INVESTIGATION OF ENERGY TRANSFER IN PEPTIDES BY EXCITATION DIFFERENCE SPECTRA TECHNIQUES

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Received May 6, 1968

The investigation of energy transfer in proteins is a subject of continuing interest (Teale and Weber, 1959; Yeargers, et al, 1966; Konev, 1967; Rabinovitch, 1968; Steiner, 1968). Since earlier studies dealt almost exclusively with energy transfer at the singlet state level we have little information about energy transfer at the triplet state level. In this communication we show how fluorescence and phosphorescence excitation difference spectra can be used to study energy transfer processes at both the singlet state and triplet state levels. Because of the close proximity of the two aromatic residues in L-tryptophyl-L-tyrosine (Trp-Tyr) we expected tyrosine to tryptophan energy transfer would occur with high efficiency at the singlet and/or triplet state levels and therefore this system was chosen for our initial studies.

The phosphorescence spectra of Trp-Tyr, and of a 1:1 mixture of tryptophan and tyrosine, in 50% ethylene glycol-water (EGW) glass, are shown in Fig. 1. Relative to the mixture, the tyrosine emission in Trp-Tyr is almost completely quenched, but the intensity of the tryptophan phosphorescence is enhanced by about 36%. Although these data do not prove it, they strongly suggest that the tyrosine emission in Trp-Tyr is being quenched by energy transfer to tryptophan. This suggestion is further supported by a comparison of the phosphorescence excitation (PE) spectra of free tryptophan with that of Trp-Tyr (see Fig. 2) which clearly indicates that the PE spectrum of Trp-Tyr is significantly altered from that of free tryptophan. We now want to show how these PE

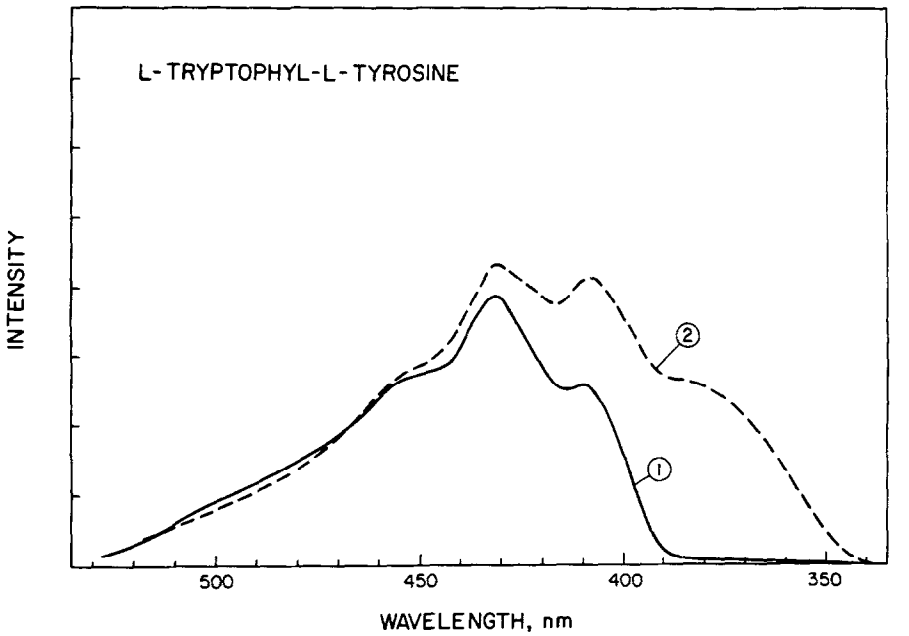

Figure 1. 77°K Phosphorescence Emission Spectra. Curve 1: 3.3×10^{-6}M L-tryptophyl-L-tyrosine in EGW; Curve 2: 1:1 mixture of tryptophan and tyrosine in EGW, each at 3.3×10^{-6}M. The excitation wavelength was 280 nm. Spectra are not corrected for detector response (RCA 7265 photomultiplier).

spectra can be used to evaluate the importance of tyrosine to tryptophan energy transfer in Trp-Tyr.

