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Analysis of Binding Interaction between *N,N*-Bis(Phosphinomethyl)Amines as a New Class of 1-Aminophosphinic Acids and Bovine Serum Albumin Using Fluorescence Spectroscopy

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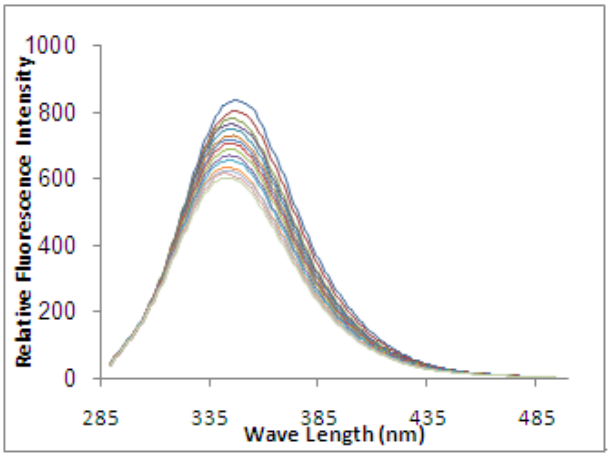
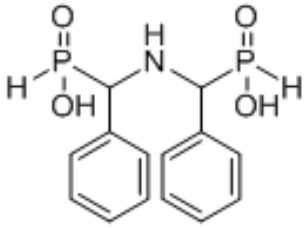
Abstract C_2-symmetric *N,N*-bis(phosphinomethyl) amines have been synthesized and their interaction with bovine serum albumin (BSA) was investigated using fluorescence quenching technique. The fluorescence quenching of BSA during its binding to C_2-symmetric *N,N*-bis(phosphinomethyl)amines molecules indicated the occurrence of energy transfer between ligand and protein. The experimental results showed that the formation of aminophosphinic acid-BSA complex and non-radiative energy transferring result in the fluorescence quenching. The binding parameters including binding constant K_A and the corresponding thermodynamic parameters were calculated at different temperatures. The thermodynamic investigation showed that the binding process of the C_2-symmetric *N,N*-bis(phosphinomethyl)amines molecules to BSA was a spontaneous molecular interaction procedure in which Gibbs free energy decreased and entropy increased. The hydrophobic interaction force plays a major role in stabilizing of the C_2-symmetric *N,N*-bis(phosphinomethyl)amine-BSA complex. The synchronous fluorescence spectroscopy was used to study the effect of the C_2-symmetric *N,N*-bis(phosphinomethyl)amine on the conformation of BSA. The results obtained from synchronous fluorescence spectra showed that the C_2-symmetric *N,N*-bis(phosphinomethyl)amines did not cause considerable conformational changes in BSA.

Keywords C_2-Symmetric *N,N*-Bis(Phosphinomethyl)Amine, Bovine Serum Albumin (BSA), Fluorescence Spectroscopy, Thermodynamic Parameters

Graphical Abstract



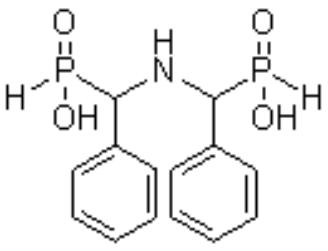


The binding interactions of the water-soluble aminoalkylphosphinic acids APA **1-4** to bovine serum albumin (BSA) shows that the interaction process was spontaneous and interaction forces were found to be hydrophobic .

1. Introduction

The binding of a drug with protein greatly influences the metabolism, distribution, absorption, and excretion properties of drugs [1]. Serum albumin (SA), one of the most important blood plasma proteins, has been extensively studied in the interaction of a drug with proteins that shows an extra ordinary ability to reversibily bind a wide range of endogenous and exogenous compounds that regulate plasma concentrations [2]. Recently, attention has been focused on the interaction between SA and biologically active molecules by their relationship studies between the structure of these compounds and their affinities toward serum albumin [3-8].

Aminophosphinic acids have attracted considerable attention because of their considerable biological activity. [9] 1-Aminophosphinic acids are phosphorus analogues of natural amino acids and are expected to show strong coordination abilitywith metals. It has been indicated that 1-aminophosphinic acid derivatives are selective inhibitors of various proteolytic enzymes, particularly metalloproteases.[10-15] It has been well known that the amino acids are the main elements of proteins and they generally play an important physiological role in life process. However, for a long times many researches were only focused on the biological activities of aminophosphinic acids and did not consider their targeting of biological tissues [16-18]. The structure of the phosphinic functional group mimicsthetransition state of peptide hydrolysis and the symmetric nature of the phosphinic acid derivatives are expected to benefit in their binding to the homodimer of HIV-protease having C_2-axis symmetry [19]. During past decade, our laboratories have reported the novel methods for the synthesis of 1-amino-*H*-phosphinic acids [20-24]. Recently we reported the synthesis and complexation properties of *N,N*-bis(phosphinomethyl)amines as a novel 1-amino-*H*-phosphinic acid containing two phosphinic moieties with C_2-symmetry axis [25-27]. As an extension of our previous studies on the ineraction of SA with phosphinic acids [28],we have now reported the interaction of novel 1-amino-*H*-phosphinic acid containing two phosphinic moieties with C_2-symmetry axis with BSA using fluorescence spectroscopic technique (Scheme 1). The BSA was selected as an appropriate model protein because of its low cost, readily availability, and unusual ligand-binding properties.



