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Photophysics of indole-2-carboxylic acid (I2C) and indole-5-carboxylic acid (I5C): Heavy atom effect

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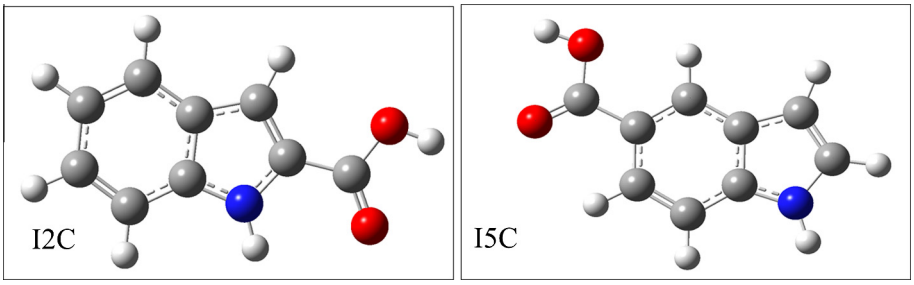
HIGHLIGHTS

- Photophysical parameters of carboxylic indole derivatives have been determined.
- Phosphorescence lifetime of I2C and I5C in water was 912 and 56 μs, respectively.
- The triplet quantum yields of aqueous I2C and I5C were similar to that of indole.
- Temperature dependence of I2C and I5C triplet state quenching by KI was determined.
- The ATR-FTIR spectra of I2C and I5C in the solid state have been recorded.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study the effect of carboxylic group substitution in the 2 and 5 position of indole ring on the photophysics of the parent indole chromophore has been studied. The photophysical parameters crucial in triplet state decay mechanism of aqueous indole-2-carboxylic acid (I2C) and indole-5-carboxylic acid (I5C) have been determined applying our previously proposed methodology based on the heavy atom effect and fluorescence and phosphorescence decay kinetics [Kowalska-Baron et al., 2012]. The determined time-resolved phosphorescence spectra of I2C and I5C are red-shifted as compared to that of the parent indole. This red-shift was especially evident in the case of I2C and may indicate the possibility of hydrogen bonded complex formation incorporating carbonyl C=O, the NH group of I2C and, possibly, surrounding water molecules. The possibility of the excited state charge transfer process and the subsequent electronic charge redistribution in such a hydrogen bonded complex may also be postulated. The resulting stabilization of the I2C triplet state is manifested by its relatively long phosphorescence lifetime in aqueous solution (912 μs). The relatively short phosphorescence lifetime of I5C (56 μs) may be the consequence of more effective ground-state quenching of I5C triplet state. This hypothesis may be strengthened by the significantly larger value of the determined rate constant of I5C triplet state quenching by its ground-state (4.4×10^8 M^{-1} s^{-1}) as compared to that for indole (6.8×10^7 M^{-1} s^{-1}) and I2C (2.3×10^7 - M^{-1} s^{-1}). The determined bimolecular rate constant for triplet state quenching by iodide $k_q^{T_1}$ is equal to 1×10^4 M^{-1} s^{-1}; 6×10^3 M^{-1} s^{-1} and 2.7×10^4 M^{-1} s^{-1} for indole, I2C and I5C, respectively. In order to obtain a better insight into iodide quenching of I2C and I5C triplet states in aqueous solution, the temperature dependence of the bimolecular rate constants for iodide quenching of the triplet states has been expressed in Arrhenius form. The linearity of the obtained Arrhenius plots clearly indicated the existence of one temperature-dependent non-radiative process for the de-excitation of I2C and I5C triplet state in the presence of iodide. This process may be attributed to the solute-quenching by iodide and, most probably, proceeds via reversibly formed exciplex. The activation energies obtained from linear Arrhenius plots (1.89 kcal/mol for I5C; 2.55 kcal/mol for I2C) are smaller as compared to that for diffusion controlled

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reactions in aqueous solution (about 4 kcal/mol), which may indicate the great importance of the electrostatic interactions between solute and iodide ions in lowering the energy barrier needed for the formation of the triplet-quencher complex. Based on the theoretical predictions (at the DFT(CAM-B3LYP)/6-31 + G(d,p) level of theory) and careful analysis of the obtained FTIR spectra it may be concluded that in the solid state I2C and I5C molecules form associates by intermolecular NH···O=C and OH···O=C hydrogen bonding interactions, whereas the existence of intramolecular NH···O=C interactions in the solid state of I2C and I5C is highly unlikely.

Introduction

Remarkable sensitivity of indole to the local changes in its environment allows to use this chromophore of the side chain of tryptophan, as a natural spectroscopic probe in the studies of protein structure and dynamics. The importance of these studies is undisputed due to the fact that biological functions of proteins are determined by their structural flexibility and molecular dynamics. Since conformational changes of proteins may occur within a broad timescale of femtoseconds to seconds, fluorescence and phosphorescence spectroscopy has a great potential and applicability for studying structural flexibility and monitoring internal dynamics of tryptophan containing proteins.

