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Short Communication

Excitation energy transfer in wool keratin

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There has been considerable interest shown in the possibility of energy transfer in proteins because of the importance of such a process in determining the photochemical properties of these molecules. The majority of quanta in the 250 - 300 nm region are absorbed by the amino acids phenylalanine, tyrosine and tryptophan. However, in protein molecules the luminescence observed is not simply a summation of the emissions from these three component amino acids but is largely attributable to tryptophan. It has been suggested that emission from tyrosine is quenched in proteins; however, the fulfillment of the donor–acceptor conditions for energy migration from phenylalanine to tyrosine and from tyrosine to tryptophan provides a further explanation for the observed protein luminescence. This problem and the general luminescence properties of proteins have been extensively reviewed by Konev [1] and Longworth [2]. It would appear that in some proteins energy transfer plays a significant role while in others it does not.

We have found that the fluorescence and phosphorescence from wool keratin at liquid nitrogen and room temperatures is similar to that of tryptophan in a solid poly(vinyl alcohol) (PVA) film and present quantum yield evidence which shows that excitation energy transfer occurs in the solid protein both at 77 K and at room temperature.

Materials and methods

dl-Tryptophan (BDH), sodium 1-naphthylamine-4-sulphonate (Hopkin and Williams) and poly(vinyl alcohol) (Matheson, Coleman and Bell) were used without further purification. A 20 μm Merino wool, in fabric form, was used after thorough cleaning by Soxhlet extraction.

Fluorescence and phosphorescence emission spectra were recorded on a spectrofluorophosphorimeter constructed in these laboratories [3]. The instrument is fully electronically corrected for both excitation beam intensity variation and emission beam monochromator/photomultiplier combination.

Fluorescence and phosphorescence quantum yields were determined

by the method of Parker and Rees [4]. The standard used was sodium 1-naphthylamine-4-sulphonate in a PVA film whose quantum yield of fluorescence was taken to be 1, the value it has been found to have in glycerol [5, 6]. The emission yields of tryptophan in PVA films at room temperature when compared to this standard were found to have values of $\Phi_F = 0.55$ and $\Phi_P = 0.03$. The luminescence from wool was compared to the emission from tryptophan–PVA films.

The u.v.–visible absorption spectrum of wool keratin was recorded on an Olympus Double Beam Microspectrophotometer Model DMSP-II using a spot size of approximately 6 μm. The tryptophan content of the wool keratin was determined independently by both the method of Cegarra and Gacen [7] and Opienska-Blauth *et al.* [8]. Both methods find a tryptophan content of 8.6 mg/g dry wool which is in good agreement with determinations carried out by Graham *et al.* [9] on other wools. The fraction of quanta absorbed by tryptophan was calculated from the wool tryptophan content using the extinction coefficient determined from a tryptophan aqueous solution. At 280 nm, the excitation wavelength used for this work, the fraction of quanta absorbed by tryptophan in wool keratin was 0.13.

Results and Discussion

Quantum yields of luminescence at 77 K

The total emission spectrum from wool keratin is presented in Fig. 1. The emission is basically that of tryptophan, as demonstrated by comparison with the emission from tryptophan in PVA film (shown in the same Figure). The phosphorescence emission from wool keratin at 77 K, also shown in Fig. 1, has a component below 400 nm which is not tryptophan-like but exhibits spectral properties characteristic of tyrosine phosphorescence emissions. A small tyrosyl contribution has been found for many globular proteins [2]. The decay of phosphorescence from wool is similar to that from tryptophan in PVA films, predominantly obeying first-order kinetics with a lifetime of 4.76 s. Similar emission spectral evidence in the past has been interpreted by some workers [10, 11] as evidence of energy transfer from tyrosine to tryptophan; however, others [12, 13] have criticized this interpretation on the grounds that tyrosine fluorescence and phosphorescence are preferentially quenched in proteins.

The luminescence emission quantum yields are listed in Table 1 where the yields have been calculated both per quanta absorbed by wool and per quanta absorbed by tryptophan. When calculated per quantum absorbed by tryptophan, quantum yields significantly greater than one are obtained for both total luminescence and fluorescence while the phosphorescence quantum yield is almost one. These quantum yields provide convincing evidence of energy transfer to tryptophan in wool keratin at 77 K.

Quantum yields of luminescence at room temperature

The total luminescence emission and phosphorescence from wool keratin at room temperature is given in Fig. 2.

The total luminescence is basically the fluorescence of tryptophan with

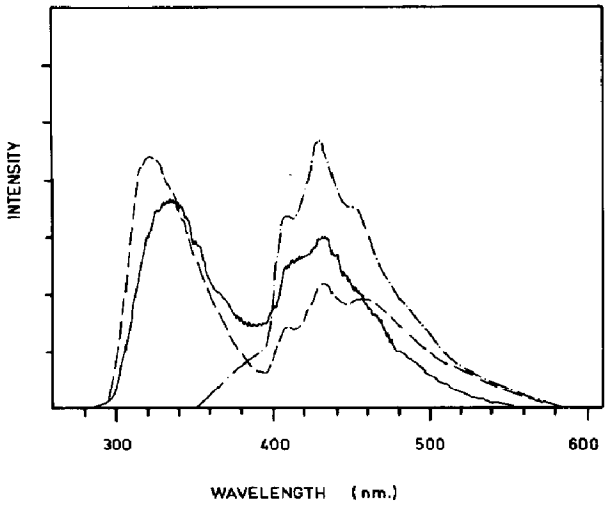

Fig. 1. Luminescence emissions at 77 K. ——, Total emission from wool keratin; — · — · —, phosphorescence emission from wool keratin; — — — —, total luminescence emission from tryptophan in a PVA film. Spectra recorded at different gains.