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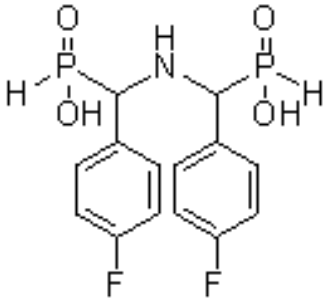

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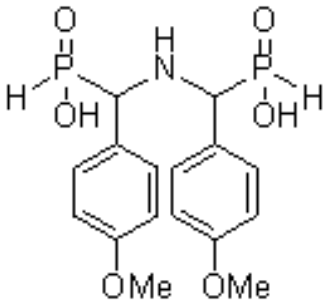
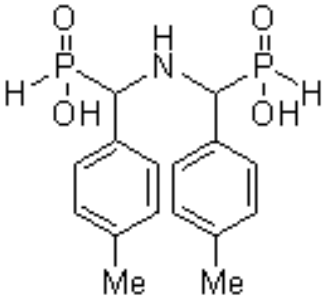


APA-3



APA-4

Scheme 1. Structures of APA **1-4**

2. Materials and Methods

2.1. Materials and Apparatus

All chemicals were commercial products and distilled or recrystallized before use. Bovine serum albumin was purchased from Sigma-Aldrich and used without further purification. The solutions of BSA were prepared in 0.05 M sodium phosphate (mixture of Na_2HPO_4 and NaH_2PO_4) buffer pH 6.4 containing 0.005 M NaCl. The BSA solution was prepared based on its molecular weight of 65000. The exact concentration of BSA was determined spectrophotometrically using molecular absorption coefficient of $\varepsilon_{280\ nm}$ = 43800 $M^{-1}cm^{-1}$ [29]. A 0.5 mM solution of the amino-*H*-phosphinic acid was prepared in 0.05 M sodium phosphate buffer pH 6.4 containing 0.005 M NaCl. The NMR spectra were taken with a 250 and 400 BruckerAvance instrument with the chemical shifts being reported as δ ppm and couplings expressed in Hertz. Merck Silica-gel 60 F254 plates (No. 5744) were used for the preparative TLC. All fluoresence measurements were carried out on a Cary Eclipse recording spectrofluorimeter (VARIAN) equipped with 1.0 cm quartz cells and the thermostat bath, the widths of both the excitation and the emission slits were set at 5.0 nm with a nominal resolution of 0.5 nm. Appropriate blanks corresponding to the buffer were subtracted to correct background of fluorescence. A UV-Vis Ultraspec 4000 recording spectrophotometer (Phramacia Biotech) was used for scanning the UV spectrum equipped with 1.0 cm quartz cells and a slit width of 5 nm with a nominal resolution of 0.5 nm.

2.1.1 General procedure for the preparation of 1-aminophosphinic acids (APA 1-4)):

The compounds were obtained according to the method of our previously published article [25-27]. The aldehyde (3 mmol) was added to ammonium hydroxide (30%, 15 mL) and the solution was stirred for 5 h at reflux. During this time, a white precipitate formed. The precipitate was removed by filtration and was dried. The solid was dissolved in 5 mL of ethanol and hypophosphorus acid (5 mmol-anhydrous) was added to this mixture and the resulting solution was stirred for 2-12 h at reflux. The solvent was evaporated and the mixture resolved in acetone by heating. Dropwise addition of water gave the crude product as a white solid. The crude product was washed with ethanol and dried in air at room temperature to give product **APA 1-4**.

2.1.1.1 {[(Hydroxyphosphinoyl-phenyl-methyl-(-amino]-phenyl-methyl}-phosphinicacid (R*,R*-APA-1):

White solid; mp: 222–224°C; ^{1}H NMR (D_2O-250 MHz): 3.79 (2H, d, J = 13.5 Hz, –CHP), 6.83, (2H, d, J_{HP} = 552 Hz), 6.85–7.40 (10H, m); ^{13}C NMR (D_2O-NaOD-62.9 MHz): 61 (dd, J_{CP} = 98.5 and 14.1 Hz), 127.0, 127.5, 127.7, 128.5, 128.7, 128.9, 134.9, 136.5; ^{31}P NMR (D_2O/H_3PO_4-101.2 MHz):17.89 ppm; IR (KBr): 3650–2120 (–OH), 1250 (P=O), 1055–710 (P–O) cm^{-1}; Anal. Calcd for $C_{14}H_{17}NO_4P_2$: C, 51.68; H, 5.27; N, 4.31. Found: C, 51.55; H, 5.20; N, 4.56; Calcd. Mass: 326.0711; Found: 326.0742.

2.1.1.2. ((4-Fluoro-phenyl)-{[(4-fluoro-phenyl)-hydroxyphosphinoyl-methyl]-amino}- methyl)-phosphinic acid (R*,R*-APA-2):

White solid; mp: 230–232°C; ^{1}H NMR (D_2O-250 MHz): 3.98 (2H, d, J = 13.5 Hz, –CHP), 6.99 (2H, d, J_{HP} = 552 Hz), 7.01–7.15 (m, 8H); ^{13}C NMR (D_2O-NaOD-62.9 MHz): 60.1 (dd, J_{CP} = 98.5 and 14.1 Hz), 115.4 (d, J_{CP}=21.4 Hz), 130.1–130.5 (m, Ar), 162.1 (d, J_{CP} = 243.2 Hz); ^{31}PNMR (D_2O/H_3PO_4-101.2 MHz): 17.22 ppm; IR (KBr): 3650–2120 (-OH), 1240 (P=O), 1180–580 (P–O) cm^{-1}; Anal. Calcd for $C_{14}H_{15}F_2NO_4P_2$: C, 46.53; H, 4.19; N, 3.88. Found: C, 46.56; H, 4.12; N, 3.72; Calcd. Mass: 384.0342; Found: 384.0327.