Fluorescence from tryptophan containing proteins is a well-established phenomenon and has been exploited for structural and dynamics studies for several decades. As compared to fluorescence, phosphorescence offers some advantage over fluorescence which justifies the revival and growing interest in the application of phosphorescence spectroscopy as a powerful means of gaining insight into the dynamics of conformational changes in proteins. First of all, the long phosphorescence lifetime permits the monitoring of processes in proteins that occur on the millisecond-second range, which is very relevant to folding processes and enzymatic activity and which conventional fluorescence spectroscopy cannot access. Long phosphorescence lifetime also makes phosphorescence considerably more sensitive to quenching (comparing with fluorescence), an attribute that can be used for studies of protein conformation and flexibility. Since phosphorescence spectra are resolved, it is often possible to distinguish the emission of individual tryptophans, which is in contrast with fluorescence: the resolution of fluorescence from individual tryptophans is often obscured by the broad fluorescence emission band [1,2]. Moreover, the use of phosphorescence is advantageous in *in vivo* measurements because it enables omitting the high fluorescence background which arises from the native fluorescence from cells [1,3].

Although the above mentioned advantages, as compared to fluorescence, protein phosphorescence receives much less attention. There are many reasons for this, from which the most important are: low triplet quantum yield, exceptional sensitivity to quenching, significant difficulty in performing of phosphorescence measurements requiring efficient deoxygenation of the samples. A very important factor, which contributes to the relatively low popularity of phosphorescence techniques in the studies on protein structure and dynamics is the existing in the literature discrepancy regarding photophysical parameters crucial in indole triplet state decay kinetics, such as triplet state lifetime of indole and its derivatives [4–6], indole triplet quantum yield and the rate constant for intersystem crossing $S_1 \rightarrow T_1$ [4,7,8]. Additionally, little is known about the influence of various ionic substituents on the phosphorescence properties of indole, which also contributes to the limited application of phosphorescence spectroscopy in protein studies.

In general, the triplet state lifetimes of aqueous indoles determined from flash photolysis experiments are microsecond long [4,7,9], whereas recently reported phosphorescence lifetimes of aqueous indoles, determined from photon counting phosphorescence techniques vary from μs [6] to ms [5]. There is also a significant discrepancy in the previously reported values of indoles triplet quantum yields. For example, triplet quantum yield of aqueous tryptophan, estimated by Tsentalovich [4] using flash photolysis was 0.065 ± 0.012, which is lower than both the value of 0.18 estimated by Volkert [7] and the value of 0.12–0.14 determined by Chen and coworkers [8]. It should be noted in this point that the latter authors applied the method of Medinger and Wilkinson [10] as modified by Vander Donckt and Lietaer [11], which assumes that intersystem crossing is the only non-radiative process affected by the heavy-atom quenchers. If other non-radiative processes are introduced or enhanced in the presence of the heavy-atom quencher, the triplet yield determined by this method will be overestimated. Closer to Tsentalovich's results was the value of 0.09, calculated by Robbins et al. [12] based on fluorescence decay measurements and the value of 0.10, calculated from experimental data collected by Bent and Hayon [9]. Some inconsistence in the existing in the literature values of the rate constants for aqueous indoles triplet state quenching by their ground states may also be found. According to Tsentalovich et al. [4], the triplet state of tryptophan is quenched by its ground state with the rate constant of $1.2 \times 10^7\ M^{-1}\ s^{-1}$ and the most probable quenching mechanism is electron transfer. The above mentioned value of triplet quenching by ground-state tryptophan differs significantly from the value reported by Volkert et al. [7] ($1.1 \times 10^8\ M^{-1}\ s^{-1}$) and by Song [13] ($3.7 \times 10^8\ M^{-1}\ s^{-1}$), but is in excellent agreement with the results obtained by Strambini [5] (the reported second-order quenching rate constants for indole and NATA are $1.2 \times 10^7\ M^{-1}$ - s^{-1} and $1.7 \times 10^7\ M^{-1}\ s^{-1}$, respectively).

Only few studies have been carried out to study the effect of substituents on the photophysical properties of indole chromophore. The importance of these studies is evident, since they are expected to manifest the great sensitivity of the indole chromophore to the local changes in polarity and structural rigidity of its surroundings. Previous theoretical studies indicated that introduction of carboxylic group or other small substituents in the indole ring results in significant change in electronic charge distribution of indole chromophore, therewith both the position and the kind of substituent is important [14]. The structure and FTIR spectrum in the solid state of indole-2-carboxylic acid have been recently determined [15]. The effect of carboxyl group substitution in the 2 and 5 positions of indole ring on the absorption spectra and fluorescence properties have been studied in detail [16,17], but so far, to our knowledge, there is no work devoted to investigation of room temperature phosphorescence properties of aqueous indole derivatives with carboxyl group substituted in the 2 and 5-position of indole ring. This prompt us to apply our previously proposed methodology [18], based on both fluorescence and phosphorescence decay kinetics to determine photophysical parameters crucial in triplet state decay kinetics of aqueous indole and its derivatives: indole-2-carboxylic acid (I2C) and indole-5-carboxylic acid (I5C). This methodology, combined with efficient deoxygenation of the sample, is based on the well-known heavy atom effect: the presence of heavy atoms induces a strong spin–orbit coupling, which results in the enhancement of the intersystem