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Fluorescence and phosphorescence of tryptophan in peptides of different length and sequence

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ABSTRACT

To interpret accurately protein fluorescence and phosphorescence, it is essential to achieve a better understanding of the luminescence properties of tryptophan (Trp, or W) in peptides. In published literature data on luminescence of peptides of varied length are scarce. This article describes studies of fluorescence and phosphorescence properties of the eight Trp-containing synthetic peptides: WAK, AWK, SWA, KYLWE, AVSWK, WVSWAK, WAKLAWE, and AVSWAKLARE. The aim was to investigate which factors influence the fluorescence yield and phosphorescence-spectra and lifetimes. Absorption spectra, room temperature fluorescence emission and corresponding excitation spectra and time-resolved phosphorescence spectra (77 K) have been recorded; the dependence of the fluorescence quantum yield on the specific peptide and its variation with the wavelength of excitation has been studied. The changes in fluorescence yield and shape of phosphorescence spectra are explained in terms of internal electron and proton transfer. The structured phosphorescence spectrum originates from proton transfer occurring upon excitation of Trp, while electron transfer gives rise to a non-structured luminescence spectrum. There is also electron transfer from higher vibronic S_1 states. In the peptides there is higher probability of electron transfer than in Trp alone. The obtained data are interpreted in light of the peptides' sequence, length and conformation.

1. Introduction

Two factors have contributed to the immense popularity of luminescence measurements among workers interested in studying protein folding. First, the overwhelming contribution to the luminescence of the polypeptides and proteins which contain at least one tryptophan (Trp) residue comes from Trp itself; secondly, some of the key spectroscopic properties (lifetimes and quantum yields of emission, spectra) of the Trp molecule undergo, more often than not, large changes in response to subtle variation in its environment. When the protein under investigation contains a Trp residue whose properties in the unfolded form differ markedly from those in the folded conformation, fluorometric detection allows the experimenter to investigate folding with comparatively small quantities of the sample; accordingly, it has been suggested that, when one is dealing with a protein that does not contain an intrinsic Trp residue, it would be profitable to add one [1]. Unfortunately, an unambiguous interpretation of the photophysical data of Trp-bearing proteins has turned out to be far from simple, because even proteins with a single Trp residue fail to show a monoexponential decay of the intensity of emission (whether fluorescence or phosphorescence).

In fact, even fluorescence from solutions of Trp has been found to deviate, in general, from a monoexponential decay [2–5]. Several suggestions, including one about electron transfer from, and another about proton transfer to the N-atom of, the indole nucleus of Trp have been put forward to account for the heterogeneity of the lifetimes of Trp in solution, in proteins, and in synthetic polypeptides, but a comprehensive picture is yet to emerge.

On the other side, there are some studies of Trp phosphorescence, as a delicately sensitive monitor of protein conformation [6–8].

There appears to be a general consensus that, in order to interpret data on protein luminescence, it is essential to acquire a better understanding of similar data on Trp-containing peptides. Some excellent investigations of the fluorescence spectroscopy of peptides have appeared in the literature [1,9], but there seems to be a dearth of studies in which both fluorescence and phosphorescence of peptides of different lengths are examined, and it is clear that such data are sorely needed. The present study was undertaken with a view to taking a first step towards this goal, an aim that could not be accomplished without developing a new approach to recording time-resolved emission spectra of long-lived triplets. Fluorescence and luminescence of the eight synthesized peptides of different length,

