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Room temperature fluorescence and phosphorescence study on the interactions of iodide ions with single tryptophan containing serum albumins

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ABSTRACT

In this study, the influence of heavy-atom perturbation, induced by the addition of iodide ions, on the fluorescence and phosphorescence decay parameters of some single tryptophan containing serum albumins isolated from: human (HSA), equine (ESA) and leporine (LSA) has been studied. The obtained results indicated that, there exist two distinct conformations of the proteins with different exposure to the quencher. In addition, the Stern–Volmer plots indicated saturation of iodide ions in the binding region. Therefore, to determine quenching parameter, we proposed alternative quenching model and we have performed a global analysis of each conformer to define the effect of iodide ions in the cavity by determining the value of the association constant. The possible quenching mechanism may be based on long-range through-space interactions between the buried chromophore and quencher in the aqueous phase. The discrepancies of the decay parameters between the albumins studied may be related with the accumulation of positive charge at the main and the back entrance to the Drug Site 1 where tryptophan residue is located.

1. Introduction

Structural flexibility of proteins is strongly correlated with their biological functions. Slow collective motions associated with transitions between conformational substates in the submicrosecond to second time domain are considered to be directly linked to enzymatic activity, signal transduction and protein–protein interactions [1]. Despite their biological significance, these slow proteins motions are still poorly characterized. One approach to gain insight into the structural fluctuations of proteins is to monitor tryptophan fluorescence and phosphorescence quenching by solutes of various molecular sizes. Luminescence quenching of tryptophan containing proteins by small solutes has been widely used in the structural and dynamical studies of proteins, since it can provide information on the location of tryptophan residue and its accessibility to the quenching moiety. Moreover, the fluorescence and phosphorescence quenching studies have a potential for revealing (uncovering) structural changes in the conformation of polypeptide that permit diffusion of solutes through well-packed internal regions of the proteins [1–4].

Various mechanisms have been proposed to account for protein fluorescence and phosphorescence quenching by added solutes. The first one assumes diffusive penetration of quencher through the protein matrix into its proximity [3], which sometimes requires transient formation of channels or the opening of gates of commensurate size to facilitate the quencher to penetrate the protein (so called protein-gated quencher penetration model). A key feature of the penetration gating mechanism is that it relies on segmental protein motions and therefore is intimately connected to structural flexibility [1]. Alternative quenching mechanisms involve either diffusion of quencher to the protein surface followed by long-range interactions with the buried chromophore (external quenching) or transient local unfolding or large amplitude structural motions of the protein that bring the normally buried chromophore on the surface, both of which permit efficient reaction with quencher in the solvent. An additional contribution to quenching may come from quencher binding to the protein surface [5]. Internal migration of a quencher and partial unfolding transitions bringing tryptophan to the aqueous interface, both require protein structural fluctuations of relatively large amplitude that presumably occur in the microsecond time domain or slower.

It should be underlined that the contributions from gating and binding tend to saturate at high quencher concentrations. As a consequence, these processes give rise to nonlinear, downward-curving Stern–Volmer plots [1,3,5].

The general feature of protein structural motions which are required for quencher access in the protein-gated quencher penetration model is

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their sensitivity to solvent viscosity and to the increase in the local rigidity induced by ligand binding (the increase in solvent viscosity and the local rigidity caused by ligand binding is supposed to dampen structural fluctuations in the protein and therefore reduce the rate of internal migration of the quencher within protein) [3]. A decisive test for discriminating quencher penetration from alternative quenching mechanisms is the dependence of the penetration rate on quencher size [2].

External quenching of protein luminescence by freely diffusing quencher in the aqueous phase is not dependent on the size and the charge of the quencher. Moreover, it is also insensitive to either solvent viscosity or to the structural dynamics of proteins [6]. Long-range external quenching can be either dynamic or static or both, depending whether it involves, respectively, collisions with or reversible quencher binding to the protein surface in the proximity of chromophore [2]. In the worst cases binding may even cause perturbations of the native structure [4]. Partial unfolding transitions leading to transient exposure of internal chromophores to the protein surface would lead to interactions with quencher in the aqueous phase with little discrimination on quencher size and charge [6].

In this study we have examined the effect of iodide ions on the determined fluorescence and phosphorescence decay parameters of some single tryptophan containing serum albumins isolated from: human (HSA), equine (ESA) and leporine (LSA).

Relatively few spectroscopic studies have been devoted to the interactions of iodide ions with protein tryptophan. Previously [7] it has been found that the mechanism of the iodide fluorescence quenching of NATA (*N*-acetyl-L-tryptophanamide), which is considered as the representative of tryptophan residues in proteins, can be explained by the short range heavy atom exchange interactions between the singlet excited tryptophan and iodide causing the increase of the ISC process. These interactions require close contact between the reactants, in the range of 4–6 Å.

