essential for physiological processes such as reproduction, cell proliferation and differentiation, vision, and embryonic development [1]. However, extensive research has proved that RAs are among the most potent known animal teratogens [2]. Exposure of embryos to exogenous RAs causes a spectrum of malformations, including defects of the neural tube and central nervous system; skeleton, palate, ear, and other craniofacial malformations; defects in the heart, thymus, and urogenital system; as well as missing or duplicate limbs and digits in offspring of humans, rodents, chickens, the African clawed frog (*Xenopus laevis*), and fish [3-10]. When frogs were exposed to RAs during metamorphosis, a suite of abnormalities, such as reductions and deletions of the hind limb, bony triangles, and eye deformities, could occur [11-15]. These abnormalities observed in the laboratory are similar to those found in eutrophic habitats [16,17].

Human serum albumin (HSA) is the most abundant protein in plasma, which functions in the maintenance of colloid osmotic blood pressure and in the binding and transportation of various ligands such as fatty acids, hormones, and drugs, then transports them between tissues and organs [18]. It has been shown that the distribution, free concentration, and metabolism of various ligands can be significantly altered as a result of their binding to HAS [19]. Ligand interactions at the protein binding level will in most cases significantly affect the apparent distribution volume of the ligands and also affect the elimination rate of ligands. Therefore, investigating the interactions of RAs and HSA are significant for knowing their transports and distributions in the body and clarifying their action mechanisms. As of yet, however, no work has been reported for the mechanism of these interactions and the detailed physicochemical characterizations of RAs binding to HSA.

In this paper, we present a spectroscopic analysis of the interaction of HSA with four RAs (Figure 1) such as Retinoids (RED), Acitren (ACE), all-trans-Retinal (REA) and 4-keto all-trans-retinoic acid (REO) in aqueous solution at physiological conditions, using constant protein concentration and various RAs compositions. Structural information regarding RAs binding mode and the effects of RAs–HSA complexation on the protein stability and secondary structure are reported here.

Experimental

Materials

HSA (fatty acid content <0.05%), Retinoids, Acitren, all-trans-Retinal, 4-keto all-trans-retinoic acid were purchased from the Sigma Chemical Company and used without further purification. All other reagents were of analytical reagent grade. Ultrapure water was used throughout the experiments. The solution of HSA was prepared in phosphate buffers (1 μ M, pH 7.40).

Apparatus and methods

All the steady-state fluorescence spectra were recorded on a LS-50 B spectrofluorimeter (Perkin Elmer, USA) equipped with 1.0 cm quartz

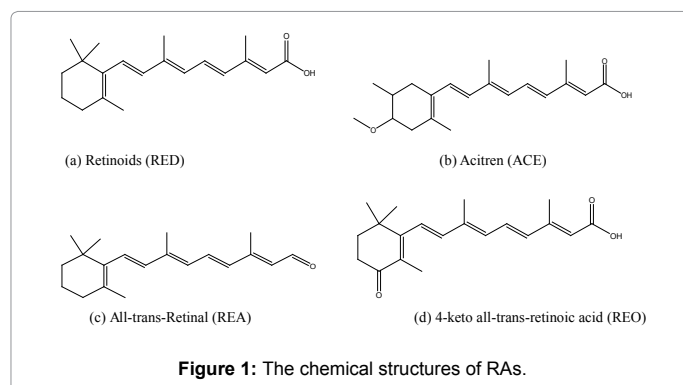


Figure 1: The chemical structures of RAs.

***Corresponding author:** Guoxiang Wang, Jiangsu Key Laboratory of Environment Change and Ecological Construction, Jiangsu Center for Collaborative Innovation in Geographical Information Resource Development and Application, College of Geographical Science, Nanjing Normal University, Nanjing 210023, China, Tel: +8602585898176; E-mail: huangheyong@njnu.edu.cn

Received March 29, 2017; Accepted April 04, 2017; Published April 07, 2017

Citation: Huang H, Liu X, Han R, Wang G (2017) Study on the Interaction of Retinoic Acids to Human Serum Albumin by Fluorescence and Circular Dichroism Spectroscopy. J Environ Anal Chem 4: 192. doi:10.4172/2380-2391.1000192

Copyright: © 2017 Huang H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

cells. The excitation wavelength was 280 nm and the slit widths of emission and excitation were set 5.0-5.0 nm.

Synchronous fluorescence spectra of were measured under the same conditions as the steady-state fluorescence. For synchronous fluorescence, $\Delta\lambda$ ($\lambda_{em}-\lambda_{ex}$) were set at 15 nm and 60 nm, respectively.

The fluorescence lifetime measurements were done in a Horiba Jobin Yvon Fluoro Max-4 time correlation single photon counting (TCSPC) system, using a 265 nm nanosecond diode laser. The time ranges are 0.11 ns/channel, in 4096 effective channels. Data were globally fitted to the appropriate exponential model after deconvolution of the instrument response function by an iterative deconvolution technique, using the IBH DAS6 fluorescence decay analysis software.

CD measurements were taken with an Applied Photophysics Chriascan circular dichroism spectrometer using a 1 cm quartz cell. The wavelength interval is 200-260 nm.