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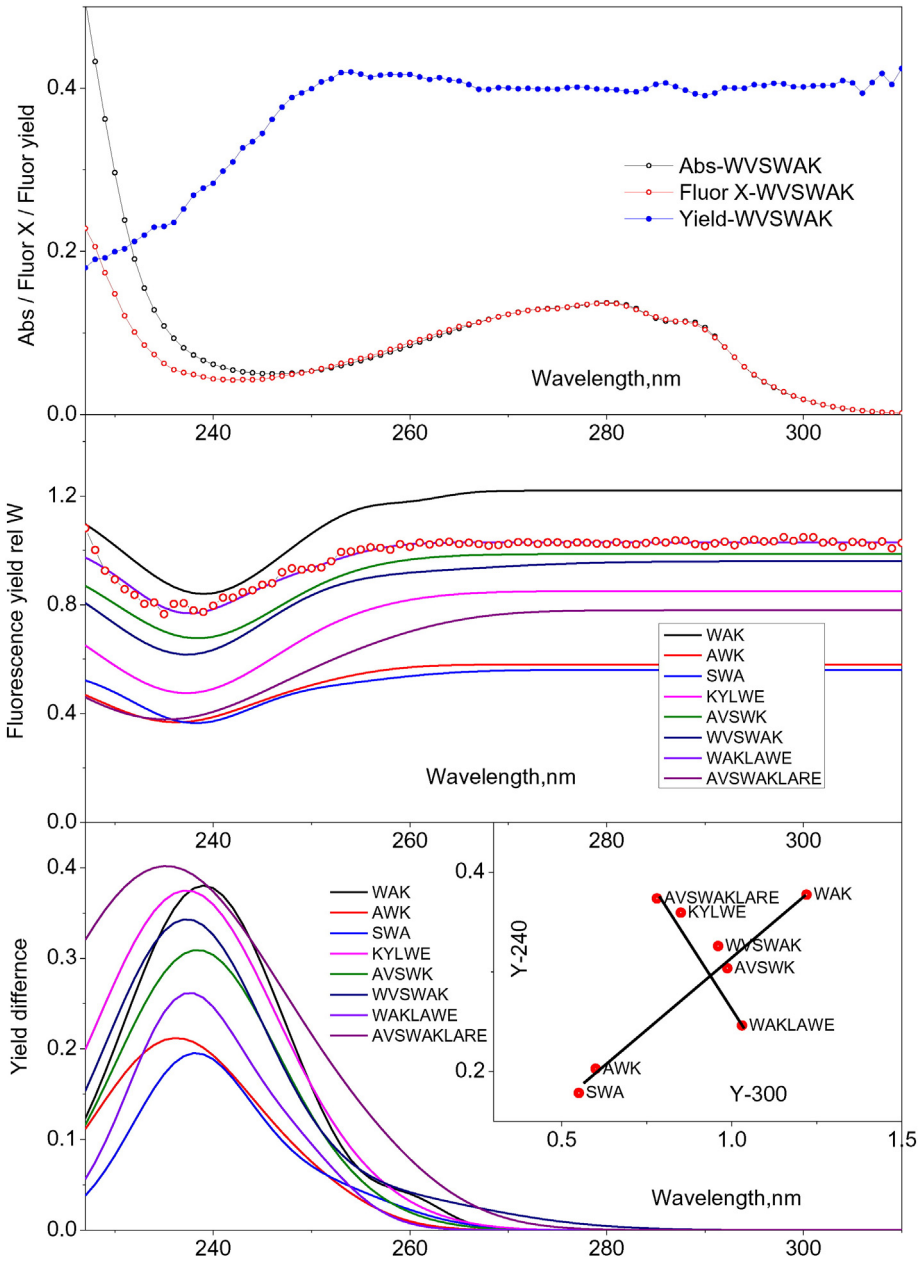

Fig. 1. Upper: Absorption, corrected fluorescence excitation and relative fluorescence yield of sample WVSWAK. Middle: Fluorescence yields of all samples, divided by that of tryptophan (circles), as a function of excitation wavelength. Lower: The graphs in the middle inset are subtracted from their respective terminal levels; and inset: The heights at 240 nm in relation to the yield at 280 nm.

containing three to ten amino acids with Trp at different positions in the peptide, have been investigated. The fluorescence and phosphorescence luminescence properties of the peptides are related to their sequence, length and conformation.

2. Materials and Methods

2.1. Materials

Peptides were synthesized by China Peptides Co., Ltd. (Shanghai, China) with >99% purity. All other reagents were of analytical grade.

Three peptides are composed of three amino acids: WAK, AWK and SWA. One of them, Trp (W) is located at the end of peptide, while at the other end is a charged amino acid (K) and in the middle is a non-polar amino acid (A). For the other two peptides Trp is located in the middle of peptide, one at the end being non-polar amino acid and one at the other end being a polar or charged, basic, amino acid.One of the two five-amino acid peptides, KYLWE, contains Tyr (Y) separated from Trp by one amino acid. Y is less strictly polar, and the other amino acids are non-polar or charged, one basic and one acidic located at the two peptide ends. The second five-amino acid peptide, AVSWK, contains Trp at the same position as the other five amino-acid peptide, and also the non-polar amino acids, one neutral/polar and one charged (basic) amino acid at the end of peptide. In the six amino-acid peptide, WVSWAK, and in the seven amino-acid peptide, WAKLAWE, there are two Trps, one of them being at the peptide end; the other amino acids are non-polar or charged, basic or acidic at the peptide end. The ten amino acid peptide, AVSWAKLARE, contains one Trp inside the peptide, the other amino acids being non-polar, polar and charged, two basic and one acidic. The sequence of this peptide is the same as the WAKLAWE, except for the amino acid next to the end amino acid, R, which is polar and basic, instead of W in the former amino acid. Thus in these peptides