2.1.1.3. [{[hydroxyphosphinoyl-(4-Mthoxy-phenyl)-methyl]--amino}-(4-Mthoxy-phenyl)methyl]-phosphinic acid (R*,R*-APA-3):

White solid; mp: 212–214°C; ^{1}H NMR (D_2O-250 MHz): 3.63 (s, 6H), 3.73 (2H, d, J = 14.5 Hz, –CHP), 6.86 (2H, d, J_{HP} = 550 Hz), 6.82 (4H, d, J = 8.0 Hz), 6.90 (4H, d, J = 8.0 Hz); ^{13}C NMR (D_2O–NaOD-62.9 MHz): 55.3, 59.2–61.5 (m, CHP), 114.8, 120.4, 130.1 (d, J_{CP} = 5.5 Hz), 159.7; ^{31}P NMR (D_2O/H_3PO_4-101.2 MHz): 17.81 ppm; IR (KBr): 3650–2320 (-OH), 1248 (P=O), 1150–650 (P–O) cm^{-1}. Anal. Calcd for $C_{16}H_{21}NO_6P_2$: C, 49.86; H, 5.50; N, 3.64. Found: C, 49.71; H, 5.43; N, 3.52; Calcd. Mass: 386.1004; Found: 386.0826.

2.1.1.4. {[(Hydroxyphosphinoyl-p-tolyl-methyl)-amino]-p-tolyl-methyl}-phosphinic acid (R*,R*-APA-4):

White solid; mp: 228–230°C;^{1}H NMR (D_2O-250 MHz): 2.18 (s, 6H), 3.33 (2H, d, J = 14.0 Hz, –CHP), 6.74 (2H, d, J_{HP} = 551 Hz), 6.75–7.17 (8H, m); ^{13}C NMR (D_2O–NaOD-62.9 MHz): 20.1, 60.53 (dd, J_{CP}=98.7 , 14.2 Hz), 128.4 (d, J_{CP} = 5.7 Hz), 128.8 (d, J_{CP} = 5.7 Hz), 129.0, 129.2, 131.6, 133.3, 137.6 (d, J_{CP} = 3.1 Hz), 137.9 (d, J_{CP} = 3.1 Hz); ^{31}P NMR (D_2O/H_3PO_4-101.2 MHz): 18.24 ppm; IR (KBr): 3650–2220 (-OH), 1251 (P=O), 1185–610 (P–O) cm^{-1}. Anal. Calcd for $C_{16}H_{21}NO_4P_2$: C, 54.38; H, 5.99; N, 3.96. Found: C, 54.30; H, 5.85; N, 4.05; Calcd. Mass: 354.1024; Found: 354.1026.

2.2. Procedures

Fluorescence spectra recorded from 285 to 500 nm with the excitation wavelength at 280 nm. Both excitation and emission slit widths were 5 nm. First 600 μL of solution containing constant concentration of BSA (4 μM) was titrated by the successive addition of APA **1-4** solution (concentration was varied from 0 to 32.71 μM). Titration was done manually via a micro syringe and the fluorescence intensity of BSA measured in the absence and the presence

of the APA **1-4**. All experiments were measured at three temperatures.

3. Results and Discussion

The fluorescence measurements can provide valuable information about the molecular environment in the vicinity of the fluorophore groups. In Fig.1, the effect of amino-*H*-phosphinic acids (APA **1-4**) on the BSA fluorescence spectra is shown. As it can be seen from Fig.1, the fluorescence intensity of BSA was gradually decreased upon increasing the concentration of aminophosphinic acids, indicating the binding of APA **1-4** in the vicinity of the tryptophan residues of BSA. On the other hand, the maximum emission wavelength of BSA shifted slightly after addition of APA **1-4**. This slightly spectral shift showes that the binding site of APA **1-4** on BSA was very close to the tryptophan residues.

The fluorescence quenching of a compound can be caused by a variety of molecular interactions such as molecular rearrangements and collisional encounters [30]. The APA **1-4** molecules shows no fluorescence emission in the range of 300-500 nm, thus the contribution of the APA **1-4** could be neglected when measuring the protein fluorescence emission at the different APA **1-4** concentrations. Such decrease in the emission intensity is called fluorescence quenching. Fluorescence quenching is described by the Stern–Volmer equation (Eq. (1)).

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

Fluorescence quenching mechanism includes dynamic and static quenching. Dynamic quenching originates from the production of the collision of molecule in excited states and quencher and a nonfluorescent complex between fluorophore in the ground states and quencher leads to static quenching.