Results and Discussion

Steady-state fluorescence spectra

Fluorescence spectroscopy is widely employed to study proteins and peptides. The intrinsic fluorescence of HSA is very sensitive to its microenvironment. Actually, the intrinsic fluorescence of HSA is almost contributed by tryptophan(Trp) alone, because phenylalanine has a very low quantum yield and the fluorescence of tyrosine(Tyr) is almost totally quenched if it is ionized or near an amino group, a carboxyl group, or a tryptophan [20]. When local surroundings of HSA were altered slightly its intrinsic fluorescence would weaken obviously, such factors as protein conformational transition, biomolecule binding, and denaturation, etc. are responsible for the weakening. Figure 2A-2D showed the fluorescence emission spectra ($\lambda_{ex}=280$ nm) obtained for HSA at pH 7.40 with the addition of different RAs compounds. It can be seen that the fluorescence intensity of HSA decreased in the presence of RAs. The measurements of intensity and position (due to the change of polarity around the fluorophore molecule) are useful to learn the microenvironment of amino acid residues. The fluorescence change indicates that all four RAs compounds can change the structure of HSA.

Fluorescence quenching studies

Fluorescence quenching can be dynamic, resulting from collisional encounters between the fluorophore and the quencher, or static, resulting from the formation of a ground-state complex between the fluorophore (protein) and the quencher. In both cases, molecular contact is required between the fluorophore and the quencher for fluorescence quenching to occur [21]. Dynamic and static quenching can be distinguished by their difference depending on temperature [22]. Higher temperature results in faster diffusion and larger amounts of collisional quenching. This will typically lead to the dissociation of weakly bound complexes. Therefore, the quenching constant increases with the increase in temperature for dynamic quenching. It decreases, however, with increasing temperature for static quenching.

The fluorescence quenching data were analyzed by using the Stern-Volmer equation [23]:

$$F_0/F=1+K_{sv}[Q]=1+K_q\tau_0[Q] \quad (1)$$

where F_0 and F are the fluorescence peak area of HSA in the absence and presence of RAs, respectively. K_{sv} and K_q are the Stern-Volmer quenching constant and the quenching rate constant of HSA, respectively. τ_0 is the average lifetime of HSA without RAs ($\tau_0=10^{-8}$ s) [24].

Figure 3 shows the fluorescence quenching curves of HSA by RAs following the Stern-Volmer plots at various temperatures. For all four RAs, the fluorescence quenching curves have favorable linear relationships, and the slopes of the quenching curves decreased with the increasing of temperature. This indicates that the quenching mechanism between RAs and HSA is the static quenching. The quenching constants K_{sv} and K_q at different temperatures are listed in Table 1. For all four RAs, the K_{sv} values decrease with increasing temperature and the calculated K_q was far greater than the maximum diffusion-controlled quenching rate constant of various quenchers with the biopolymer (10^{12} L.mol⁻¹.s⁻¹). Both results imply that the quenching is not initiated by dynamic collision but formation of compound.

The K_q of RAs-HSA systems at 298 K is Acitren>Retinal>Retinoic acid>Retinoids. We can conclude that the binding force of Acitren in vivo is more stable than Retinal, retinoic acid, Retinoids.

Binding parameters of interaction

When small molecules bind independently to a set of equivalent sites on a macromolecule, the binding constant (K_b) and possible number of binding sites (n) can be determined from the following equations [25]:

$$\log[(F_0-F)/F] = \log K_b + n \log [Q] \quad (2)$$

In above equations, $[Q]$ are the RAs concentration. K_b and n are the binding constant and the number of binding sites, respectively. K_b and n can be obtained from the slope and intercept of the plots of $\log(F_0-F)/F$ versus $\log[Q]$. The binding constants K_b and binding sites n were listed in Table 2. The values of K_b indicate that there is a great binding between RAs and HSA. The number of binding sites n of RAs and HSA at is approximately 1.0, which indicates that there are 1.0 binding sites in HSA for RAs during their interaction.

Generally, the forces acting between a drug and a biomolecule may include hydrogen bonds, van der Waals forces, electrostatic and hydrophobic interactions. Ross and Subramanian have summarized thermodynamic laws to estimate the above forces as follows: If $\Delta H>0$, $\Delta S>0$, the main force is hydrophobic interaction. If $\Delta H<0$, $\Delta S>0$, the main force is electrostatic interactions. If $\Delta H<0$, $\Delta S<0$, the main forces are van der Waals and hydrogen bonds [26]. If the temperature changes little, the reaction enthalpy change is regarded as a constant. In order to get the detail information of interaction force, the reaction enthalpy change and entropy change were calculated according to the following equations [27]:

$$\ln[K_{b2}/K_{b1}] = (1/T_1 - 1/T_2)/(\Delta H^0/R) \quad (3)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln K_b \quad (4)$$

The negative of ΔG indicates that the interaction process of RAs interact with HSA are spontaneous. The positive of ΔH and ΔS indicate that hydrophobic interaction plays major role. Based on the hydrophobicity of the amino acid residues of trypsin and the bulk hydrophobic group benzene ring of RAs, it was tempting to speculate a direct hydrophobic interaction between the aromatic ring of RAs and the hydrophobic amino acid residues. The ΔS could enter into the hydrophobic pocket of trypsin. In addition, the hydrogen bonding between the OH of RAs and some carboxyl of HSA backbone or some $\pi-\pi$ stacking between aromatic ring of RAs and aromatic residues in trypsin cannot be excluded.

