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Temperature study of indole, tryptophan and N-acetyl-L-tryptophanamide (NATA) triplet-state quenching by iodide in aqueous solution

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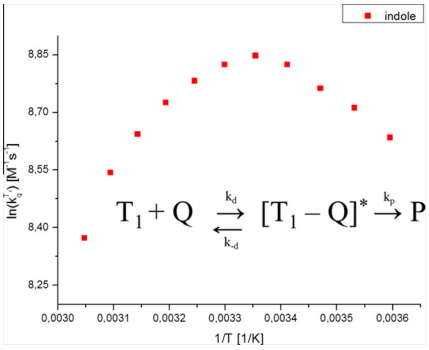
HIGHLIGHTS

- Temperature dependence of indole triplet state quenching by iodide was determined.
- Diffusion-controlled quenching by iodide proceeds via reversibly formed exciplex.
- Highly curved Arrhenius plots were obtained above 0.06 M of iodide.
- A change in the rate determining step over the temperature range was suggested.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the temperature dependence of the measured phosphorescence lifetimes of aqueous indole, tryptophan and N-acetyl-L-tryptophanamide (NATA) between 6 and 55 °C in the absence and in the presence of iodide, a suitable intersystem crossing enhancer, has been determined. The obtained results suggest the existence of one process for the temperature-dependent, non-radiative deactivation of triplet states of the aqueous indoles in the absence of iodide. This process may be associated with the high sensitivity of indole triplet state lifetime to the subtle changes in the local viscosity of the surrounding aqueous environment or may be attributed to diffusional quenching by solvent molecules and/or by possible impurities present in water. However, the steep decrease in the measured phosphorescence lifetimes of indole and tryptophan with temperature suggests that diffusion-mediated quenching processes are not prevailing. Upon increasing concentration of iodide (up to 0.1 M), the obtained Arrhenius plots for the deactivation rate ($1/\tau_{ph}$) of the triplet states of the studied indoles were linear, which provided strong support for the hypothesis of the existence of one temperature dependent non-radiative process for the de-excitation of indoles triplet state. Our results showed that this process is attributed to the diffusion-controlled solute-quenching by iodide and, most probably, proceeds via reversibly formed exciplex. At concentration of iodide higher than 0.1 M highly curved Arrhenius plots were obtained, which may indicate a change in the rate determining step with a change in temperature. This change most probably is associated with a transition from diffusion-controlled exciplex formation followed by rate-determining exciplex deactivation at high temperature.

Introduction

The strong dependence of the tryptophan room temperature phosphorescence (RTP) lifetime on the rigidity of the environment, first recognized in 1985 by Strambini and Gonnelli [1], showed the

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potential of tryptophan RTP lifetime measurements for probing slow conformational changes in proteins occurring within microsecond to second time range [2,3]. The lifetime of unquenched tryptophan in proteins at ambient temperature ranges from 0.2 ms for a solvent-exposed tryptophan residue to 4 s for one which is deeply buried within the protein interior [4].

The room temperature phosphorescence lifetime of tryptophan residues in proteins which are exposed to solvent should show similarity to that of free tryptophan in solution, therefore it is of a great importance to determine the lifetime of aqueous triplet state of indole and its derivatives, tryptophan and NATA (N-acetyl-L-tryptophanamide). The latter indole derivative is considered as the standard reference compound for tryptophan in proteins by its mimicking of the amino acid's attachment in the backbone chain.

So far, the triplet state of indoles were determined applying two different experimental techniques: flash photolysis and phosphorescence decay measurements. In flash photolysis, the triplet state lifetime is determined from triplet–triplet absorption following flashlamp excitation, while phosphorescence decay measurements are performed utilizing pulsed UV excitation and sensitive photon-counting techniques. Due to unfavorable conditions required in flash photolysis measurements (high concentration of chromophore, which may increase the possibility of the occurrence of second-order reactions, such as triplet–triplet annihilation; high-energy excitation pulse, which may contribute to the generation of various photoproducts) and since phosphorescence intensity depends on triplet state concentration, photon counting phosphorescence techniques seem to be more reliable in the determination of triplet state lifetime.

