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Spectroscopic analyses on interaction of Amantadine-Salicylaldehyde, Amantadine-5-Chloro-Salicylaldehyde and Amantadine-o-Vanillin Schiff-Bases with bovine serum albumin (BSA)

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ABSTRACT

In this work, three Tricyclo [3.3.1.1(3,7)] decane-1-amine (Amantadine) Schiff-Bases, Amantadine-Salicylaldehyde (AS), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C) and Amantadine-o-Vanillin (AS-o-V), were synthesized by direct heating reflux method in ethanol solution and characterized by infrared spectrum and elementary analysis. Fluorescence quenching was used to study the interaction of these Amantadine Schiff-Bases (AS, AS-5-C and AS-o-V) with bovine serum albumin (BSA). According to fluorescence quenching calculations the bimolecular quenching constant (K_q), apparent quenching constant (K_{SV}), effective binding constant (K_A) and corresponding dissociation constant (K_D), binding site number (n) and binding distance (r) were obtained. The results show that these Amantadine Schiff-Bases can obviously bind to BSA molecules and the binding strength order is AS < AS-5-C = AS-o-V. Synchronous fluorescence spectroscopy reveals that these Amantadine Schiff-Bases adopt different way to bind with BSA molecules. That is, the AS and AS-5-C are accessibility to tryptophan (Trp) residues more than the tyrosine (Tyr) residues, while the AS-o-V is equally close to the Tyr and Trp residues.

1. Introduction

Tricyclo [3.3.1.1(3,7)] decane-1-amine (Amantadine) is an antivirotic drug that has been used to treat influenza and Parkinson disease [1–6]. However, in recent years, influenza is often caused by viruses and germs at the same time [7,8]. Because it has only antivirotic activity, Amantadine cannot be used to treat current influenza. Therefore, it is necessary to develop the difunctional or multifunctional anti-influenza drug, which not only has antiviral activity but also does antibacterial action [9].

Aromatic Schiff-Bases are a large class of organic compounds with imino (–HC=N–) group and simple structures [10–12]. The aromatic plane structure and strong binding ability lead to bacteriostatic, fungicidal, anti-tumour, and anti-viral biological activities. Hence, many aromatic Schiff-Base compounds have developed as antibacterial, antitumour and antivirotic drugs in recent years [13–15]. 2-Hydroxybenzaldehyde (Salicylaldehyde), which has frequently been used to synthesis aromatic Schiff-Base compounds, has many biological activities, such as analgesic, anti-inflammatory, antibacterial, bactericidal and antiviral activities [16–18]. Aromatic Schiff-Bases have also been used as efficient herbicide, pesticide and bactericide [19,20]. In order to combine bacteriostatic ability and anti-viral activity in aromatic Schiff-Bases, in this work, the Salicylaldehyde and its two analogous compounds (5-Chloro-2-hydroxybenzaldehyde (5-Chloro-Salicylaldehyde) and 2-Hydroxy-3-methoxybenzaldehyde (o-Vanillin)) were used to react with Amantadine, so that three Amantadine Schiff-Bases (Amantadine-Salicylaldehyde (AS), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C) and Amantadine-o-Vanillin (AS-o-V)) were synthesized [21]. There identify was verified by elemental analysis and IR spectroscopy. The molecular structures of three synthesized Amantadine Schiff-Bases (AS, AS-5-C and AS-o-V) are given in Fig. 1.

The well known serum albumin is the most abundant proteins in the plasma [22–24], and has many physiological functions. In this work, the bovine serum albumin (BSA) was chosen as a target protein molecule because of its low cost, ready availability, ligand-binding properties [25–27]. Moreover, the molecular structure of BSA is similar to that of human serum albumin (HSA) with 76% identify, so the results of the studies conducted here are applicable to HSA [28]. Hence, the quenching of intrinsic fluorescence is used to research the interaction between three synthesized Amantadine Schiff-Bases (AS, AS-5-C and AS-o-V) and BSA molecules. The interaction strength and mode of binding were also studied by synchronous fluorescence spectroscopy.

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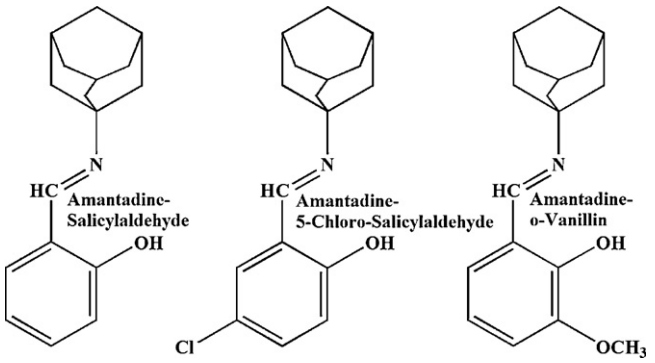

Fig. 1. Molecular structure of Amantadine-Salicylaldehyde (AS), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C) and Amantadine-o-Vanillin (AS-o-V) Schiff-Bases.

2. Experimental

2.1. Materials

Commercially prepared bovine serum albumin (BSA, purity >98%. 4.8 isoelectric point, 16% nitrogen content, sugar content 0.08% and 0.2% fat content) was obtained from Beijing Abxing Biological Technology Company and stored in refrigerator at 4.0 °C. Tricyclo [3.3.1.1(3,7)] decane-1-amine (Amantadine hydrochloride, analytical reagent grade), 2-hydroxybenzaldehyde (Salicylaldehyde, analytical reagent grade), 5-Chloro-2-hydroxybenzaldehyde (5-Chloro-Salicylaldehyde, analytical reagent grade) and 2-Hydroxy-3-methoxybenzaldehyde (o-Vanillin, analytical reagent grade) were purchased from Tianjing Tianhe Chemical Reagent Co., Ltd. All other reagents were commercial products of analytical grade and used as received. The Tris (hydroxyl-methyl) aminomethane (Tris), HCl and NaCl were all of analytical reagent grade, and double distilled water was used for all solution preparation.