It was also previously suggested that iodide ions due to their charged nature are relegated to the aqueous medium and therefore act exclusively through space [8]. It is expected that due to their shortest distance range, the interactions with iodide ions exhibit the smallest quenching effectiveness for buried tryptophan residues [4].

Previous studies have shown that when the aromatic ring is within a few Å of van der Waals contact with the iodide ions, there is an increase of up to 2–3 times in phosphorescence quantum yield followed by a considerable shortening of the triplet-state lifetime [9]. Therefore, the existence of heavy atom interactions with tryptophan residue of the proteins studied may be useful in the estimation of the location of tryptophanyl side chains with respect to the solvent-protein interface.

In this study we have examined the influence of heavy-atom perturbation, induced by the addition of iodide ions, on the fluorescence and phosphorescence decay parameters of HSA, ESA and LSA. Additionally, to rationalize the presence or absence of interactions between tryptophan residue and iodide ions we have analysed the crystallographic structure as well as the calculated electrostatic potential maps for the ground states of the proteins studied assuming that upon excitation of Trp residue ($\lambda_{ex} = 295$ nm) and intersystem crossing to the excited triplet state there are little changes in electrostatic potential in the regions of macromolecule around indole ring chromophore.

2. Materials and methods

2.1. Materials

Equine serum albumin (ESA) was purchased from Equitech. Tryptophan, human serum albumin (HSA), leporine serum albumin (LSA), potassium iodide (KI) and sodium sulfite (Na_2SO_3) were of the highest purity grade available from Sigma-Aldrich. Tryptophan, potassium iodide (KI) and sodium sulfite (Na_2SO_3) were used without further purification. Since the purity of the purchased albumins were estimated to be 96–98%, additional purification of these proteins, (i.e. the elimination of both the fatty acids which are bound to the protein [10] and dimers which are formed during protein isolation with the involvement of the unpaired Cys34) has been performed. Removal of fatty acids was accomplished by adsorption onto charcoal (Fluka, St Louis, Missouri, USA [11]). The disulfide-bridged dimer formed during protein isolation was removed by gel-filtration chromatography on an A¨ KTA FPLC system using an XK 16/100 column (Amersham Biosciences, Uppsala, Sweden) filled with Superdex 200 prep grade. The solutions of Tryptophan, HSA, ESA and LSA were prepared in 10 mM Tris-NaCl buffer (pH 7) using high-purity water (18.2 MΩ) from Milli-Q system.

The analysis of secondary and tertiary structures of the albumins studied were conducted utilizing the crystallographic structures deposited in the Brookhaven Protein Data Bank and the program PyMOL [12]. Calculation and presentation of electrostatic potential maps of the proteins were conducted utilizing the crystallographic structures deposited in the Brookhaven Protein Data Bank and the program PyMOL [12].

2.2. Methods

2.2.1. Steady-state fluorescence measurements

Steady-state fluorescence measurements were performed with a FluoroMax4 (Jobin Yvon Spex) spectrofluorimeter, using an excitation wavelength of 295 nm. All measurements were performed in a standard quartz cuvette at 20 °C.

2.2.2. Time-resolved fluorescence measurements

Fluorescence lifetime measurements were carried out at 20 °C with a FL900CDT time-correlated single photon counting fluorimeter from Edinburgh Analytical Instruments. The excitation and emission wavelengths were set to 295 nm and 340 nm, respectively. Data acquisition and analysis were performed using the software provided by Edinburgh Analytical Instrumentation.

2.2.3. Phosphorescence measurements

Phosphorescence measurements were made on a homemade system. A general description of the equipment for phosphorescence measurements has previously been given [13]. Since molecular oxygen is known to be a strong quenching agent, it was efficiently removed from the sample before the phosphorescence measurements. O_2 removal was achieved by the application of moderate vacuum and inlet of ultrapure nitrogen. The pre-purified nitrogen gas (0.1 ppm of O_2) was further purified by passing through an oxygen-trapping filter. This degassing procedure was additionally accompanied by the addition of 0.3 ml of 0.1 M Na_2SO_3 as an O_2 scavenger (final concentration of Na_2SO_3 in the sample was 0.01 M). Additionally, the presence of Na_2SO_3 stabilizes the iodide solution and prevents the formation of iodine. The sample (protein solution with 0.01 M Na_2SO_3) was placed in a quartz cuvette, which was connected to the N_2/vacuum line by tubing. Five cycles of de-oxygenation were performed. After de-oxygenation, the cuvette was moved into the phosphorimeter, while remaining attached to the tubing and allowed to equilibrate before taking measurements. The background emission was determined by measurements carried out before de-oxygenation of the sample and was subtracted from the phosphorescence decay. All phosphorescence decays, after subtraction of the backgrounds, were analysed in terms of a sum of exponential components by a nonlinear least squares fitting algorithm using the software provided by Origin Pro 8.0.

2.2.4. Fluorescence emission spectral fitting

Fluorescence emission spectra were normalized and fit by application of Franck–Condon analysis of emission bands profiles, as described