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Interaction of antihypertensive drug amiloride with metal ions in micellar medium using fluorescence spectroscopy

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ABSTRACT

Steady state and life time fluorescence spectroscopy have been employed to study the interaction of antihypertensive drug amiloride with biologically important metal ions *i.e.* Cu^{2+}, Fe^{2+}, Ni^{2+} and Zn^{2+} in various micellar media (anionic SDS (sodium dodecyl sulfate), nonionic TX-100 (triton X-100) and cationic CTAB (cetyl trimethyl ammonium bromide)). It was observed that fluorescence properties of drug remain unaltered in the absence of micellar media with increasing concentration of metal ions. However, addition of Cu^{2+}, Fe^{2+} and Ni^{2+} caused fluorescence quenching of amiloride in the presence of anionic micelle, SDS. Binding of drug with metal ions at the charged micellar interface could be the possible reason for this pH-dependent metal-mediated fluorescence quenching. There were no remarkable changes observed due to metal ions addition when drug was present in cationic and nonionic micellar medium. The binding constant and bimolecular quenching constant were evaluated and compared for the drug–metal complexes using Stern–Volmer equation and fluorescence lifetime values.

1. Introduction

Fluorescence spectroscopy is a simple, rapid, sensitive and non-destructive method which has got widespread application in biologically and environmentally important areas [1–2]. Fluorescent molecules (probes) are very sensitive to its immediate environment; therefore these probes can be used to study micellation, probe–DNA interaction, probe–serum albumin interaction, probe–metal interaction *etc.* [3–6]. The interaction of fluorescent drugs with biologically important metal ions has gained significant interest in recent years because, this study provides detailed information regarding the mechanism of action of the pharmaceutical preparation in living systems [7,8]. Fluorescence of drug mostly gets quenched in the presence of metal ions and the extent of quenching gives a detailed picture regarding their interaction, binding mechanism and bioavailability of drugs [9,10]. This can also be used for the quantitative analysis of metals.

Interactions of fluorophores with metal ions in the presence of micelles are very significant as they are the true mimic of membranes. Study using such model membrane helps to understand the mechanism of its interaction in biological systems. Surfactants self-assembled microstructures can be stabilized by polymerization method [11,12]. Micelle enhances the solubility of drug molecules and also enhances the communication between fluorophore and metal ions [10,13,14]. Micelles or surfactants are classified as four types based on their charge such as anionic, cationic, nonionic and zwitterionic [15]. Surfactant minimizes drug degradation, enhances the bioavailability of drug and protects the body from side effects of the drug by achieving the desired concentration of drug at target site. Simplicity of application and high selectivity towards metal ions makes fluorophore-micellar systems a promising candidate for metal sensing applications [16–19].

Amiloride (AMI) [3, 5-diamino-6-chloro-N-(diaminomethylene)-pyrazine-2-carboxamide], a pyrazine compound, was selected as a fluorescent probe for this study. Amiloride is a diuretic drug that is used for the treatment of hypertension [20–21]. Different studies on biochemical, cellular and animal models have shown that AMI also possesses anti-tumour and anti-metastasis activities [22]. Amiloride (AMI) structure consists of a substituted pyrazine ring where, two amino groups are present at ring positions 3 and 5, a chloride moiety is at ring position 6, and an acylguanidium group present at ring position 2. Due to the acylguanidium moiety, amiloride is a weak base with a pK_a of 8.7 and protonation occurs on the guanidine part of the molecule. In the physiological pH range (7.3–7.5), amiloride exists primarily as a monovalent cation with a positive charge resonating between the terminal amidinium fragment [21].

The structures of amiloride tautomers in aqueous solutions are shown in Fig. 1. The planar tautomers are stabilised by three intramolecular hydrogen bonds. The free-base (*i.e.* unprotonated) form of amiloride (Ia and Ib) exists primarily as the acylimino tautomer (double bond between N8 and C9), whereas the protonated form of the molecule (II) assumes the acylamino tautomeric conformation

