TRIPLET STATE ENERGY TRANSFER IN SEVERAL PROTEINS

D. A. GRAINGER AND A. M. NISHIMURA, *Department of Chemistry, Wichita State University, Wichita, Kansas 67208 U.S.A.*

ABSTRACT Energy transfer between excited triplet states of aromatic amino acid residues was observed at 1.4°K. The distance necessary for energy transfer between monomeric tyrosine and tryptophan residues was determined to be roughly 63 Å. Total phosphorescence decay rate constants for several proteins were determined while emission corresponding to tyrosine and tryptophan residues was monitored. The observed decay rate constants are interpreted in terms of intramolecular interactions of the polypeptide residues.

The source of the phosphorescent emission from excited protein triplet states has been identified as arising from the aromatic amino acid residues of the polypeptides (Debye and Edwards, 1952). The phosphorescent emissions from the amino acid residues tyrosine and tryptophan individually show characteristic spectra. The relative intensities of these emissions are used as an indication of the relative concentrations of the two moieties. But for certain proteins in which relative proportions of the component amino acids are known, even if both tyrosine and tryptophan residues are present, the characteristic emission is that of tryptophan only (Becker, 1969). This has been attributed to energy transfer from tyrosine to tryptophan residues (Rabinovitch, 1968; Maki and Zuclich, 1975). If such energy transfer can occur, there must be some critical distance between the residues such that when the distance separating the residues is less, energy transfer process competes favorably with other relaxation processes. It should be remembered that when these aromatic amino acid residues are embedded within the hydrophobic portion of the protein, the local environment is a somewhat more efficient perturber than when these monomeric species are hydrated in solution (Yeagers et al., 1966; Steiner and Kolinski, 1968; Beyer et al., 1974).

Recently triplet state studies at low temperatures have proved to be a sensitive and powerful method in the study of molecular interactions (Tinti and El-Sayed, 1971). The typically long lifetimes of the phosphorescent metastable electronic state yield a large steady-state population detectable by optical techniques. The phosphorescence relaxation rate is a function of inter- and intramolecular perturbations. Thus a careful analysis of the decay data should yield information on the environment of the aromatic

amino acid residues. A complete discussion of the method is given in our previous study (Brunk et al., 1976).

The aromatic amino acids tyrosine and tryptophan were purchased from Sigma Chemical Co. (St. Louis, Mo.). They were purified by extraction from an ammonium hydroxide-ethanol solution. The proteins, ribonuclease-A, insulin, lysozyme, and α-chymotrypsinogen were also purchased commercially.

Solutions of these amino acids and proteins were made with propylene glycol subjected to multiple vacuum distillation. These solutions were placed in a quartz ampule and immersed in liquid helium. The helium Dewar flask could be pumped, thereby achieving temperatures as low as 1.4°K. A mercury lamp was used to optically pump the triplet state of the molecules under study. The lamp was placed in a 45° configuration to a 3/4 meter Czerny-Turner spectrometer and a thermoelectrically cooled EMI 6256B photomultiplier (EMI Gencom Inc., Plainview, N.Y.).

For phosphorescence decay measurements, the spectrometer was set at the emission maxima and the excitation source was extinguished in about 2 ms by an electronically controlled mechanical shutter, or the decay was measured alternatively after a xenon flash lamp excitation of 10 μs duration. The signals were repetitively accumulated in a signal averager Tracor-Northern (Middleton, Wis.) and the resulting data were transferred directly to a minicomputer interfaced to a visual display. By a regression program, the three rate components of the total phosphorescence decay were generated.[1]

The emission spectra of the aromatic amino acid tryptophan and tyrosine at 1.4°K were broad and exhibited relatively few structural features. The emission maximum for pure tyrosine in propylene glycol was at approximately 385 nm. Pure tryptophan exhibited a maximum at 410 nm. In Table I, the phosphorescence rate constants as measured by shuttering the excitation light have been tabulated for the various mixtures and proteins. The changes in the rate constants k_1, k_2, and k_3 between the monomer and mixture represent the anisotropic molecular interaction along the spin axes of the emitting amino acid residue (Brunk et al. 1976).

The concentration of the two monomeric amino acids was varied to find the concentration at which the emission characteristic of tyrosine would become minimized by the competitive energy transfer from tyrosine to tryptophan. The critical distance thus determined from free amino acids would be extrapolated to proteins known to contain both of these aromatic amino acid residues.

The critical concentrations of the tyrosine and tryptophan were found to be 3.4 and 3.1 mmol/liter in propylene glycol, respectively. At lower concentrations the emission spectrum was a superposition of subspectra characteristic of pure tyrosine and tryptophan. At concentrations equal to or larger than the critical concentration, the spectrum was characteristic of pure tryptophan only. This has been attributed to the

[1] Power, R. K. Software for the analysis of a decay composed of a sum of first-order exponentials. In preparation.