Synchronous fluorescence spectra studies

Synchronous fluorescence spectra studies are sensitive techniques to detect the conformation changes. It is typical to study changes of the molecular microenvironment near the fluorophore groups. When $\Delta\lambda$

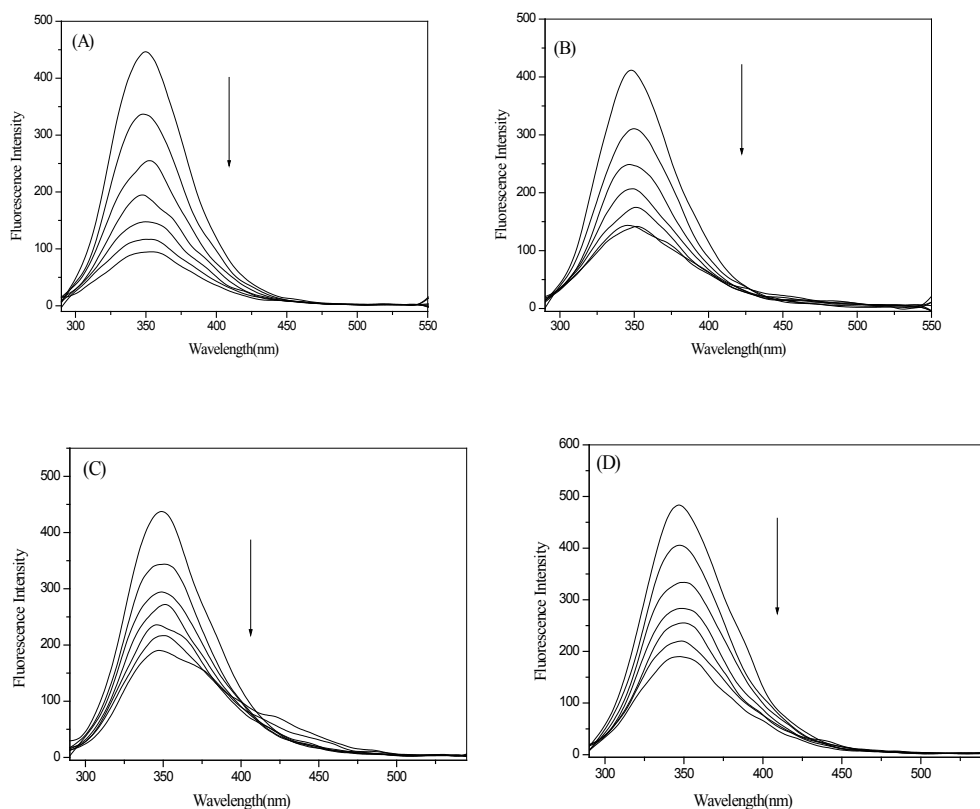


Figure 2: (A) Fluorescence spectra of HSA and RED, [RED]=0, 0.80, 1.60, 2.40, 3.20, 4.00 μM ; (B) fluorescence spectra of HSA and ACE, [ACE]=0, 0.80, 1.60, 2.40, 3.20, 4.00 μM ; (C) Fluorescence spectra of HSA and REA, [REA]=0, 0.50, 1.00, 1.50, 2.00, 2.50 μM ; (D) fluorescence spectra of HSA and REO; [REO]=0, 0.50, 1.00, 1.50, 2.00, 2.50 μM ; [HSA]= 5×10^{-6} μM , T=298 K.

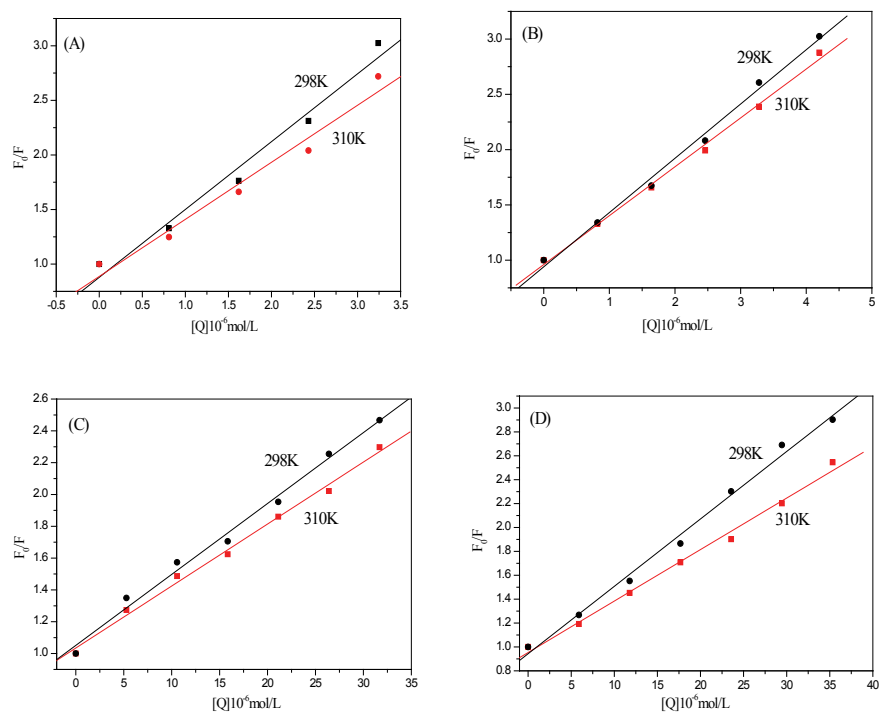


Figure 3: Emission quenching curves of HSA with increasing concentration of RAs: (A) RED; (B) ACE; (C) REA; (D) REO. at different temperatures (298 and 310 K).