One of the requirements for obtaining efficient (detectable) room temperature phosphorescence signal is the efficient population of the triplet state, which may be achieved applying so called heavy-atom induced phosphorescence methodology. This methodology 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 crossing rate in the molecule. Since heavy atoms increase both the rate of spin forbidden radiative (phosphorescence) and radiationless $T_1 \rightarrow S_0$ transitions, the decrease in phosphorescence lifetime is observed in the presence of heavy atoms. Other effects observed in the presence of heavy atoms are the increase of phosphorescence quantum yield and fluorescence quenching [5]. The increase in phosphorescence intensity results from an increase in the rate of intersystem crossing for $S_1 \rightarrow T_1$ [6–9]. It has been shown that, if unimolecular decay is the main route leading to the depopulation of the triplet state, the rate of radiationless intersystem crossing transition from a perturbed singlet state to the triplet state is more sensitive to heavy atom perturbation than the rate of spin forbidden transition from the perturbed triplet to the ground state [5].

However, efficient population of the triplet state is not the only requirement for obtaining room temperature phosphorescence: its subsequent radiation deactivation (by phosphorescence) should also be efficient, i.e. it should be able to compete with the other deactivation pathways including quenching by impurities and molecular oxygen present in the sample. Therefore, the relative importance of RTP can be increased by reducing the rates of the non-radiative deactivation pathways. This can be achieved by enhancing the rigidity of the environment-for example, by inclusion of the phosphorophore into micells or polymer-and by efficient deoxygenation of the sample [10]. Alternatively, the heavy-atom induced phosphorescence (HAIP) method may be used in order to obtain detectable phosphorescence signal from the samples in which no protective medium is needed. This methodology is often combined with efficient deoxygenation of the sample.

Studies on the temperature dependence of phosphorescence lifetime should provide information on the possible mechanism for the temperature-dependent, non-radiative decay (de-excitation) of the triplet state. The temperature dependence of the phosphorescence lifetime of indole and its derivatives may be associated with the high sensitivity of indole triplet state lifetime to the changes in the viscosity of the surrounding environment [1,3].

According to the excess-energy model [11,12], the phosphorescence lifetime in high-viscosity media is predominantly determined by a temperature-dependent non-radiative process enabled by the thermal (Arrhenius) activation of certain excited triplet-state vibrational modes of the chromophore with large spin-vibronic coupling [13]. The temperature dependence of this process is associated with the activation energy necessary to populate these vibrational modes.

In low viscosity solution, both collisional (diffusive, diffusion controlled) triplet state quenching processes (associated with solvent interactions or/and with the presence of possible impurities which may act as quenchers) and thermal activation to higher vibrational states may affect the triplet state lifetime.

In the absence of quenchers, the decrease in phosphorescence lifetime with temperature reflects an increase in the rate of non-radiative decay of the excited triplet state due to an increase in the rate of non-radiative decay to the ground state S_0 [14].

To our knowledge there is only few studies on the temperature dependence of triplet deactivation processes of indole and its derivatives. Fischer et al. [13] measured the temperature dependence of indole phosphorescence lifetime in water and in polymethyl methacrylate (PMMA). No changes in the phosphorescence lifetime of aqueous indole over the temperature range 5–45 °C has been reported, which allowed the authors to exclude the presence of diffusional quenchers. The addition of acrylamide resulted in significant decrease in phosphorescence lifetime of indole and the extent of quenching was the function of the temperature (i.e. viscosity). The observation of Fischer et al. [13] that the phosphorescence lifetime of aqueous indole is viscosity independent over a range of 2.5-fold change in viscosity is quite surprising in light of previously reported works [1,3], which suggested a correlation between viscosity and phosphorescence lifetime for the indole chromophore.

In the solid matrix of PMMA collisional triplet-state quenching processes are inhibited, which gave Fischer et al. [13] the opportunity to determine the activation energy for the excess energy triplet-state deactivation mechanism. Arrhenius plot of the phosphorescence lifetime of indole in PMMA glass showed a break at approximately 200 K between the cold temperature limit of 1.08 kcal/mol and 3.94 kcal/mol at higher temperatures.

In this study we have applied heavy-atom induced phosphorescence methodology combined with an efficient deoxygenation of the samples to determine the temperature dependence of phosphorescence lifetimes of aqueous indole, tryptophan and NATA. We have used potassium iodide, which is a well-known suitable enhancer of intersystem crossing for indole [15,16] and tryptophan [15].

Materials and methods

Materials

Indole, N-acetyl-L-tryptophanamide (NATA), L-tryptophan, potassium iodide (KI), sodium sulfite (Na_2SO_3) were of the highest purity grade available from commercial sources (Sigma–Aldrich) and were used without further purification. All solutions were freshly prepared using high-purity water (18.2 MΩ) from Milli-Q system by mixing stock solutions (10^{-3} M for indole; 1 M for KI) in aqueous medium.