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Room temperature phosphorescence study on the structural flexibility of single tryptophan containing proteins

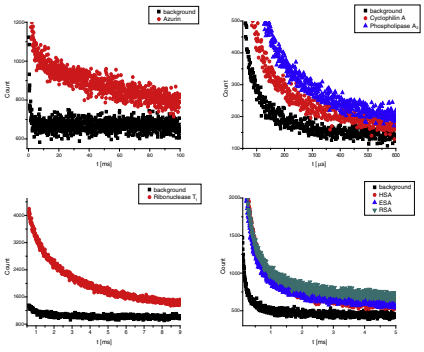
Agnieszka Kowalska-Baron *, Krystian Gałęcki, Stanisław Wysocki

Institute of General Food Chemistry, Faculty of Biotechnology and Food Sciences, Technical University of Lodz, ul. Stefanowskiego 4/10, 90-924 Lodz, Poland

HIGHLIGHTS

- Spectroscopic parameters of single tryptophan containing proteins were determined.
- Various structural indicators of protein flexibility/rigidity were analyzed.
- Long RTTP lifetimes correlate with high hydrophobicity of indole microenvironment.
- Protein RTTP lifetimes correlate with their B-factors for tryptophan residue.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we have undertaken efforts to find correlation between phosphorescence lifetimes of single tryptophan containing proteins and some structural indicators of protein flexibility/rigidity, such as the degree of tryptophan burial or its exposure to solvent, protein secondary and tertiary structure of the region of localization of tryptophan as well as B factors for tryptophan residue and its immediate surroundings. Bearing in mind that, apart from effective local viscosity of the protein/solvent matrix, the other factor that concur in determining room temperature tryptophan phosphorescence (RTTP) lifetime in proteins is the extent of intramolecular quenching by His, Cys, Tyr and Trp side chains, the crystallographic structures derived from the Brookhaven Protein Data Bank were also analyzed concentrating on the presence of potentially quenching amino acid side chains in the close proximity of the indole chromophore. The obtained results indicated that, in most cases, the phosphorescence lifetimes of tryptophan containing proteins studied tend to correlate with the above mentioned structural indicators of protein rigidity/flexibility. This correlation is expected to provide guidelines for the future development of phosphorescence lifetime-based method for the prediction of structural flexibility of proteins, which is directly linked to their biological function.

Introduction

Current views of protein structure have been created largely based on the results from X-ray crystallography and NMR studies. Although such structural determinations are extremely useful, they provide only a static or dynamically averaged model of the protein, while the macromolecular function of a protein is related to its flexibility [1]. In general, information about residue flexibility is provided by Debye–Waller factors (B-values) reported in experimental atomic-resolution structures. They represent the decrease of intensity of diffraction due to both the dynamic disorder caused

* Corresponding author. Tel.: +48 42 631 34 26; fax: +48 42 636 28 60.
E-mail address: agnieszka.kowalska-baron@p.lodz.pl (A. Kowalska-Baron).

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by the temperature-dependent vibration of the atoms and due to static disorder that is related to the orientation of the molecule [2]. As a rule highly mobile residues located usually on the surface of the protein and exposed to solvent are characterized by high B-values, while low temperature factors for the particular residue and its immediate surroundings are indicative of a rigid site in a protein. However, the experimentally determined B-value is not an absolute quantity; instead it depends on many factors such as the overall resolution of the structure, crystal contacts and on the particular refinement procedures [2].

The remarkable sensitivity of the excited state lifetime of indole chromophore to subtle changes in its local microenvironment allows to use this chromophore of the side chain of tryptophan as a natural spectroscopic probe in the studies of protein structure and dynamics. Conformational changes of proteins may occur within a broad timescale of femtoseconds to seconds [3]. Therefore, both fluorescence and phosphorescence spectroscopy have a great potential and applicability for studying structural flexibility and monitoring internal dynamics of tryptophan containing proteins [4].

The above mentioned remarkable sensitivity of room temperature tryptophan phosphorescence (RTTP) lifetime to subtle changes in the local environment of the emitting tryptophan residue, and the fact that the RTTP lifetime is the order of magnitude of the timescale of biologically relevant processes such as protein folding, enzymatic catalysis, signal transduction and protein–protein interactions make RTTP a particularly useful technique in studying protein structure and its conformational dynamics [4]. Phosphorescence lifetime has been considered to be a very sensitive monitor of protein conformation and flexibility. Numerous studies have shown the utility of the phosphorescence method based on RTTP lifetime measurements in the studies of changes in the flexibility of the proteins in solution induced by denaturation or renaturation [5–7], transition of the protein conformations into partially folded states [5,7], upon changes in the solvent viscosity [8], hydrostatic pressure [9], pH, ionic strength [5,10], binding of substrates, inhibitors or allosteric effectors [5,10], removal of cations from the active center of the enzyme [5], limited proteolysis [5], association and aggregations [5,11]. Moreover, RTTP has the potential of providing a reporter signal free of the autofluorescence from other proteins in the cellular environment [12].

