Pharmaceutica Analytica Acta
ISSN: 2153-2435

Albani, Pharm Anal Acta 2015, 6:12
DOI: 10.4172/2153-2435.1000452

Research Article Open Access

Fluorescence Study of Bovine β-Lactoglobulin

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Abstract

β-Lactoglobulin consists of a single polypeptide of 162 amino acid residues (Mr=18,400). Tertiary structure of β-lactoglobulin possesses a pocket (calyx) where hydrophobic ligands can easily bind. The protein normally exists as a dimer, each monomer having one free cysteine and two disulphide bridges. Quaternary structure of the protein varies with the pH. For example, at pH 2, β-lactoglobulin is in a molten globule state, stable although partially unfolded, and at pH 12 the protein is denatured. At some pH, mixtures of both monomeric and dimeric forms are found.

Keywords: Amino acid; Protein; β-lactoglobulin; Calcofluor white

Introduction

β-Lactoglobulin contains 2 Trp residues, Trp 19 present in a hydrophobic pocket and Trp 61 present at the surface of the protein near the pocket [1-5]. In order to find out whether both Trp residues contribute to β-lactoglobulin fluorescence or not, time-resolved studies and static quenching were performed in presence of high concentrations of calcofluor white, a fluorophore that is specific to both carbohydrate residues and hydrophobic sites in proteins. When protein fluorescence occurs from both intrinsic and surface Trp residues, recorded emission spectrum will be the result of the contribution of each residue to the total fluorescence [6]. Addition of high calcofluor white concentrations to proteins (ratio 10 to 1) allows total quenching of protein extrinsic tryptophan residue without any denaturation. In this case, binding of calcofluor white at high concentration on the protein, will yield a fluorescence emission spectrum that characterizes Trp residue embedded in the protein core and thus with an emission peak that is shifted to shorter wavelengths compared to the spectrum recorded in absence of calcofluor white [7,8].

Addition of high calcofluor white concentrations to β-lactoglobulin quenches fluorescence emission intensity without modifying position of the spectrum peak (331 nm). Also, difference between the two spectra (in absence and presence of calcofluor white) yields an emission spectrum with a peak located at 332 nm and not at 340 or 345 nm, an emission peak characteristic of Trp residue present at the protein surface. This result clearly indicates that Trp 61 residue does not contribute to the protein emission. These results were obtained at different pH including pH 2 and pH 7, where β-lactoglobulin is in a dimeric state. Thus, absence of fluorescence from Trp 61 residue is independent of the ratio monomer/dimer present in solution and of any possible differences in the local structure of the protein around Trp residues. Also, since identical results were obtained whether the protein is in a 100% monomeric state (pH 2) or when it is in a dimeric state (pH 7), absence of Trp 61 residue fluorescence cannot be attributed to the self-quenching of Trp 61 by the nearby Trp 61 of the other monomer, in the β-lactoglobulin dimeric form [9]. Absence of emission of β-lactoglobulin Trp 61 residue can be explained by the fact that Trp 61 residue is not necessarily excited, as the result of its close interaction with other amino acids whether cysteine disulfide bridge (Cys66-Cys160 disulfide moiety) ($\approx$ 3.7 Å) or other amino acids [10].

Fluorescence excitation spectrum of β-lactoglobulin in solution recorded in absence and presence of calcofluor white at pH 2 and 7 displays a peak position at 283 nm. Also, global shape of the spectra is the same whether calcofluor white is present or not. These results mean that structural rearrangements within β-lactoglobulin are not occurring upon calcofluor white binding. Energy transfer between Trp 19 residue and calcofluor white occurs with 100% efficiency, i.e., the two fluorophores are very close one to each other (<5 Å). This energy transfer is not Forster type [9].

Binding of calcofluor white to β-lactoglobulin induces a decrease in the fluorescence intensities of both emission and excitation peaks of Trp 19 residue and an increase of calcofluor white fluorescence emission. Analysis of the data obtained from Trp residue or calcofluor white allows obtaining a dissociation constant of β-lactoglobulin-calcofluor white complex equal to 8.45 + 0.05 μM [9].

Fluorescence intensity decay I (λ,t), of β-lactoglobulin Trp 19 residue at pH 2 (monomeric state) can be adequately represented as

$$I(\lambda,t) = 0{,}140\ e^{-t/0.48} + 0{,}697\ e^{-t/1.49} + 0{,}163\ e^{-t/4.29}$$

Where 0.140, 0.697 and 0.163 are the pre-exponential factors, 0.48, 1.49 and 4.29 ns are the decay times and λ is the emission wavelength (330 nm) (χ^2=1.054).

In presence of 116 μM calcofluor, fluorescence intensity decay can be described as

$$I(\lambda,\tau) = 0.175\ e^{-t/0.73} + 0.745\ e^{-t/2.032} + 0.098\ e^{-t/5.26}$$

Where 0.175, 0.745 and 0.098 are the pre-exponential factors, 0.73, 2.032 and 5.26 ns are the decay times and λ is the emission wavelength (330 nm) (χ^2=0.99).

At pH 8 and at 330 nm, fluorescence intensity decay I (λ,t), of β-lactoglobulin Trp 19 residue can be adequately represented as

$$I(\lambda,\tau) = 0.2707\ e^{-t/0.67} + 0.5606\ e^{-t/1.83} + 0.1687\ e^{-t/5.12}$$

(χ^2=0.98).

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Received November 03, 2015; **Accepted** December 07, 2015; **Published** December 10, 2015

Citation: Albani JR (2015) Fluorescence Study of Bovine β-Lactoglobulin. Pharm Anal Acta 6: 452. doi:10.4172/21532435.1000452

In presence of 178 μM calcofluor, fluorescence intensity decay can be described as

$I(\lambda,\tau) = 0.2403\ e\text{-}t/0.45+0.4758\ e\text{-}t/1.6+0.1839\ e\text{-}t/4.73$

(χ^2=1.3).

Very close values for lifetimes were obtained at the different studied pHs (2 to 12) and where β-lactoglobulin is at different quaternary structure or present in solution in a mixture of dimers and monomers. Our data are interpreted as the results of emission occurring from different substructures of the tryptophan, reached at the excited state. The populations of these substructures characterized by the pre-exponential parameters of the fluorescence lifetimes are dependent on the microenvironment of the fluorophore and on the local protein structure. These populations are modified with the pH as the result of a local structural modification [9].

Conclusion

This work is in good agreement with those recently published concerning origin of tryptophan fluorescence in proteins. Emission and excitation spectra characterize global conformation of the protein. Three lifetimes are in general observed for Trp residue(s) in proteins, two of them are inherent to the tryptophan itself independently of the structure surrounding it while the third one is is generated by the interaction between Trp residue(s) and neighboring amino acids. Also, one should consider tryptophan structure and properties as different from those of NATA and indole. Finally, as it is the case for tryptophan free in solution, tryptophan in proteins cannot be described as a simple structure with one electronic distribution, but should be described as composed by three different substructures, each composed by the tryptophan backbone with its specific electronic distribution [11-15].

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