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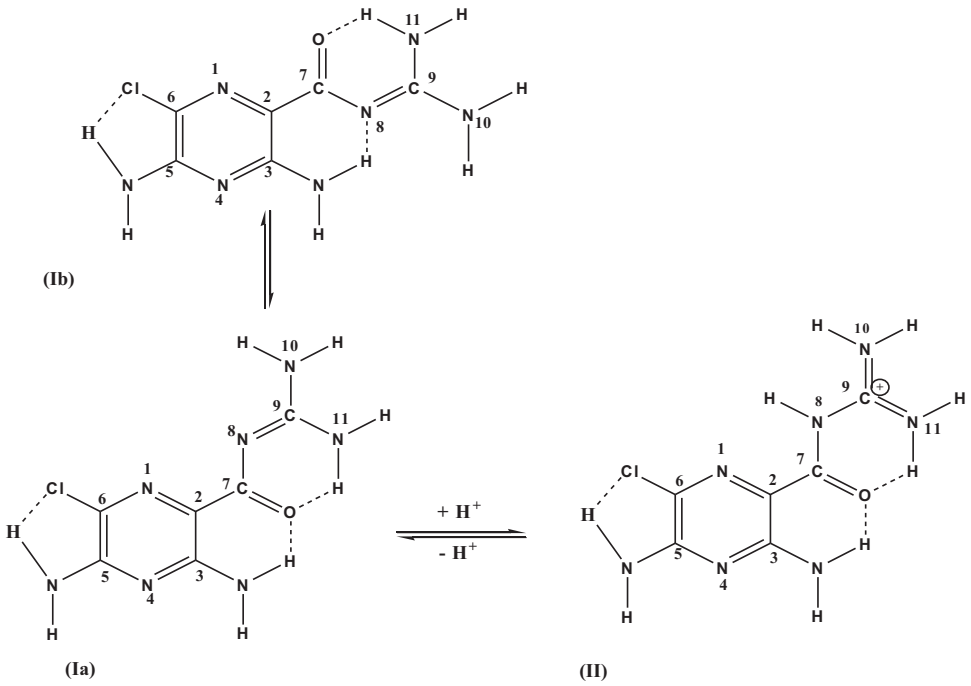

Fig. 1. The structures of amiloride tautomers in aqueous solution.

(resonating double bond between C9 and N10 and N11). Tautomer II represents the most popular moiety in aqueous solution with a pH < 8 and is the biologically active isomer [21].

Four biologically important metal ions, such as Cu^{2+}, Fe^{2+}, Zn^{2+} and Ni^{2+} were selected for this study. Iron is the most abundant transition metal that plays numerous essential roles in the human body, such as oxygen delivery, electron transport and many enzymatic reactions [23–25]. Copper is one of the essential trace metal in biological systems. It plays important roles in biological processes like, embryonic development, mitochondrial respiration, regulation of hemoglobin levels and neuronal functions [26,27]. Nickel is found in enzymes like urease and in many biologically active complexes which plays important role in antibacterial, antifungal and antimicrobial activity [28,29]. Zinc is the second most abundant metal in biological systems because it is present in active centers of many enzymes [30]. Deficiency of these metal ions causes anemia-like symptoms and depresses immunity.

Present study discusses a detailed investigation of amiloride interaction with metal ions in various micellar media at three different pH. The method provides simple way to measure concentration of metal ions in biological systems. Fluorescence response of amiloride with metal ions in the presence of anionic micelles is interesting as the cationic drug senses metal ions only in the presence of anionic micelle. The response is different in different pH as the molecule is capable of forming intra molecular hydrogen bonding. This idea is furthermore beneficial because rather than synthesizing new molecules or probes for metal sensing applications, we can utilize the already available probes as this provides simple and inexpensive route of analysis.

2. Experimental

2.1. Materials

Amiloride (AMI) was purchased from Sigma-Aldrich. Sodium dodecyl sulfate (SDS), cetyl trimethylammonium bromide (CTAB), triton X-100 (TX-100), $CuSO_4$, $FeSO_4$, $NiSO_4$, $ZnSO_4$, NaOH and HCL were purchased from S.D. fine chemicals and used as received. Triply distilled water was used throughout the study (conductance-0.18 μΩ). All the experiments were performed using freshly prepared solutions.

Higher concentrations of stock solutions were prepared and diluted further to get required concentrations. For steady state fluorescence study, concentration of amiloride was kept constant at 6×10^{-6} M and concentration of surfactants were maintained well above their critical micellar concentrations ([SDS] = 10 mM, [CTAB] = 1.5 mM, [TX-100] = 0.22 mM). The concentrations of metal ions were varied from 10 ppm to100 ppm. pH of the solutions were adjusted by dil. HCl and dil. NaOH. Solutions were kept for 24 h at room temperature and atmospheric pressure to attain equilibrium. All the experiments were performed in triplicates to check reproducibility in the results.

2.2. Method

Fluorescence spectra were recorded using JASCO FP 8300 spectrofluorimeter, equipped with a xenon lamp as the source. Scan speed of 1000 nm s^{-1} was maintained for all measurements. The slit width for both excitation and emission was kept at 2.5 nm. Amiloride was excited at 364 nm and emission was measured from 370 nm to 500 nm. The fluorescence life time measurements were carried out on Horiba Jobin Yvon IBH, UK, with picosecond laser diodes as excitation source. The decay curves were fitted using IBH-DAS6 software. Sample was excited using a 375 nm diode laser. The lifetime data were fitted using minimum number of exponential. Quality of each fitting was judged by χ^2 values and residual plot. When χ^2 value goes unfavorable (Ideally $\chi^2 \sim 1$) curve fitting is performed using higher exponentials.

3. Results and discussion

Amiloride (AMI) absorbance spectrum consists of three peaks in the ultraviolet region (211 nm, 284 nm and 361 nm). This molecule does not absorb light in the visible range of wavelengths. AMI is a highly fluorescent molecule with an emission maximum at 417 nm (broad blue fluorescence) when excited at 361 nm.