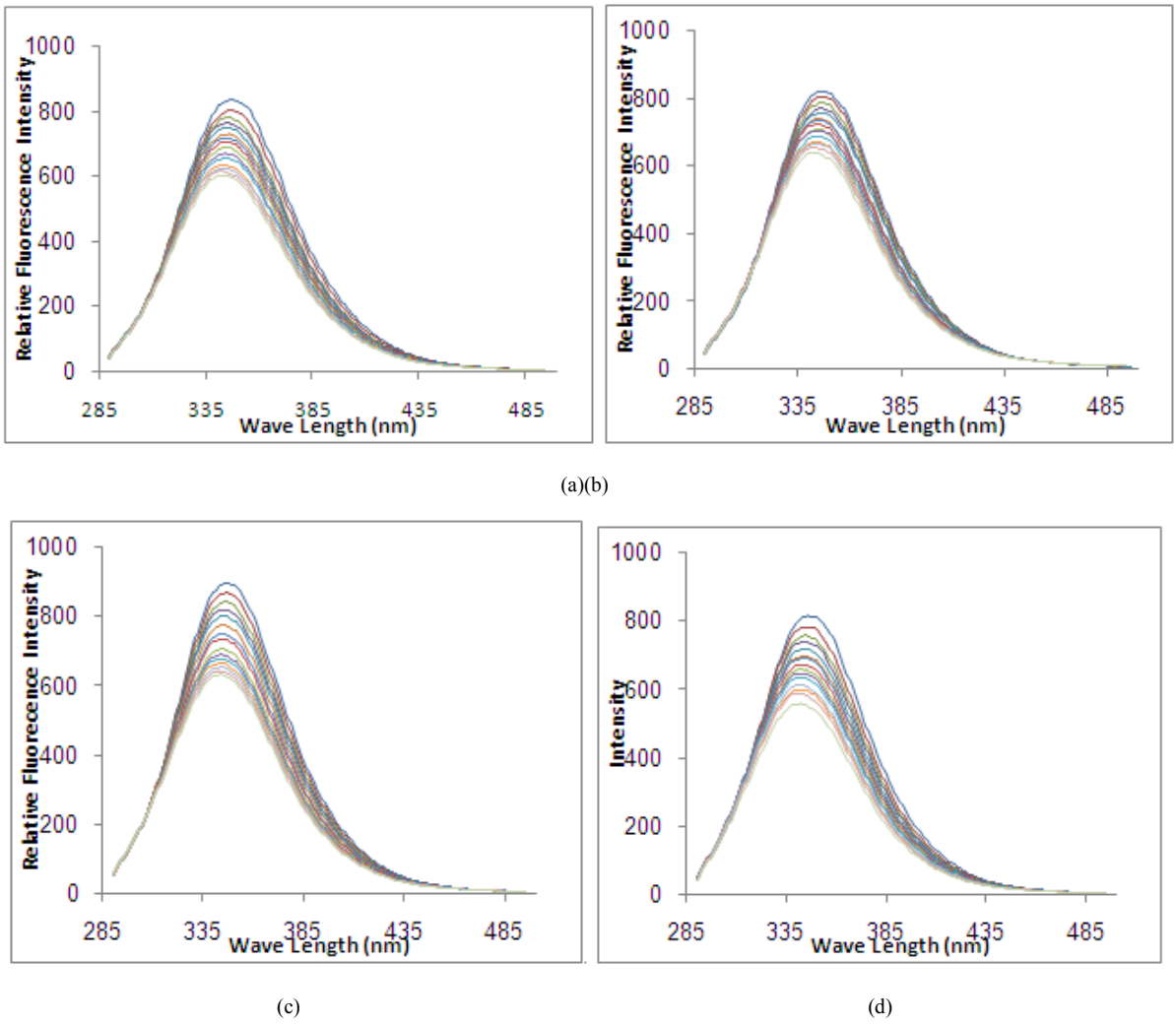


Figure 1. BSA fluorescence spectrum at the excitation wavelength of 280 nm, T= 298 K, C_{BSA} = 4.0 μM, $C_{APA\ 1\text{-}4}$ = 0-32.71 μM. a) APA-**1**, b) APA-**2**, c) APA-**3**, d) APA-**4**. The fluorescence intensity was decreased by increasing the concentration of ligands.

In Fig. 2, the curves of F_0/F versus [Q] were plotted according to Eq. (1). The results show a good linearity when the concentrations of APA **1-4** compounds ranged from 0.0 to 32.7 μM at three different temperatures. The quenching constant and quenching rate constant were calculated and reported in Table 1 considering the fluorescence lifetime of the biopolymer was 10^{-8} s [31]. Since the bimolecular quenching rate constants (k_q) are larger than the limiting diffusion rate constant of the biopolymer (2.0×10^{10} $M^{-1}s^{-1}$) [32] and the static quenching constants decreased with the increasing of temperature, therefore, the static quenching could be the main mechanism of the fluorescence quenching of BSA by APA **2** and APA **3** compounds. On the other hand, in the case of APA **1** and APA **4** the dynamic quenching is the main mechanism of the fluorescence quenching.