Compound	T(K)	K_{sv} (L/mol)	K_q (L/mol)	R
Retinoids	298	2.98×10^4	2.98×10^{12}	0.9975
	310	2.40×10^4	2.40×10^{12}	0.9994
Acitren	298	4.22×10^4	4.22×10^{12}	0.9990
	310	2.45×10^4	2.45×10^{12}	0.9986
Retinal	298	3.95×10^3	3.95×10^{11}	0.9993
	310	3.33×10^3	3.33×10^{11}	0.9980
Retinoic acid	298	3.88×10^3	3.88×10^{11}	0.9977
	310	3.44×10^3	3.44×10^{11}	0.9988

Table 1: Stern-Volmer quenching constants of the interaction of RAs–HSA systems at different temperatures.

Compound	T(K)	K_s (L/mol)	n	R	ΔH (KJ/mol)	ΔG (KJ/mol)	ΔS (J/mol.K)
Retinoids	298	2.74×10^4	1.302	0.9990	44.12	-25.32	233.02
	310	5.46×10^4	1.370	0.9982		-27.02	
Acitren	298	8.64×10^3	1.056	0.9990	49.85	-22.46	242.65
	310	1.88×10^4	1.110	0.9983		-24.38	
Retinal	298	5.21×10^3	0.847	0.9963	26.37	-21.20	159.63
	310	7.86×10^3	0.795	0.9910		-22.22	
retinoic acid	298	1.73×10^4	1.136	0.9987	12.27	-24.18	122.32
	310	2.09×10^4	1.129	0.9984		-24.65	

Table 2: Thermo dynamic parameters of the interaction of chlorophenols–trypsin systems at pH 7.40.

sets at 20 nm and 80 nm, the synchronous fluorescence spectra give the typical characteristics of tyrosine (Tyr) and tryptophan (Trp) residues [28]. Figure 4 shows the fluorescence intensity of both Tyr and Trp decreased regularly with the addition of RED, ACE, REA and DEO. Both of the fluorescence decrease of Trp residues are obvious greater than Tyr residues. It is concluded the binding sites are all near the Trp residues [29]. Absence of shifts implies that the interaction does not alter notably the polarity around these residues.

(B) synchronous fluorescence spectra of HSA and ACE,

[ACE]=0, 0.80, 1.60, 2.40, 3.20, 4.00 μ M;

(C) synchronous Fluorescence spectra of HSA and REA,

[REA]=0, 0.50, 1.00, 1.50, 2.00, 2.50 μ M;

(D) synchronous fluorescence spectra of HSA and REO;

[REO]=0, 0.50, 1.00, 1.50, 2.00, 2.50 μ M;

[HSA]= 5×10^{-6} μ M, T=298 K.

Time-resolved fluorescence spectra

Time-resolved fluorescence measurements can give information about the conformational heterogeneity of proteins. In order to further substantiate the quenching mechanism of the four RAs to HSA, fluorescence lifetime of the tryptophan of HSA were ascertained in the absence and presence of the four RAs. The fluorescence decay of HSA is reported to be triple-exponential in the literature [30]. The average fluorescence decay lifetime could be calculated according to following equations [31,32]:

$$f_i = B_i \tau_i / \sum_{i=1}^3 B_i \tau_i \quad (5)$$

$$\langle \tau \rangle = \sum_{i=1}^3 f_i \tau_i \quad (6)$$

$$I_{\lambda,t} = \sum_{i=1}^3 f_i \exp(-t/\tau_i) \quad (7)$$

In above equations, τ is the lifetimes of the different components to the total decay, $\langle \tau \rangle$ is the average fluorescence decay lifetime, f is a sufficient contribution of each component to total fluorescence intensity and the values of $f_1 + f_2 + f_3 = 1$, B is the relative contributions.

The fluorescence decay of the RAs–HSA system were shown in Figure 5A-5D and Table 3. Experiment data present the free BHb with decay lifetime is 4.76 ns. With addition of RED, ACE, REA and DEO, the average life time changes to 4.85, 4.94, 4.96 and 4.78 ns. The changes display the interaction of HSA with RED, ACE, REA and DEO. The electron transfer between Trp and RED, the same for ACE, REA and DEO, may accompany the lifetime change in the Trp fluorescence decay. It is fairly well possible that the interaction process alters the protein conformation assisting the quenching process, leading to protein denaturation. It is important to note that the observed triple exponential decay reflects the existence of several Trp in the protein, with different decay times, resulting from different environments [33-35].