To obtain a phosphorescence excitation spectrum (PEλ) of a sample the phosphorescence intensity is monitored at some fixed wavelength, λ, while the wavelength of excitation is varied continuously through the absorption region of the sample (Kearns and Case, 1966; Marchetti and Kearns, 1967). Under appropriate conditions the PE spectrum of an optically dilute, single component system, is directly proportional to the sample absorption spectrum. In a two component system where absorption of light by both components leads to phosphorescence at the wavelength monitored, the PE spectrum will be the sum of the absorption spectra of both components, weighted for their relative contributions to the intensity of phosphorescence monitored at wavelength λ. It is this property of excitation spectra which enables us to study tyrosine to tryptophan energy transfer.

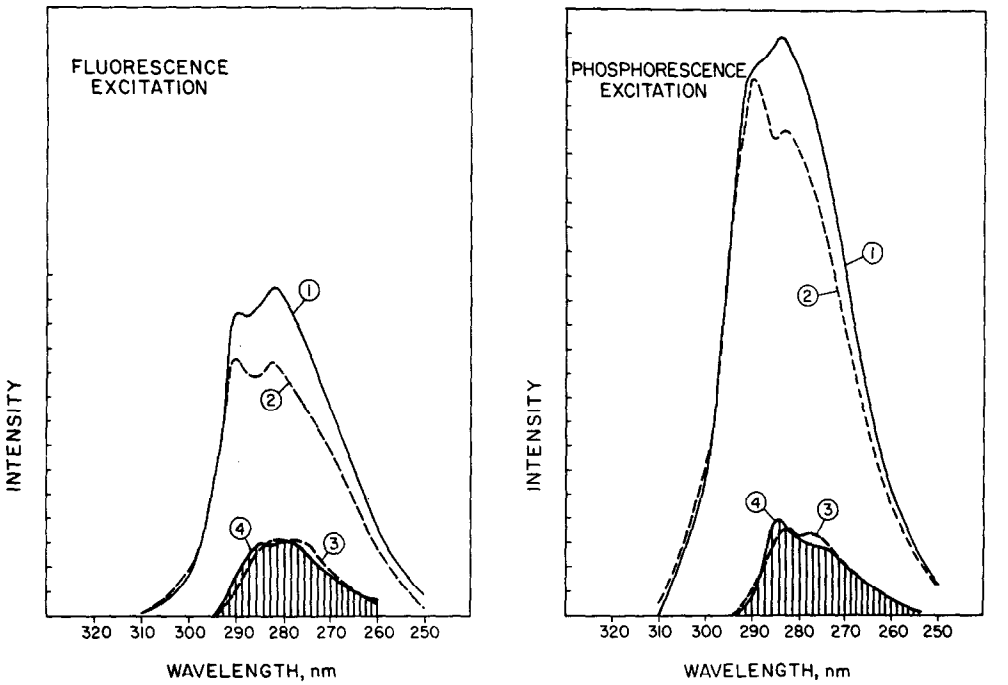

Figure 2. Fluorescence and Phosphorescence Excitation Spectra. Curve 1: 3.3×10^{-6} M L-tryptophyl-L-tyrosine; Curve 2: free tryptophan; Curve 3: free tyrosine; Curve 4: excitation difference spectrum. The phosphorescence excitation spectra were obtained at 77°K, and the fluorescence excitation spectra were obtained at room temperature. Spectra are not corrected for the wavelength dependence of the intensity of the exciting light (450 watt Xenon lamp).