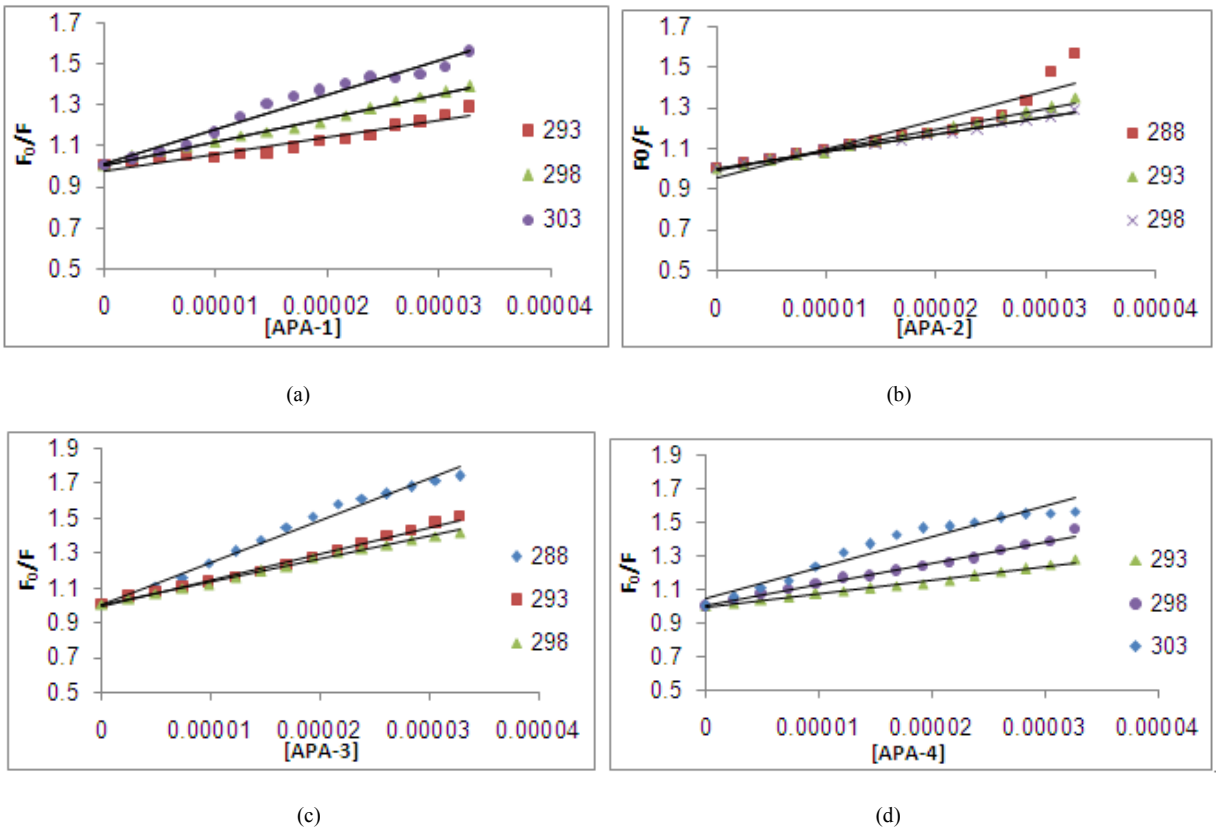


Figure 2. Stern-Volmer plots for obtainingStern-Volmer quenching constant and bimolecular quenching constant at different temperatures for , a) APA-**1**, b) APA-**2**, c) APA-**3**, d) APA-**4**

Table 1. Stern-Volmer quenching constant and bimolecular quenching constant at different temperatures

Compd	T (° K)	Stern-Volmer equation	K_{sv} ($\times10^4$ M^{-1})	K_q ($\times10^{12}$ M^{-1})	R^2
APA-**1**	293	$F_0/F = 5179[Q] + 0.9766$	0.82	0.82	0.9331
	298	$F_0/F = 15620[Q] + 1.0043$	1.56	1.56	0.9955
	303	$F_0/F = 16870[Q] + 1.1008$	1.69	1.69	0.9701
APA-**2**	288	$F_0/F = 14290[Q] + 0.9504$	1.43	1.43	0.8508
	293	$F_0/F = 10290[Q] + 0.9879$	1.03	1.03	0.9919
	298	$F_0/F = 8369[Q] + 1.0009$	0.84	0.84	0.9947
APA-**3**	288	$F_0/F = 24130[Q] + 1.0063$	2.41	2.41	0.9867
	293	$F_0/F = 15380[Q] + 0.9872$	1.54	1.54	0.9911
	298	$F_0/F = 13190[Q] + 0.9998$	1.32	9.48	0.9959
APA-**4**	293	$F_0/F = 8873[Q] + 0.9887$	0.85	0.85	0.9910
	298	$F_0/F = 12690[Q] + 1.0035$	1.27	1.27	0.9876
	303	$F_0/F = 18120[Q] + 1.0506$	1.81	1.81	0.9422

For the static quenching, the association constant (K_A), and the number of binding sites (n) can be obtained from the regression curve based on the following equation (Eq. (2)).

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

By applying a linear plot of log $(F_0-F)/F$ versus log[Q] using least squares analysis, the number of binding sites n and the binding constant K_A can be obtained. The binding parameters are shown in Table 2. The results show that the interaction between the APA **1-4** compounds and BSA is in accordance with the site-binding model. Because the values of n were close to 1, it showed strong binding forces. It also indicated that there was only one binding site for APA **1-4** compounds on BSA. Moreover, comparing of the n values of APA compounds showed a strong binding force in APA **1-4**. When the value of K_A decreased with the increase in temperature for APA-**2** and APA-**4**, it implied that the BSA-APA-**2,4** complexes were unstable.

The interaction of small molecules and macromolecules involves different natures such as electrostatic interactions, hydrogen bonds, hydrophobic forces, and Van der Waals interactions. In order to recognize the interaction of APA **1-4** compounds with BSA, the thermodynamic parameters including Gibbs free energy change (ΔG), enthalpy change (ΔH), and entropy change (ΔS) were calculated by the Van't Hoff equation (Eq. (3)) [33].

$$LnK = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \text{ and } \quad \Delta G = \Delta H - T\,\Delta S \quad (3)$$

According to the Van't Hoff equation, ΔH and ΔS can be obtained from the slope and intercept of the plot of ln K versus $1/T$, respectively. The results are shown in Table 3. The sign and magnitude of the thermodynamic parameters can represent different kinds of interaction that may take place in protein association process [34]. The negative sign for ΔGmeans that the interaction process is spontaneous for the APA **1-4**.The main binding force between APA **1** and APA **3** and BSA was a hydrophobic force due to both ΔH and ΔS >0. The negative ΔH and ΔS value for APA-**2** and APA-**4**indicates thathydrogen bond played major role (maybe due to the presence of fluoro and methoxy group at para position of aromatic ring) in the APA-BSA binding interaction and contributed to the stability of the complex [35].

Table 2. Binding constants between BSA and the compounds APA **1-4** examined at different temperatures

Compd	T (° K)	Double logarithm equation	K_a (1×10^4 mol^{-1})	n	R^2
APA-**1**	288	Ln $(F_0-F)/F$= 0.9582 Ln[Q] + 8.2457	0.38	0.9582	0.9893
	293	Ln $(F_0-F)/F$= 1.0234 Ln [Q] + 9.0144	0.82	1.0234	0.9110
	303	Ln $(F_0-F)/F$= 1.1002 Ln [Q] + 10.8490	5.15	1.1002	0.9793
APA-**2**	293	Ln $(F_0-F)/F$= 1.1080Ln [Q] + 10.3260	3.05	1.1080	0.9972
	298	Ln $(F_0-F)/F$= 0.9697 Ln [Q] + 8.7110	0.61	0.9697	0.9961
	303	Ln $(F_0-F)/F$= 0.8287 Ln [Q] + 7.6361	0.21	0.8287	0.9819
APA-**3**	293	Ln $(F_0-F)/F$= 0.9488 Ln [Q] + 9.0190	0.83	0.9488	0.9794
	298	Ln $(F_0-F)/F$= 1.0104 Ln [Q] + 9.5966	1.47	1.0104	0.9971
	303	Ln $(F_0-F)/F$= 1.1764 Ln [Q] + 11.8900	14.60	1.1764	0.9515
APA-**4**	288	Ln $(F_0-F)/F$= 1.2662 Ln [Q] + 12.1700	19.3	1.2662	0.9869
	293	Ln $(F_0-F)/F$= 0.8698 Ln [Q] + 9.0855	0.88	1.0136	0.9948
	298	Ln $(F_0-F)/F$= 0.8992 Ln [Q] + 8.3698	0.43	0.8992	0.9931

Table 3. Thermodynamic parameters of the binding process BSA with APA **1-4**.