TABLE 1

Quantum yields of luminescence from wool keratin at 77 K

Parameter	Φ per wool	Φ per tryptophan	τ
Total luminescence	0.33	2.45	
Phosphorescence emission	0.13	0.98	4.76 s (1st order)
Fluorescence emission	0.20	1.47	not measurable

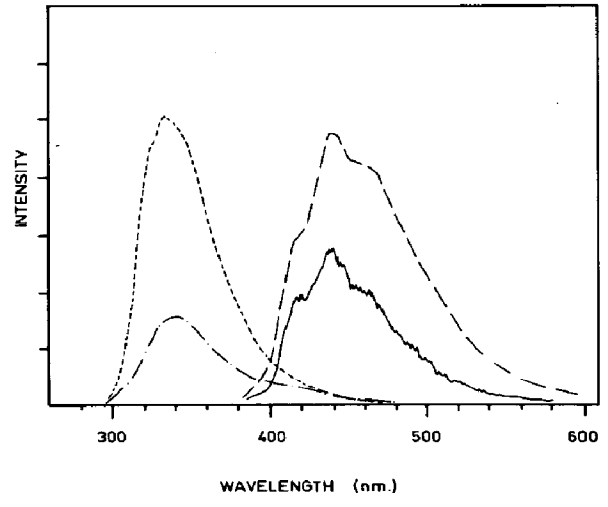

Fig. 2. Luminescence emissions at room temperature. — · — · —, Total emission from wool keratin; ———, phosphorescence emission from keratin; ·····, total emission from tryptophan–PVA film; — — — —, phosphorescence emission from tryptophan–PVA film. Spectra recorded at different gains.

TABLE 2

Quantum yields of luminescence from wool keratin at room temperature

Parameter	Φ per wool	Φ per tryptophan	τ*
Fluorescence:			
(a) Lab. atmos.	0.082	0.630	not measurable
(b) dry N_2	0.050	0.380	not measurable
Phosphorescence:			
(a) Lab. atmos.	0.0018	0.014	80 ms (1st)
(b) Dry N_2	0.0135	0.104	450 ms (2nd)

*Defined as time to decay to 0.37 remaining.

a small component, at longer wavelengths, due to tryptophan phosphorescence. This phosphorescence was recorded, at a higher gain (× 50), using the rotating shutter technique to eliminate fluorescence. At the time of writing, phosphorescence emission from wool keratin at room temperature has not been reported elsewhere.

The quantum yields of fluorescence and phosphorescence are listed in Table 2. The fluorescence emission quantum yield is in reasonable agreement with earlier work by Konev [1] calculated per quantum absorbed by keratin. When calculated per quantum absorbed by tryptophan, the fluorescence yields are greater than the value of 0.14 for aqueous environments [14] but approaching the value of 0.55 for tryptophan in PVA films.

A significant increase in phosphorescence emission can be observed from wool keratin after equilibrating in dry nitrogen. Fluorescence emission decreases under the same conditions. Presumably, under dry N_2, the plasticizer water is removed, the structure becomes far more rigid and intersystem crossing is relatively enhanced. In addition, the absence of oxygen increases the life-time of the phosphorescence emission and changes the apparent rate order from first to second. It would seem that the rate determining step, under dry nitrogen, is a radiationless triplet–triplet quenching while in the presence of oxygen the triplet state is quenched in a first order reaction possibly forming singlet excited oxygen or by direct reaction as proposed by Pailthorpe *et al.* [15].

The quantum yield of phosphorescence per quantum absorbed by tryptophan in keratin (0.104) is significantly higher than the phosphorescence from tryptophan in PVA films (0.03) under the same conditions. It can be concluded, therefore, that energy transfer to tryptophan in keratin also occurs at room temperature.

Conclusion

Evidence has been presented which shows that excitation energy transfer takes place in wool keratin both at 77 K and at room temperature.

1 S. V. Konev, in Fluorescence and phosphorescence of proteins and nucleic acids, Plenum Press, New York, 1967.
2 J. W. Longworth, in R. F. Steiner and I. Weinryb (eds.), Excited states of proteins and nucleic acids, Plenum Press, New York, 1971.
3 M. T. Pailthorpe, J. Sci. Instrum., in press.
4 C. A. Parker and W. T. Rees, Analyst, 85 (1960) 587.
5 E. J. Bowen and S. F. A. Miskin, J. Chem. Soc., (1959) 3172.
6 J. G. Calvert and J. N. Pitts, Jr., in Photochemistry, Wiley, New York, 1966, p. 799.
7 J. Cegarra and J. Gacen, J. Soc. Dyers Colour., 84 (1968) 216.
8 J. Opienska-Blauth, M. Charezinski and M. Berbec, Analyt. Biochem., 6 (1963) 69.
9 C. E. Graham, E. P. Smith, S. W. Hier and D. Klein, J. Biol. Chem., 168 (1947) 711.
10 Yu. S. Vladimirov and S. V. Konev, Biofizika, 4 (1959) 533.
11 L. Augenstein and J. Nag-Chaudhuri, Nature, 203 (1964) 1145.
12 F. W. J. Teale, Biochem. J., 76 (1960) 387.
13 T. Truong, R. Bersohn, P. Brumer, C. K. Luk and T. Tao, J. Biol. Chem., 242 (1967) 2979.
14 J. Eisinger and G. Navon, J. Chem. Phys., 50 (1969) 2069.
15 M. T. Pailthorpe, J. P. Bonjour and C. H. Nicholls, Photochem. Photobiol., 17 (1973) 209.