However, as compared to fluorescence, phosphorescence emission from protein tryptophan remains relatively unexplored in terms of investigation of dynamic protein structure–function relationships. There are many other reasons for the relatively low popularity of phosphorescence spectroscopy in protein studies, from which the most important are: low tryptophan triplet quantum yield, its exceptional sensitivity to quenching as well as 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 [13–15], indoles triplet quantum yield and the rate constant for intersystem crossing $S_1 \rightarrow T_1$ [13,16,17]. In addition, the wide application of phosphorescence spectroscopy in protein studies is also limited by the absence in the instrumentation market of mass-produced phosphorimeters suited for recording RTTP [18].

The usefulness of phosphorescence approach in the protein studies depends critically on the correct interpretation of the measured phosphorescence lifetime. Such an interpretation, however, is very difficult since, the triplet state lifetime, being extremely sensitive to the local environment of the emitting residue, is affected by many factors such as solvent viscosity, proximity of charges and quenchers (both intermolecular and intramolecular quenchers) as well as the rigidity of the residue [4].

In the absence of quenchers the phosphorescence lifetime of tryptophan is largely dominated by the effective viscosity of the medium. As a result, the intrinsic phosphorescence lifetime of tryptophan residues in proteins reflects the local flexibility of the protein matrix [19]. A wealth of observations with tryptophan residues in proteins provide strong support for a correlation between tryptophan phosphorescence lifetime and the local viscosity of protein. For example, tryptophan residues that occupy very mobile protein sites such as residues exposed to aqueous phase or located on the surface of the macromolecule are characterized by short-lived RTTP. Conversely, long-lived RTTP in proteins is a prerogative of residues buried deeply inside macromolecule's interior, in regions of protein that are disposed in rigid hydrophobic core of the globule.

The dominating quenching mechanism of protein tryptophan phosphorescence in the oxygen-free medium is deactivation of triplet-excited states due to collisions of the indole ring chromophore with the surrounding structural elements of the macromolecule. The triplet states of tryptophan residues located at the periphery of the globule in the highly mobile environment are deactivated mainly via a nonradiative mechanism resulting in the effective dynamic quenching of RTTP. Moreover, in cases when the indole ring of the tryptophan residue is in close contact with side chains of some phosphorescence–quenching amino acids (AAs), then static (intramolecular) quenching may be present along with the dynamic quenching of the RTTP. Quenching capabilities of various amino acids have been recently evaluated (based on the magnitude of the determined Stern–Volmer rate constants) by the group of Gonnelli and Strambini [20]. They have concluded that among the AAs studied, quenching reactions at 20 °C were quite effective with His, Tyr, Trp, cysteine and cysteine (with rate enhancement of 20 and 50 times when the site chains of Tyr and His, respectively, are in the ionized form [20]). Additionally, a special role in the intramolecular quenching is played by disulfide groups, which quenching ability have been associated with their capability to accept electron from triplet state tryptophan [21].

Static quenching of RTTP by the side chains of the a potentially quenching amino acids (AAs) is possible only if the distance between the indole ring and the quenching group does not exceed several Å. Moreover, the quenching efficiency depends also on the orientation of the quenching group relative to the tryptophan residue indole ring. Therefore, the presence of the potential RTTP quenching residues in the tryptophan microenvironment does not necessarily results in phosphorescence quenching [21]. Intramolecular quenching may occur not only if the indole ring chromophore is in permanent contact with the quenching AA residues (static quenching), but also if it is transiently brought into contact with the quencher by protein structural fluctuations. Consequently, efficiency of the quenching by the AAs side chains which are located at the proximity of indole ring is also determined to a great extent by intramolecular mobility of the chromophore microenvironment [21].

The aim of this study is to test the utility of the phosphorescence lifetime as the direct indicator of structural flexibility/rigidity of tryptophan containing proteins based on the correlation between the determined phosphorescence lifetimes of tryptophan residues and their temperature factors, which are considered as a measure of residues flexibility. For this purpose, a series of commercially available single tryptophan containing proteins were selected to test the importance of factors such as the degree of tryptophan burial and its structural rigidity. Protein systems were selected to satisfy the following criteria: (1) Phosphorescence emission arises from a single and structurally identified tryptophan residue (2) Protein systems provide varying degrees