Circular Dichroism (CD) studies

CD spectroscopy is an optical technique to provide information of the secondary and tertiary structures of protein. Figure 6 shows the CD spectra of RAs–HSA. There are two negative bands at 209 and 222 nm and a positive band at 215 nm of HAS. The 209 nm band corresponds to π - π^* transition and α -helix, the 222 nm band due to π - π^* transition for both the α -helix and random coil, and the 215 nm band corresponds to β -sheet.

Table 4 reveals effects of RAs on the percentage of secondary elements using CDNN program. The degrees of increasing in the percentage of α -helix (RED 26.00%, ACE 25.00%, REA 25.68% and DEO 24.11%, respectively) are observed, the degrees of reduction in the percentage of β -sheet (RED 22.28%, ACE 23.33%, REA 22.96% and DEO 24.38%, respectively) are observed. The results indicate that the binding of RED, ACE, REA and DEO induce some secondary structure changes in HSA and increase of α -helix content but loss of β -sheet content protein. The decrease in β -sheet content suggests that RED, ACE, REA and DEO combine with the amino acid residues of the main polypeptide chain and further cause partial unfolding of HSA.

Conclusions

The interactions between RAs and HSA have been investigated by fluorescence and CD spectroscopy. The linearity of modified Stern–Volmer plots indicated that RAs could bind to one class of sites on HSA.

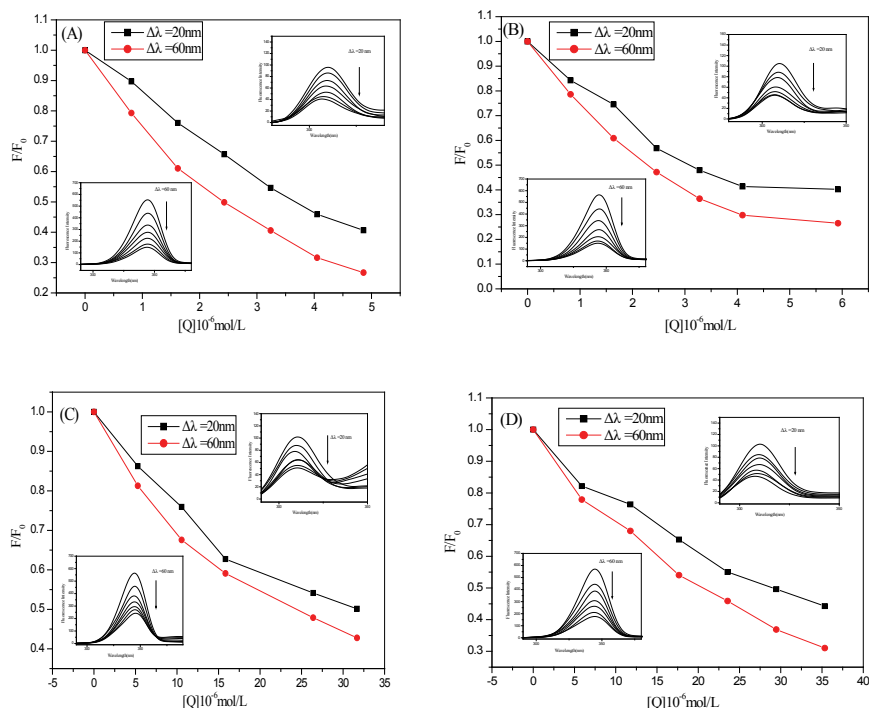


Figure 4: (A) synchronous fluorescence spectra of HSA and RED. $[RED] = 0, 0.80, 1.60, 2.40, 3.20, 4.00 \mu\text{M}$; (B) synchronous fluorescence spectra of HSA and ACE, $[ACE] = 0, 0.80, 1.60, 2.40, 3.20, 4.00 \mu\text{M}$; (C) synchronous Fluorescence spectra of HSA and REA, $[REA] = 0, 0.50, 1.00, 1.50, 2.00, 2.50 \mu\text{M}$; (D) synchronous fluorescence spectra of HSA and REO; $[REO] = 0, 0.50, 1.00, 1.50, 2.00, 2.50 \mu\text{M}$; $[HSA] = 5 \times 10^{-6} \mu\text{M}$, $T = 298 \text{ K}$.

