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# The fluorescence spectroscopic studies on the interaction of novel aminophosphinic acids with bovine serum albumin

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ABSTRACT

Six novel aminomethylphosphinic acids have been synthesized and characterized. The interaction between the aminophosphinic acids and bovine serum albumin (BSA) was investigated using fluorescence spectroscopy. The experimental results showed that the fluorescence quenching of BSA by aminophosphinic acids is a result of the formation of aminophosphinic acid–BSA complex; static quenching and non-radiative energy transferring were confirmed to result in the fluorescence quenching. The number of binding sites $n$, the apparent binding constant $K_A$ and the corresponding thermodynamic parameters were calculated at different temperatures. The process of binding of the aminophosphinic acid molecules to BSA was a spontaneous molecular interaction procedure in which entropy increased and Gibbs free energy decreased. Hydrophobic interaction force plays a major role in stabilizing the complex. The effect of aminophosphinic acids on the conformation of BSA was analyzed using synchronous fluorescence spectroscopy.



## 1. Introduction

It has been well known that the amino acids are the main components of various proteins and they generally play an important physiological role in life process. Aminophosphinic acids have attracted considerable attention because of their significant biological activity [1]. 1-aminophosphinic acids are phosphorus analogues of natural amino acids and are selective inhibitors of various proteolytic enzymes, particularly metalloproteases [2–4]. Therefore, aminophosphinic acids have already been researched purposively and developed as potential antibacterial, antitumour and antivirotic materials in recent years. Much consideration has been given to interest in the aminophosphinic acid ligands and their complexes because of their novel structures and properties [5–11]. The design of potent and specific enzyme inhibitors with significant pharmacological activity and low toxicity requires the knowledge of interaction of the compounds with proteins to provide insight about the mechanism of their biological activity. However, for a long times many researches were only focused on the bioactivities of aminophosphinic acids and did not pay attention to their targeting of biological tissue.

Serum albumin (SA), the most abundant protein in blood plasma, is one of the most important protein that has been extensively studied. SA exhibits an exceptional ability to reversibily bind a wide range of endogenous and exogenous compounds and regulate free plasma concentrations [12]. The binding of protein with a drug greatly affects on the absorption, distribution, metabolism and excretion properties of typical drugs [13]. Thus it is important and necessary to study the interaction of drugs with serum albumin at molecular levels. In recent years, attention has been focused on the interaction between SA and foreign molecules by examining the relationship between the structure of these compounds and their affinities toward serum albumin [14–19]. Therefore, the binding properties of chemicals with SA are clearly important for providing a pathway to the pharmacokinetic and pharmacodynamic characteristics of these substances in various tissues. Bovine serum albumin (BSA) has been proven to have high homology and similarity to human serum albumin (HSA) both in sequence and conformation [20].

Over the past several years, our laboratories have reported novel methods for the synthesis of 1-amino-*H*-phosphinic acids [21–25]. 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 [26–28]. As an extension of previous studies, we have now prepared and characterized a series of novel 1-aminophosphinic acids and their interaction with BSA has been explored by using fluorescence spectroscpic technique (Scheme 1). In this paper, BSA was selected as a suitable model protein because of its low cost, readily avilablility, and unusual ligand-binding properties.

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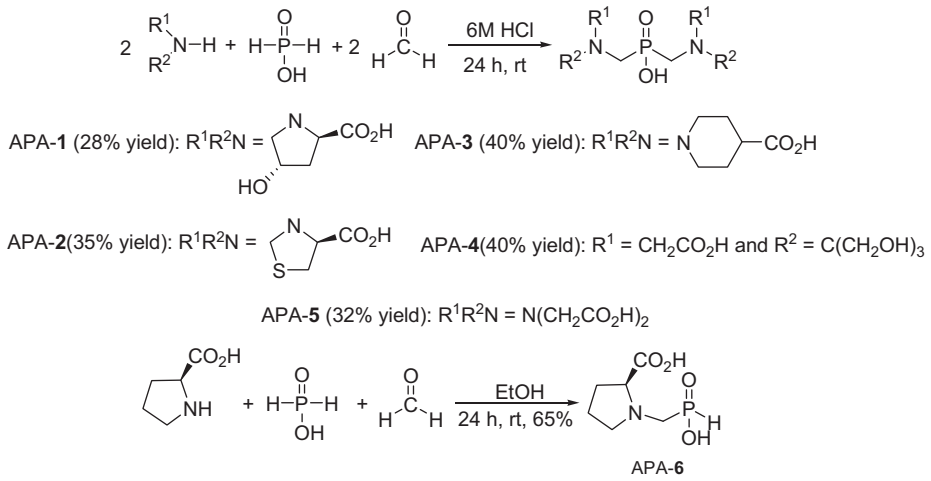


**Scheme 1.** Synthesis route of APA **1–6**.

## 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 ( ≥ 98% lyophilized powderd, Cat. No. A7030) and used without further purification. The solutions of BSA were prepared in 0.05 M sodium phosphate 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]. The aminophosphinic acid solutions were 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 Brucker Avance instrument with the chemical shifts being reported as $\delta$ 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 substracted 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 (1–5)