Compd	T (° K)	ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})	ΔG (KJ mol^{-1})
APA-**1**	288	127.16	509.63	-19.61
	293			-22.16
	303			-27.26
APA-**2**	283	-192.14	-581.88	-21.65
	298			-18.74
	303			-15.83
APA-**3**	283	211.18	793.50	-21.31
	298			-25.28
	303			-29.25
APA-**4**	288	-272.67	-848.69	-26.23
	293			-26.50
	298			-26.47

BSA has two tryptophan residues (Trp134 and Trp213); Trp134 is mainly exposed to the hydrophilic environment and Trp213 is located deep in the hydrophobic matrix of protein. According to the values of the thermodynamic parameters, the APA-**1** and APA-**3** compounds tended to bind in the vicinity of Trp213, however, for APA-2 and APA-4 the contribution of Trp134 in the binding process could not be completely excluded.

The fluorescence quenching of BSA during its binding to APA **1-4** molecules indicated the occurrence of energy transfer between ligand and protein. Fluorescence resonance energy transfer (FRET) is a non radiative interaction between the different electronic excited states of molecules. Generally, FRET occurs whenever the emission spectrum of a fluorophore (donor) overlaps with the absorption spectrum of another molecule (acceptor). The main factors affecting on the extent of energy transfer are the distance and orientation of transition dipole moment between the donor and acceptor and the extent of spectral overlap between the donor emission and the acceptor absorption [7]. FRET is an important technique for investigating a variety of biological phenomena including energy transfer process [36]. According to the Förster's theory, the efficiency of energy transfer E is describe by the following Eq. (4).

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (4)$$

Where r is the distance from the donor to the acceptor and R_0 is the Förster critical distance at which 50% of the excitation energy is transferred to acceptor and can be calculated from the donor emission and acceptor absorption spectra using the Förster formula Eq. (5)

$$R_0^6 = 8.8\times10^{-25} k^2 n^{-4} \varphi J \quad (5)$$

$$J = \frac{\sum \varepsilon(\lambda) F(\lambda) \lambda^4 \Delta\lambda}{\sum F(\lambda) \Delta\lambda} \quad (6)$$

In Eq. (5), K^2 is the orientation factor describing the geometry of the transition dipoles of donor and acceptor and K^2 = 2/3 for random orientation as in fluids; n isthe average refractive index of medium in the wavelength range where spectral overlap is significant (n = 1.336 [37]); Φ isthe fluorescence quantum yield of the donor; J expresses the spectral overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor, which could be calculated by Eq. (6), where, $F(\lambda)$ is the corrected fluorescence intensity of the donor in the wavelength range λ, ε (λ) is the molar absorptivity of the acceptor at wavelength λ.

Fig. 3 shows the spectral overlap between absorption spectrum of the APA compounds with fluorescence spectrum of BSA in the wavelength range of 300-500 nm. It was reported that K^2 = 2/3, Φ = 0.118 and n = 1.336 for BSA [38]. As shown in Table 4, the distances between the tryptophan residues of BSA (donors) and the APA **1-4** (acceptor) were all less than 7 nm, which illustrated that energy is transfer from BSA to APA **1-4** compounds with high possibility