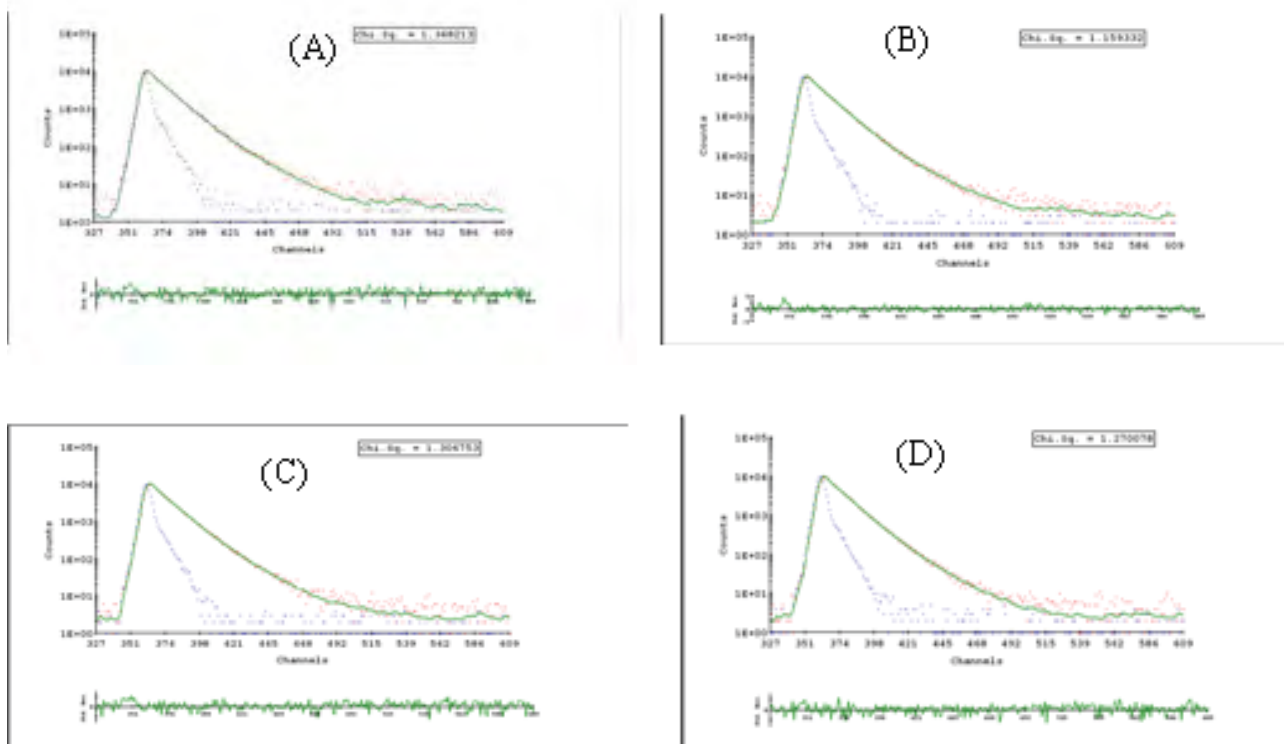


Figure 5: Fluorescence decay profiles of RAs–HSA system in aqueous solution. (A) RED–HSA; (B) ACE–HSA; (C) REA–HSA; (D) REO–HSA.

Sample	C _{RAS} (10 ⁻⁶ mol/L)	f ₁	f ₂	f ₃	τ ₁ (ns)	τ ₂ (ns)	τ ₃ (ns)	<τ> (ns)
HSA	0.00	0.21	0.48	0.31	1.33	4.10	8.12	4.76
HSA+Retinoids	2.00	0.23	0.46	0.32	1.37	4.18	8.15	4.85
HSA+Acitren	2.00	0.24	0.48	0.33	1.40	4.05	8.02	4.94
HSA+Retinal	2.00	0.23	0.51	0.32	1.35	4.04	8.11	4.96
HSA+retinoic acid	2.00	0.26	0.47	0.31	1.38	4.02	8.16	4.78

Table 3: Fluorescence decay fitting parameters for the interaction of the HSA and RAs.

