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The characterization of the fluorescence of L-histidine in simulated body fluid

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Abstract

In addition to being an essential natural amino acid, L-histidine is biologically important in the dismutation of superoxide radical ($O_2^{\bullet -}$) by superoxide dismutase (SOD). In this work, fluorescence and absorptiometric techniques were used to characterize the photo-phenomenon and optical properties of this compound in a simulated body fluid (SBF). L-Histidine fluoresces at 360 nm when excited at 220 nm. Its molar absorptivity, ε, is $4.8 \times 10^3\ M^{-1}\ cm^{-1}$. The observed bimolecular quenching rate constant, k_q, of $7.5 \times 10^8\ M^{-1}\ s^{-1}$, by hydrogen peroxide, suggests a non-diffusional activation-controlled mechanism with a rate constant, k_a, of $8.55 \times 10^8\ M^{-1}\ s^{-1}$ and an electron transfer rate constant, k_{ET} of $6.06 \times 10^8\ s^{-1}$. The determined radiative and non-radiative rate constants, 4.73×10^7 and $2.9 \times 10^8\ s^{-1}$, respectively, suggests that the deactivation of the thermally excited L-histidine is by non-radiative route rather than by normal fluorescence, which accounts for the low quenching constant, K_{SV}, of $2.22\ M^{-1}$ that was obtained. The solvent reorganization energy, λ_s, and the reaction free energy change, ΔG, of 1.48 and −5.62 eV, respectively, suggest that the electron transfer reaction in the L-histidine–H_2O_2 reaction is through a solvent separated mechanism.

Keywords: Fluorescence; L-Histidine; Simulated body fluid; Activation-controlled; Solvent reorganization energy

1. Introduction

L-Histidine is one of the naturally occurring amino acids. It plays a very active role in many biological systems [1–3]. Its relevance in the overall structure and activity of the metalloenzyme, superoxide dismutase (SOD), in the dismutation of debilitating superoxide is well documented [4–7]. The nitrogens on the imidazole moiety of this amino acid, which is a vital component of superoxide dismutase, is ligated to copper and zinc ions as shown in Fig. 1. These metallic sites act as the catalytic centers for the superoxide dismutation reactions. In order to fully understand the mechanism by which this amino acid performs its multifarious biological functions, its optical and physico-chemical properties must be known and be controllable for more efficient tailoring of its activities for specific functions.

Fluorescence techniques have always been found to be quite sensitively reliable in its application in the study and the understanding of chemical and biological reactions. Data obtained by fluorescence techniques have been found reliable in obtaining not only the binding constants between molecules but also in the determination of the rate of such binding as well as the resulting mechanism. This is especially important in the binding of bio-molecules to enzymes for catalysis in bio-activities [8–16]. Fluorescence quenching studies have been used extensively in this type of studies. We have therefore carried out fluorescence quenching experiments of L-histidine using hydrogen peroxide as a quencher molecule, in other to obtain vital physico-chemical and optical properties of this compound.

SOD is a cytosolic metalloenzyme and its reactions are carried out in that medium. Therefore, in other to obtain spectrochemical properties that is meaningfully consistent with what is obtained in the cytosol, spectrochemical experiments were conducted in a simulated body fluid (SBF) that is known to efficiently mimic biological fluid [17]. The observed pH of the SBF medium used in this work is 7.4, which is very close to the biological pH. In this medium and at this pH, L-histidine exists essentially as a neutral compound as shown in Fig. 2. Its isoelectronic point (p*I*) at this pH is known to be about 7.5 [18], which is in close agreement with the pH of the SBF medium used in this work.

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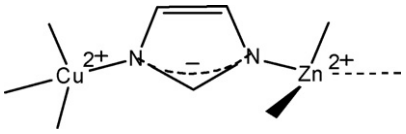

Fig. 1. Structure of the imidazole moiety of L-histidine showing the ligated metal ions on the nitrogen atoms.

2. Experimental

2.1. Chemicals

ACS certified grade L-histidine was obtained from J.T. Baker so also is a 99.0% purity quinine sulfate. A 30.7% analytical reagent grade hydrogen peroxide and a 95–98% pure sulfuric acid were obtained from Sigma–Aldrich. Cetyltrimethylamonium bromide (CTAB) (99.0% purity), magnesium chloride (99.0% pure) and ACS reagent grade disodium phosphate (Na_2HPO_4) were obtained from Acros. Potassium chloride (99.4% pure), calcium chloride (99.0% pure) and sodium bicarbonate ($NaHCO_3$) (~100.0% pure) were obtained from Fisher chemicals and sodium chloride of 99.98% purity was obtained from Thorn Smith chemist. All chemicals were used as received.