Consider an experiment in which the phosphorescence of a sample containing both tryptophan and tyrosine residues is monitored at a wavelength at which only tryptophan phosphorescence is observed. In the absence of tyrosine to tryptophan energy transfer the resulting PE spectrum should be identical to the excitation spectrum for free tryptophan residues (Fig. 2), since it will contain no tyrosine absorption component. If, however, light absorbed by tyrosine produces tryptophan triplets which then phosphoresce, the resulting PE spectrum will be the sum of the excitation spectra for tyrosine and tryptophan. From a determination of this tyrosine contribution to the PE spectrum the importance of tyrosine to tryptophan energy transfer can be evaluated. With Trp-Tyr there is negligible tyrosine phosphorescence, thus the phosphorescence excitation spectrum monitored

at 430 nm (PE 430 spectrum) will contain contributions only from:

(1) tryptophan residues which have been excited by direct absorption of light and which emit at 430 nm (denoted as the "tryptophan contribution") and

(2) tyrosine residues which absorbed light but transferred their excitation energy to tryptophan residues which then emit at 430 nm (denoted as the "non-trivial tyrosine contribution").

To evaluate this "non-trivial tyrosine contribution" arising from tyrosine to tryptophan energy transfer we must subtract the "tryptophan contribution" from the Trp-Tyr PE 430 spectrum. This was accomplished in the following manner. We assumed that the shape of the "tryptophan contribution" to the PE 430 spectrum of Trp-Tyr is the same as that observed for free tryptophan, or for N-acetyl-tryptophan amide (the latter two compounds have identical PE spectra in 50% EGW). Since the long wavelength region (310-295 nm) of the PE 430 spectrum of Trp-Tyr is associated only with tryptophan absorption, we estimated the "tryptophan contribution" by matching the long wavelength portion of a PE spectrum for free tryptophan (curve 2 in Fig. 2) with the corresponding portion of the PE spectrum of Trp-Tyr (curve 1 in Fig. 2). The PE difference spectrum, obtained by subtracting the free tryptophan PE curve from the Trp-Tyr PE spectrum, gives directly the "non-trivial tyrosine contribution" to the Trp-Tyr PE spectrum. As expected, the PE difference spectrum (curve 4, Fig. 2) closely resembles the PE spectrum for free tyrosine (curve 3, Fig. 2), with good agreement between peak positions. That these two spectra do not have identical shapes is undoubtedly due, in part, to the small unavoidable errors inherent in calculating difference spectra.

From a consideration of the relative magnitude of the extinction coefficients of tyrosine ($\epsilon_{280}=1.1\times10^3$) and tryptophan ($\epsilon_{280}=3.9\times10^3$), in Trp-Tyr (Cassen and Kearns), we estimated the "non-trivial tyrosine contribution" to the PE 430 spectrum of Trp-Tyr should be ~20%, assuming 100% efficient energy transfer at the singlet state level. The PE difference spectrum indicates that there is approximately a 17±2% "non-trivial tyrosine contribution" to the Trp-Tyr PE 430 spectrum, but does not allow us to distinguish between singlet and triplet energy transfer.

To determine whether energy transfer was occurring at the excited singlet, or at the triplet state level, we also measured

the fluorescence excitation (FE) spectra of Trp-Tyr, tyrosine, and tryptophan, by monitoring the fluorescence at 360 or 320 nm (Fig. 2). From the FE difference spectrum we found that the "non-trivial tyrosine contribution" to the Trp-Tyr FE spectrum was about 23±3%. This is in good agreement with the theoretical value of 20% indicating that tyrosine to tryptophan energy transfer in Trp-Tyr occurs at the excited singlet state level, with essentially unit efficiency.

We have described a method by which excitation spectra may be used to provide information about energy transfer, independent of a knowledge of luminescence efficiencies and intersystem crossing yields of the emitting substance. We have applied this method to a study of energy transfer in Trp-Tyr and have shown that significant tyrosine to tryptophan energy transfer occurs at the excited singlet state level. This method is currently being employed to study energy transfer in enzymes and results will be presented shortly (Cassen and Kearns).

Acknowledgement

The support of a Public Health Service Grant is gratefully acknowledged.

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