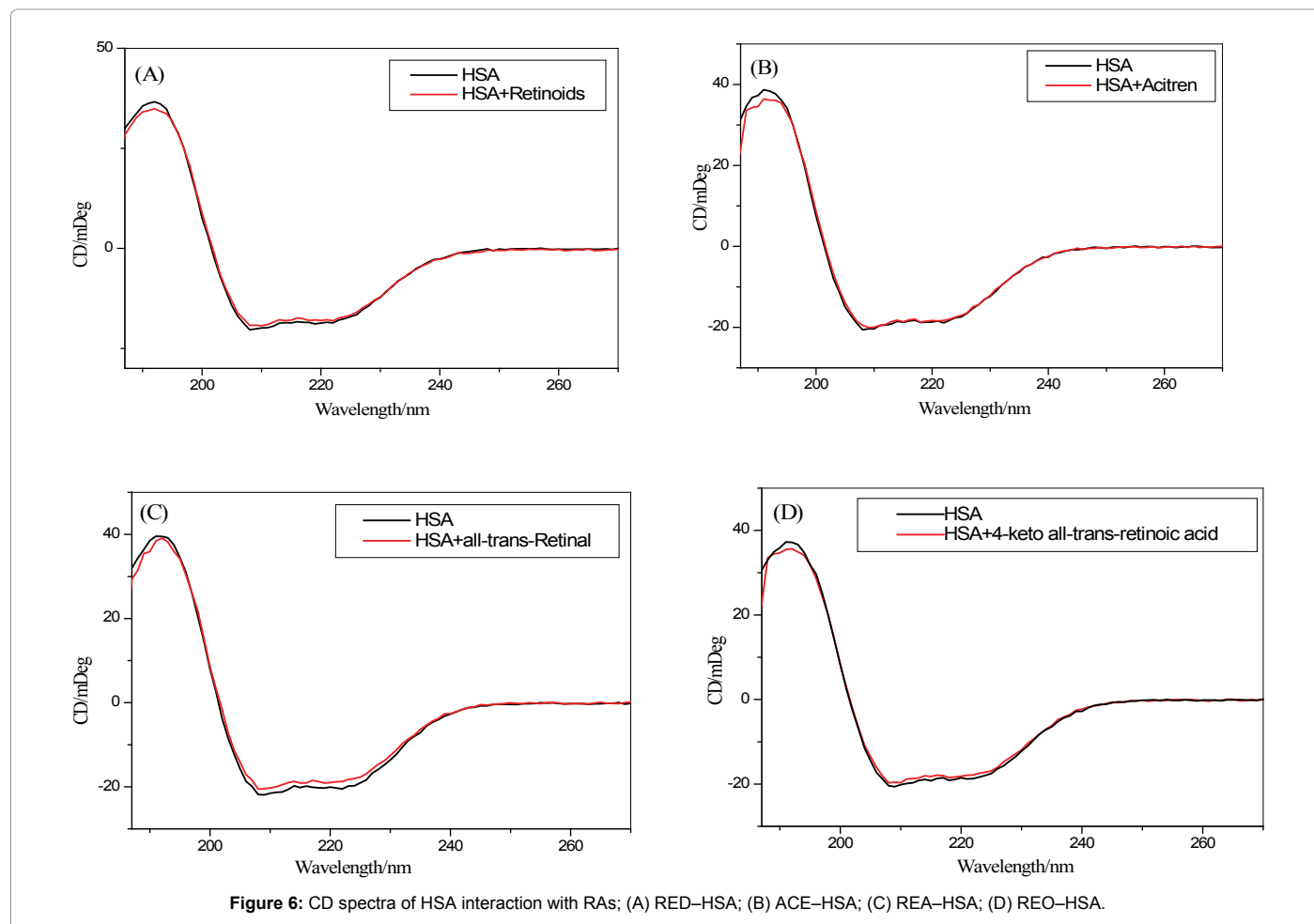


Figure 6: CD spectra of HSA interaction with RAs; (A) RED-HSA; (B) ACE-HSA; (C) REA-HSA; (D) REO-HSA.

Sample	α-Helix%	β-Sheet%	β-Turn%	Random%
HSA	21.86	27.24	16.94	34.05
HSA+Retinoids	26.00	22.28	16.90	34.73
HSA+Acitren	25.00	23.33	16.85	34.72
HSA+Retinal	25.68	22.96	16.95	34.08
HSA+retinoic acid	24.11	24.38	18.87	34.74

Table 4: Secondary structural of HSA interaction with four RAs from CD data using CDNN (program).

The thermodynamic parameters of the binding reactions indicated the presences of hydrophobic forces and hydrogen interactions in the RAs-HSA interactions. Additionally, the results showed that the binding of the three RAs to HSA induced small conformational changes in the overall structure of HSA, which was further proved by the quantitative analysis data of the CD spectra. This will provide important insight into the interactions of the physiologically important protein HSA with globally pervasive contaminants RAs.

Acknowledgements

This work was financially supported by the Natural Science Foundation of China (Grant No. 41173078) and Taihu Lake Water Environment Comprehensive Treatment Projects of Jiangsu Province (Grant No. TH2014402). The authors are grateful to all the people who provided help in our research process.

References

- Gudas LJ, Sporn MB, Robert AB (1994) Cellular biology and biochemistry of the retinoids. Raven Press, New York, USA.