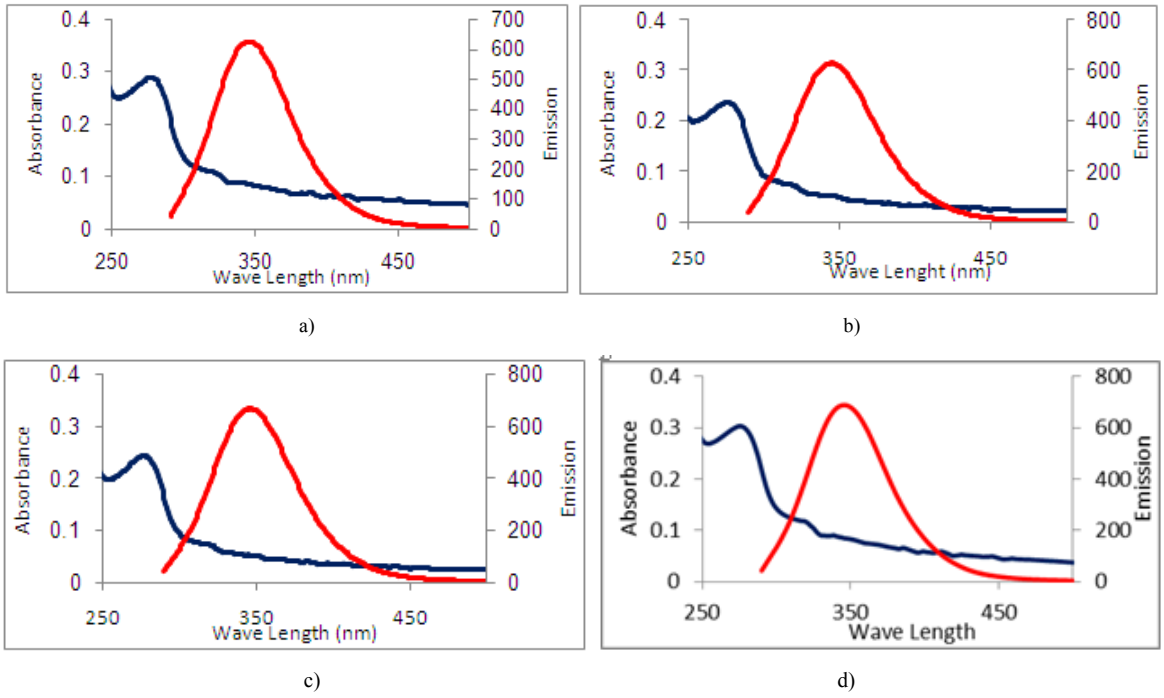


Figure 3: The overlap of the fluorescence spectrum of BSA at the excitation wavelength 280 nm, and the absorption spectrum of the APA **1-4** compounds a) APA-**1**, b) APA-**2**, c) APA-**3**, d) APA-**4**.

Table 4. Parameters of energy transfer between APA **1-4** compounds and BSA

Compd	J. cm^3 dm^3 mol^{-1}	R_0, nm	E	r, nm
APA-**1**	3.22×10^{-14}	3.06	0.18	3.94
APA-**2**	1.90×10^{-14}	2.80	0.18	3.62
APA-**3**	1.96×10^{-14}	4.15	0.12	5.77
APA-**4**	3.20×10^{-14}	3.05	0.10	4.42

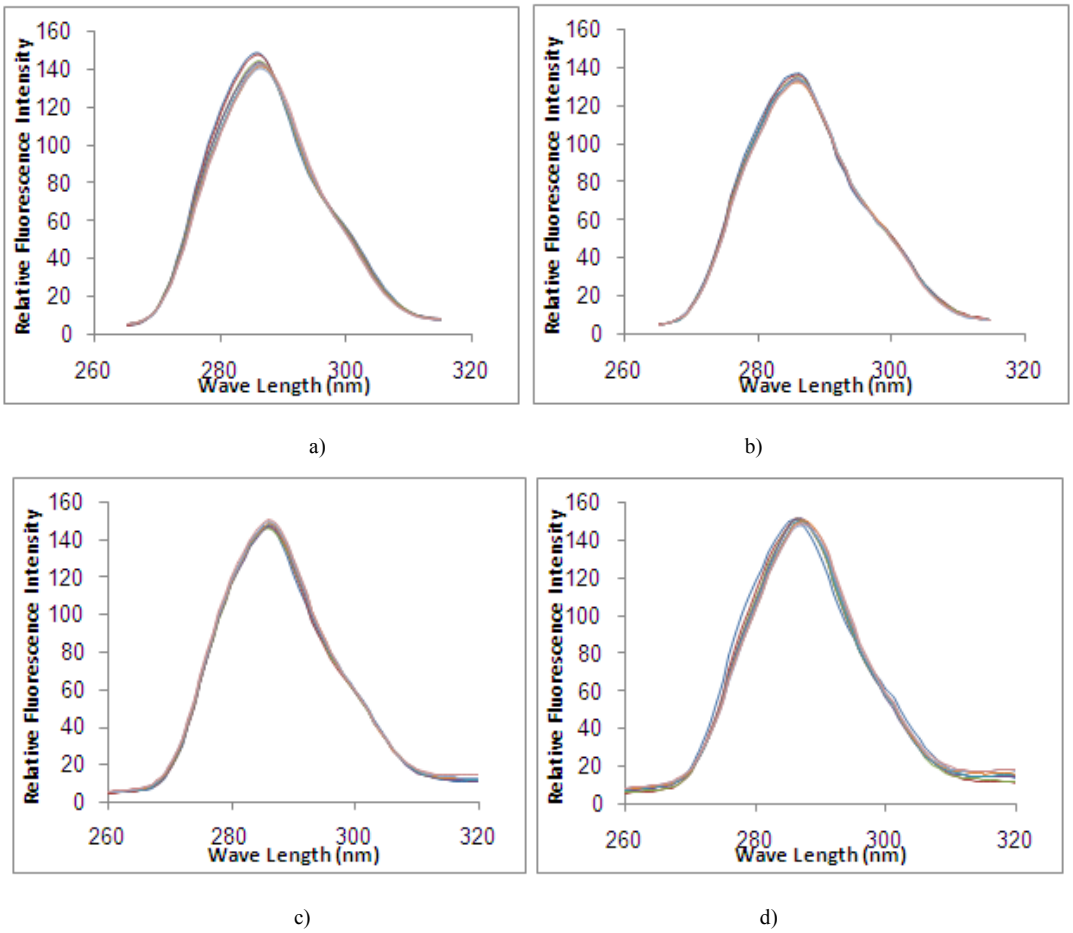


Figure 4. Effect of the APA **1-4** (a-d) on the synchronous fluorescence spectra of BSA. Δλ= 15 nm C_{BSA} = 4 μM, $C_{APA\text{-}1\text{-}4}$ = from 0 to 32.71 μM. The fluorescence intensity was decreased by increasing the concentration of ligands

The synchronous fluorescence is another spectroscopic technique which provides information about the microenvironment of the fluorophore groups and has several advantages, such as sensitivity, spectral simplification, spectral bandwidth reduction and avoiding different perturbing effects [38]. Moreover, the shift of the maximum emission wavelength correlates with changes of the polarity around the fluorophores. In the synchronous fluorescence spectra of BSA, the difference between excitation and emission ($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) reflected the conformational changes of BSA. The fluorescence of BSA comes from the tyrosine, tryptophan and phenylalanine residues. When the $\Delta\lambda$ = 60 nm, synchronous fluorescence offers information about characteristics of the tryptophan residues, while when the $\Delta\lambda$ = 15 nm it provides characteristic information about the tyrosine residues [39]. The fluorescence spectra of BSA at various concentrations of APA **1-4** for the tyrosine residues and the tryptophan residues are shown in Figures 4 and 5, respectively. It can be seen from Figures 4 and 5 that the quenching effects of the APA compounds for tryptophan residues are more than that for tyrosine residues. This result indicated that the bound APA molecules are closer to the tryptophan residues than to the tyrosine residues. Although the emission intensities at $\Delta\lambda$ = 60 and $\Delta\lambda$ = 15 nm decreased, the change in emission wavelengths for the APA **1-4** compounds was not detected. The results indicated that binding of the aminophosphinic acids compounds APA **1-4** to BSA did not cause any alterations in the conformation of the tryptophan or tyrosine residues.