2.2. Apparatus and instruments

The fluorescence measurements were performed on a fluorophotometer (Cary 300, Varian Company, USA) and the UV–vis absorption spectra were recorded with an UV–vis spectrophotometer (Cary 50, Varian Company, USA). The compositions of synthesized Amantadine Schiff-Bases were determined by using an elementalanalyser (Perkin-Elmer 2400, PerkinElmer Company, USA). Their structures were analyzed by Fourier transform infrared spectrophotometer (Spectrum 100, Perkin-Elmer Company, USA) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy (Mercury-Vx300, Varian Company, USA). The solution pH value was measured with a pH meter.

2.3. Syntheses of Amantadine Schiff-Bases (AS, AS-5-C and AS-o-V)

Three Amantadine Schiff-Bases, Amantadine-Salicylaldehyde (AS, $C_{17}H_{21}NO$), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C, $C_{17}H_{20}NOCl$) and Amantadine-o-Vanillin (AS-o-V, $C_{18}H_{23}NO_2$), were synthesized, respectively, through refluxing, distillation and filtration of mixed ethanol solutions of Amantadine and Salicylaldehyde, 5-Chloro-Salicylaldehyde and o-Vanillin. 4.1296 g (0.022 mol) Amantadine hydrochloride and 1.2343 g (0.022 mol) KOH were added to 50 mL ethanol in a 150 mL beaker and then stirred for 24 h. The produced white precipitates (KCl) were filtered out and the clear liquid was transferred to a 250 mL three-necked flask. During the refluxing at 95 °C in oil bath, 50 mL Salicylaldehyde (2.4424 g, 0.020 mol) ethanol solution was added dropwise to the above Amantadine ethanol solution. After 3.0 h reflux, the mixed ethanol solution was concentrated to about 10 mL through reduced pressure distillation. After naturally cooling to room temperature, the AS Schiff-Base (pale yellow) microcrystals appeared in the bottom of flask. The sample was filtrated and washed with ethanol three times and dried at 120 °C to constant weight.

AS-5-C (yellow microcrystals) and AS-o-V (deep yellow microcrystals) Schiff-Bases were synthesized using similar methods described above. The elemental analysis of these compounds is given in Table 1, and the FT-IR and ^{1}H NMR results are given in Fig. 2 and Tables 2 and 3, respectively.

2.4. Measurement of binding parameters

For each of the three Amantadine Schiff-Bases (AS, AS-5-C and AS-o-V), the binding parameters with BSA molecules were measured by fluorescence spectroscopy. BSA and Amantadine

Table 1
Elementary analyses of Amantadine-Salicylaldehyde (AS), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C) and Amantadine-o-Vanillin (AS-o-V) Schiff-Bases.

Schiff-Bases	Molecular formula	Molecular weight	Colour	Melting point (°C)	C (%)		H (%)		N (%)	
Amantadine-Salicylaldehyde	$C_{17}H_{21}NO$	255.34	Pale yellow	92 –93	Calcd. Found	79.96 79.94	Calcd. Found	8.29 8.31	Calcd. Found	5.49 5.53
Amantadine-5-Chloro-Salicylaldehyde	$C_{17}H_{20}NOCl$	289.79	Yellow	130 –133	Calcd. Found	70.46 70.43	Calcd. Found	6.96 6.98	Calcd. Found	4.83 4.89
Amantadine-o-Vanillin	$C_{18}H_{23}NO_2$	285.37	Deep yellow	104 –107	Calcd. Found	75.75 75.72	Calcd. Found	8.12 8.10	Calcd. Found	4.91 4.96

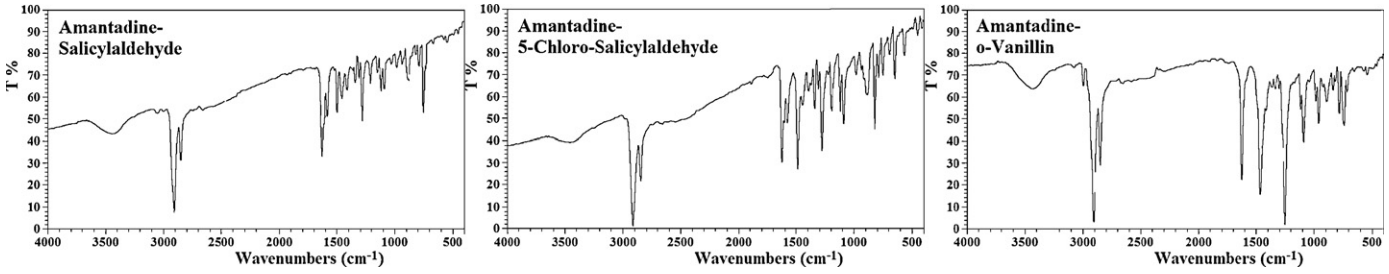

Fig. 2. Infrared spectra of Amantadine-Salicylaldehyde (AS), Amantadine-5-Chloro-Salicylaldehyde (AS-5-C) and Amantadine-o-Vanillin (AS-o-V) Schiff-Bases (with potassium bromide (KBr) pellet at room temperature (about 25 °C)).