2. Bryant SV, Gardiner DM (1992) Retinoic acid, local cell-cell interactions, and pattern formation in vertebrate limbs. *Dev Biol* 152: 1-25.
3. Edward J, Lammer MD, Diane T, Chen MD, Richard M (1985) Retinoic acid embryopathy. *J Med* 313: 837-841.
4. Rutledge JC, Shourbaji AG, Hughes LA, Polifka JE, Cruz YP (1994) Limb and lower-body duplications induced by retinoic acid in mice. *Proc Natl Acad Sci* 91: 5436-5440.
5. Kessel M, Gruss P (1991) Homeotic transformations of murine vertebrae and concomitant alteration of Hox codes induced by retinoic acid. *Cell* 67: 89-104.
6. Niederreither K, Ward SJ, Chambon P (1996) Morphological and molecular characterization of retinoic acid-induced limb duplications in mice. *Dev Biol* 176: 185-198.
7. Thaller C, Eichele G (1987) Identification and spatial distribution of retinoids in the developing chick limb bud. *Nature* 327: 625-628.
8. Durston AJ, Timmermans JPM, Hage WJ, Hendriks HFJ, Vries NJD, et al. (1989) Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* 340: 140-144.
9. Sive HL, Draper BW, Harland RM, Weintraub H (1990) Identification of a retinoic acid-sensitive period during primary axis formation in *Xenopus laevis*. *Genes Dev* 4: 932-942.
10. Herrmann K (1995) Teratogenic effects of retinoic acid and related substances on the early development of the zebrafish (*Brachydanio rerio*) as assessed by a novel scoring system. *Toxicol In Vitro* 9: 267-283.
11. Scadding SR, Maden M (1986) Comparison of the effects of vitamin A on limb development and regeneration in *Xenopus laevis* tadpoles. *J Embryol Exp Morphol* 91: 35-53.
12. Scadding SR, Maden M (1986) The effects of local application of retinoic acid on limb development and regeneration in tadpoles of *Xenopus laevis*. *J Embryol Exp Morphol* 91: 55-63.
13. Maden M (1993) The homeotic transformation of tails into limbs in *Rana temporaria* by retinoids. *Dev Biol* 159: 379-391.
14. Degitz SJ, Kosian PA, Makynen EA, Jensen KM, Ankley GT (2000) Stage- and Species-Specific Developmental Toxicity of All-Trans Retinoic Acid in Four Native North American Ranids and *Xenopus laevis*. *Toxicol Sci* 57: 264-274.
15. Alsop DH, Brown SB (2004) Dietary Retinoic Acid Induces Hindlimb and Eye Deformities in *Xenopus laevis*. *Environ Sci Technol* 38: 6290-6299.
16. Gardiner DM, Hoppe DM (1999) Environmentally induced limb malformations in mink frogs (*Rana septentrionalis*). *J Exp Zool* 284: 207-216.
17. Gardiner D, Ndayibagira A, Grun F, Blumberg B (2003) Deformed frogs and environmental retinoids. *Pure Appl Chem* 75: 2263-2273.
18. Zolse G, Falcioni G, Bertoli E, Galeazzi R, Wozniak M, et al. (2000) Steady-state and time resolved fluorescence of albumins interacting with N-oleylethanolamine, a component of the endogenous N-acylethanolamines. *Proteins: Struct Funct Genet* 40: 39-48.
19. Soares S, Mateus N, Freitas VD (2007) Interaction of Different Polyphenols with Bovine Serum Albumin (BSA) and Human Salivary α -Amylase (HSA) by Fluorescence Quenching. *J Agric Food Chem* 55: 6726-6735.
20. Chen GZ, Huang XZ, Xu JG, Zheng ZZ, Wang ZB (1990) Fluorescence study on the interaction of human serum albumin with loureirin B. *Science Press* 24: 547-557.
21. Gentili PL, Qrtica F, Favaro G (2008) *J Phys Chem B* 112: 16793-16801.
22. Zhang YZ, Zhou B, Zhang XP, Huang P, Li CH, et al. (2009) 163: 1345-1352.
23. Teng Y, Ji FY, Li C, Yu ZH, Liu RT (2011) *J Lumin* 131: 2661-2667.
24. Gonzalez-Jimenez J, Cortijo M (2002) Urea-Induced Denaturation of Human Serum Albumin Labeled with Acrylodan. *J Protein Chem* 21: 75-79.
25. Zhou JH, Wu XH, Yang C, Gu XT, Zhou L (2009) *Spectrochimica Acta Part A-molecular and Biomolecular Spectroscopy* 72: 151-155.
26. Wu D, Xu G, Sun Y, Zhang H, Mao H (2007) *Biomacromolecules* 8: 708-712.
27. Szabo AG, Krajcarski D, Zuker M (1984) Conformational heterogeneity in hemoglobin as determined by picosecond fluorescence decay measurements of the tryptophan residues. *Chem Phys Lett* 108: 145-149.
28. Sentchouk VV, Bodaryuk EV (2007) *J Appl Spectrosc* 74: 731-747.
29. Mahato M, Pai P, Kamilya T, Talapatra GB (2010) *J Phys Chem B* 114: 7062-7070.
30. Lakowicz R (2006) *Novel Fluorophores*. Springer Science & Business Media. New York, USA, pp: 675-703.
31. Paramaguru G, Kathiravan A, Selvaraj S, Venuvanaligam P, Renganathan R (2010) *J Hazard Mater* 175: 985-991.
32. Wu PG, Rice KG, Brand L, Lee YC (1991) *Proc Natl Acad Sci* 88: 9355-9359.
33. Li R, Nagai Y, Nagai M (2000) Changes of tyrosine and tryptophan residues in human hemoglobin by oxygen binding: near- and far-UV circular dichroism of isolated chains and recombined hemoglobin. *J Inorg Biochem* 82: 93-101.
34. Sareh S, Jamshidkhan C (2010) *Int J Biol Macromol* 47: 558-569.
35. Bolanos-Garcia VM, Ramos S, Castillo R, Mas-Oliva J (2001) *J Phys Chem B* 105: 5757-5765.

Citation: Huang H, Liu X, Han R, Wang G (2017) Study on the Interaction of Retinoic Acids to Human Serum Albumin by Fluorescence and Circular Dichroism Spectroscopy. *J Environ Anal Chem* 4: 192. doi:[10.4172/2380-2391.1000192](https://doi.org/10.4172/2380-2391.1000192)

OMICS International: Open Access Publication Benefits & Features

Unique features:

- Increased global visibility of articles through worldwide distribution and indexing
- Showcasing recent research output in a timely and updated manner
- Special issues on the current trends of scientific research

Special features:

- 700+ Open Access Journals
- 50,000+ editorial team
- Rapid review process
- Quality and quick editorial, review and publication processing
- Indexing at major indexing services
- Sharing Option: Social Networking Enabled
- Authors, Reviewers and Editors rewarded with online Scientific Credits
- Better discount for your subsequent articles

Submit your manuscript at: <http://www.omicsonline.org/submission>