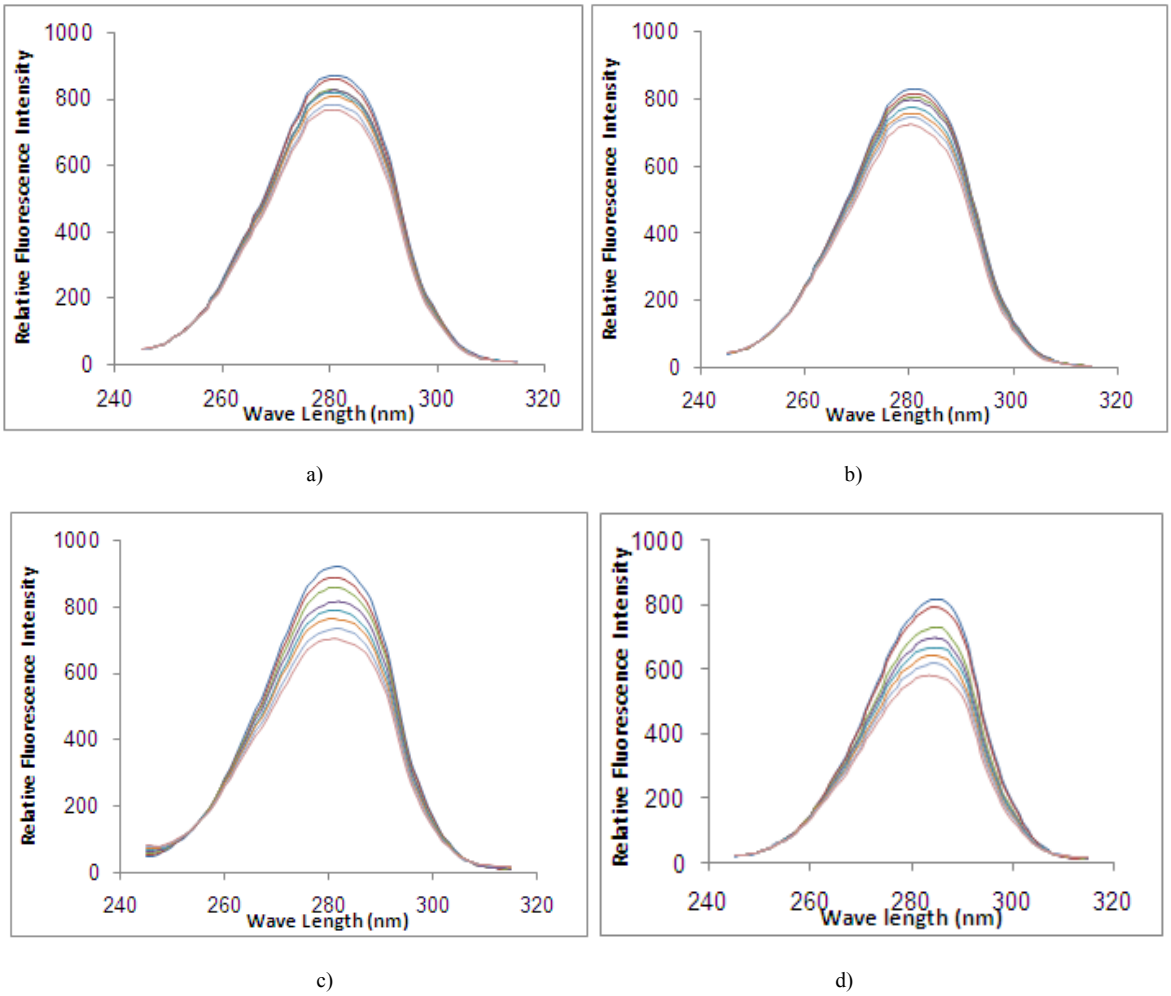


Figure 5. Effect of the APA **1-4** (a-d) on the synchronous fluorescence spectra of BSA. Δλ= 60 nm C_{BSA} = 4 μM, $C_{APA\text{-}1\text{-}4}$ = from 0 to 32.71 μM. The fluorescence intensity was decreased by increasing the concentration of ligands

4. Conclusions

The current article provided valuable information about the interaction between aminophosphinic acids (APA **1-4**) as important phosphorus analogous of natural amino acids and BSA as one of the carrier protein. The applied fluorescence method was easy to operate and reliable, practical and simple. The binding and quenching parameters were determined by the Stern-Volmer plots. The results indicated that the quenching mechanism of fluorescence of BSA by APA **1-4** is static and dynamic quenching process. Thermodynamic parameters suggested that hydrophobic forces played an important role during the formation of BSA-APA **1** and **3** complexes because the values of both ΔH and ΔS were positive. The negative ΔH and ΔS value for APA **2** and **4**indicate thathydrogen bond played major role in the APA-BSA binding interaction and contributed to the stability of the complex. Furthermore, the energy transfer from BSA to the APA **1-4** compounds was differed according to the calculated distances. The results obtained from synchronous fluorescence spectra showed that the APA **1-4** compounds did not cause considerable conformational changes in BSA and the binding site is more likely in the vicinity of the tryptophan residues than tyrosine residues.

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