The amine (20 mmol) was added to a mixture of HCl 6 M (4 mL) and hypophosphorus acid (2.7 g, 10 mmol) and the solution was stirred for 1 h at room temperature (in the case of APA-5 reaction carried out at reflux). Formaldehyde 37% (40 mmol, 3.2 g) was added dropwise over 1 h to the mixture and stirred for 24 h at room tepearture (only for APA-4 and APA-5 at reflux). Acetone (150 mL) was added to the mixture dropwise slowly under vigorous stirring. During this time, a white precipitate formed. The precipitate was removed by filtration and for further purification the solid was dissolved in 3 mL of water and acetone (50 mL) was added to the solution under vigorous stirring. A fine, white powder was obtained which was filtered off and washed with acetone (3 × 15 mL). Finally the products were dried on air at room temperature. Yields: 28–40%

*2.1.1.1. Bis-[(L-thiazolidino-4-carboxylic acid)-N-methyl]-phosphinic acid (APA-1).* White solid; mp: 87–89 °C; $^1$H NMR ($D_2O$—250 MHz): 3.32–3.39 (4H, m, $CH_2$–P). 3.42–3.47 (4H, dd, ring S–$CH_2$–CH, $^2J_{HH}$=12 Hz, $^3J_{HH}$=8 Hz,), 4.39 (2H, d, $^2J_{HH}$=10.4 Hz, ring N–$CH_2$–S). 4.56 (2H, dd, $^3J_{HH}$=7.6 Hz, $^3J_{HH}$=5.2 Hz, N–CH–$CH_2$), 4.70 (2H, d, $^2J_{HH}$=10.4 Hz, ring N–$CH_2$–S); $^{13}$C NMR ($D_2O$—62.9 MHz): 31.6 (s, $CH_2$–S), 53.5 (d, P–$CH_2$ $^1J_{CP}$=97.5 Hz), 60.0 (d, N–CH, $^3J_{CP}$=5.0 Hz), 70.8 (d, N–$CH_2$–S, $^3J_{CP}$=5.0 Hz), 170.7 (s, C=O); $^{31}$P NMR ($D_2O$/$H_3PO_4$—101.2 MHz): 42.28 ppm; Anal. Calcd for $C_{10}H_{17}N_2O_6PS_2$: C, 33.70; H, 4.81; N, 7.87. Found: C, 34.01; H, 5.02; N, 7.69.

*2.1.1.2. APA-2.* White solid; mp: 249–251 °C; $^1$H NMR ($D_2O$—250 MHz): 2.15–2.23 (2H, m, ring N–$CH_2$–CH), 2.38–2.45 (2H, m, ring N–$CH_2$–CH), 3.26 (2H, d, $^3J_{HH}$=12.8 Hz, ring N–CH–$CH_2$), 3.48–3.72 (4H, m, ring CH–$CH_2$–CH), 4.00 (2H, m. CH–OH), 4.58 (4H, d, $^2J_{HP}$= 11.5 Hz, $CH_2$–P).

$^{31}$P NMR ($D_2O$/$H_3PO_4$—101.2 MHz): 15.15 ppm;

$^{13}$C NMR ($D_2O$—62.9 MHz) 37, 55.9 (d, $^1J_{CP}$=93.7 Hz, P–$CH_2$), 64.3 (d, $^3J_{CP}$=3.1 Hz, N–$CH_2$), 68.6 (d, $^3J_{CP}$=5.0 Hz, N–CH), 68.9, 170.7 (C=O); Anal. Calcd for $C_{12}H_{21}O_8N_2P$: C, 40.89; H, 6.01; N, 7.95. Found: C, 40.54; H, 5.95; N, 7.80.

*2.1.1.3. (APA-3).* White solid; mp: 115–118 °C; $^1$H NMR ($D_2O$—250 MHz): 2.82 (s, 4H, $CH_2$–P), 3.32 (s, 12H, $CH_2OH$), 3.60 (s, 4H, N–$CH_2CO_2H$); $^{13}$C NMR ($D_2O$—62.9 MHz): 47.4 (d, $^1J_{CP}$=98.7 Hz, P–$CH_2$), 52.7, 60.4, 64.6 (d, $^3J_{CP}$=3.8 Hz, N–$CH_2$), 182.0 (C=O); $^{31}$P NMR ($D_2O$/$H_3PO_4$—101.2 MHz): 40.25 ppm; Anal. Calcd for $C_{14}H_{29}O_{12}N_2P$: C, 33.95; H, 6.89; N, 6.60. Found: C, 33.70; H, 6.56; N, 6.48.

*2.1.1.4. (APA-4).* White solid; mp: 281–284 °C; $^1$H NMR (DMSO-d6–250 MHz) 1.95 (4H, s, $CH_2$P), 3.18–3.57 (18H, m, ring), 12.53 (1H, br); $^{13}$C NMR ($D_2O$—62.9 MHz): 25.3, 37.6, 54.5, 55.8 (d, $^1J_{CP}$=96.2 Hz, $CH_2$–P), 177 (C=O); $^{31}$P NMR (DMSO-d6–101.2 MHz): 11.16 ppm; Anal. Calcd for C14H26NO6P: C, 44.28; H, 8.06; N, 8.61. Found: C, 44.50; H, 8.30; N, 8.42.

*2.1.1.5. (APA-5).* White solid, mp: 203–205 °C (Lit mp: 205 °C) [29] $^1$H–NMR ($D_2O$/TMS—400 MHz): 3.56 (4H, d, $^2$JHP=9.6 Hz,