2.2. Simulated body fluid preparation

This fluid system which was used as the solvent for all experiments performed in this work was prepared in accordance with literature directions [19,20]. Briefly, the following compounds were dissolved in 500 mL of triply distilled deiozed water: NaCl (3.9681 g); KCl (0.1864 g); $CaCl_2$ (0.1387 g); $MgCl_2$ (0.1307 g); $NaHCO_3$ (0.1764 g) and Na_2HPO_4 (0.0710 g). These compounds give an ionic concentration of Na^+ (143 mM), K^+ (5.0 mM), Mg^{2+} (1.5 mM), Ca^{2+} (2.5 mM), Cl^- (148.8 mM), HCO_3^- (4.2 mM), and HPO_4^- (1.0 mM). The ionic strength was calculated to be 0.16 M and the pH of the solution is about 7.4.

2.3. Optical measurements

All fluorescence measurements were performed using a Perkin-Elmer's Luminescence Spectrophotometer, Model LS 50B. The L-histidine exhibits complex fluorescence spectra due to the presence of the imidazole moiety [21]. For this reason the excitation and emission wavelength of this compound were determined by varying the excitation wavelength from 200 to 250 nm and observing the resulting fluorescence intensity. This way the excitation wavelength that gave the maximum emission intensity was obtained at 220 nm as can be seen in Fig. 3. The wavelength (220 nm) was used for all further excitation experiments in this work. The excitation and emission slits were kept constant at 3.0 nm. At this excitation wavelength, the fluorescence of this amino acid was observed at 360 nm. In all experiments, unless otherwise specified, the concentration of L-histidine was kept constant at 2.5×10^{-4} M and the concentration of the quencher, hydrogen peroxide, was varied from 0.0185 to 0.1294 M. All experiments were conducted at room temperature ($25 \pm 0.2\,^{\circ}C$). The fluorescence spectra from which the fluorescence intensities were obtained were uncorrected.

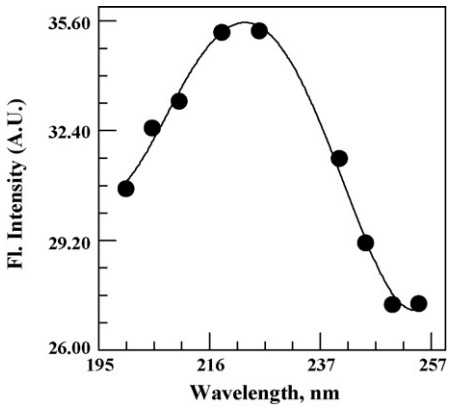

Fig. 3. Plot of the observed fluorescence intensity of L-histidine as a function of the exciting wavelength.

The absorptiometric experiments were performed using a Cary Spectrophotometer, Model 1E, supplied by Varian Analytical Instruments. Absorptiometric spectrum of this compound whose λ_{max} is observed at 211 nm was also obtained using a 1.0 cm cuvette. This absorption wavelength of L-histidine is in good agreement with that observed by Tatischeff et al. [21]. The molar absorptivity, ε, of L-histidine in SBF was determined by plotting the ratio of the observed absorbance, A, taken at λ_{max} to the concentration, C, as a function of the respective wavelengths encompassing the absorbance spectrum in accordance with Beer–Lambert's law (A/bC). b is the solution thickness or light path length, was 1.0 cm. The ε was taken at the peak of the plot at which $\lambda = \lambda_{max}$.

2.4. Refractive index determination

The refractive index, n, of SBF and 0.05 M H_2SO_4 solutions were determined using the digital Abbe Leica Refractometer

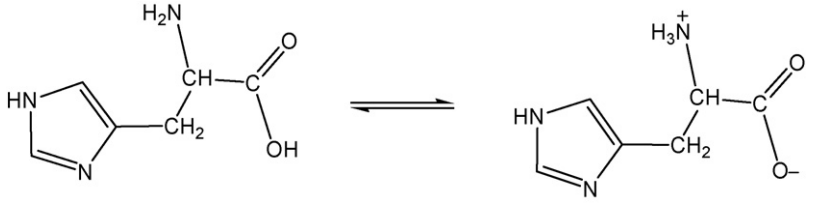

Fig. 2. Structure of L-histidine at isoelectronic point (pI) of 7.59.