TABLE I

TOTAL PHOSPHORESCENCE DECAY RATE CONSTANTS (±10%) FOR TYROSINE, TRYPTOPHAN, AND SEVERAL PROTEINS IN PROPYLENE GLYCOL SOLVENT OBSERVED AT TWO WAVELENGTHS.

	385 nm			410 nm		
	k_1	k_2	k_3	k_1	k_2	k_3
			s^{-1}			
Tyrosine (2.8 m*M*)	2.1	0.80	0.49			
Tryptophan (2.8 m*M*)				5.7	1.0	0.23
Tyrosine-tryptophan mixture*	4.4	1.1	0.30	6.4	1.4	0.31
Ribonuclease‡	13.	1.2	0.34			
Insulin‡	7.7	1.5	0.28			
α-chymotrypsinogen§	>1,000	1.4	0.38	5.7	1.1	0.32
Lysozyme ‖	150	8.2	0.54	9.1	3.5	0.72

*Concentration of 3.4 to 3.1 mM for tyrosine to tryptophan, respectively.
‡Contains tyrosine only. From Becker (1969).
§Contains 2.5% tyrosine and 5% tryptophan. From Becker (1969).
‖ Contains 11% tryptophan and tyrosine. From Becker (1969).

energy transfer between the two aromatic amino acid residues. The distance between the amino acid molecules at which energy transfer occurs at the critical concentration has been estimated to be about 63 Å. This is in reasonably good agreement with past studies. For example, in a model system of binary phenol and indole mixture, the interaction distance has been found to be roughly 16 Å (Weber and Teale, 1965; Weber, 1966). Rabinovitch has extrapolated the interaction distance using electron spin resonance techniques of tyrosine and tryptophan to be about 160 Å (1968).

As shown in Table I, the increase in the rate constants in the binary mixture reflects the competitive radiationless energy transfer process, which becomes important at this critical concentration. The tyrosine residues in ribonuclease and insulin exhibit larger rate constants than the pure monomers, due to the increased molecular interactions of nearby residues. Past studies have indicated that in α-chymotrypsinogen only tryptophan phosphorescence has been observed (Becker, 1969). The extremely rapid decay of the tyrosine phosphorescence emission may indicate that the residue might be in an highly interactive environment, whereas the relatively unchanged rate constants for tryptophan residues indicate very little interaction with tyrosine and/or other residues.

The majority of tyrosine residues in lysozyme probably is less perturbed than the corresponding residue in α-chymotrypsinogen. The tryptophan moiety appears to be in an interactive environment and the energy transfer process may be the cause for the increase in the tryptophan phosphorescence rate constant. Alternatively this increase may be due to molecular interactions at the vicinity of the tryptophan residues.

The sensitivity of the total phosphorescence rate constants to molecular perturbations and energy transfer processes makes the triplet state spectroscopic technique a

useful probe in the study of molecular interaction. We are extending the study to other proteins containing tyrosine and tryptophan residues.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this work. We also thank the National Institutes of Health (GM 21770) for supporting this research.

Received for publication 24 June 1977 and in revised form 1 August 1977.

REFERENCES

BECKER, R. S. 1969. *In* Theory and Interpretation of Fluorescence and Phosphorescence. John Wiley & Sons, Inc., New York.

BEYER, C. F., W. A. GIBBONS, and L. C. CRAIG. 1974. Heterogeneous tryptophan environments in the cyclic peptides tyrosidines B and C. *J. Biol. Chem.* **249**:3204.

BRUNK, G. R., K. A. MARTIN, and A. M. NISHIMURA. 1976. A study of solvent effects on the phosphorescence properties of flavins. *Biophys. J.* **16**:1373.

DEBYE, P., and J. O. EDWARDS. 1952. A note on the phosphorescence of proteins. *Science* (Wash. D.C.). **116**:143.

MAKI, A. H., and J. ZUCLICH. 1975. Protein Triplet States. *Top. Curr. Chem.* **54**:115–163.

RABINOVITCH, B. 1968. Transference of triplet state excitation energy from tyrosine to tryptophan amino acids in simple and mixed solutions. *Arch. Biochem. Biophys.* **124**:258.

STEINER, R. F., and R. KOLINSKI. 1968. The phosphorescence of oligopeptides containing tryptophan and tyrosine. *Biochemistry.* **7**:1014.

TINTI, D. S., and M. A. EL-SAYED. 1971. New techniques in triplet state phosphorescence spectroscopy: application to the emission to 2,3-dichloroquinoxaline. *J. Chem. Phys.* **54**:2529.

WEBER, G., and F. W. J. TEALE. 1965. Interaction of proteins with radiation. *In* The Proteins. H. Neurath, editor. Academic Press, Inc., New York. 360–443.

WEBER, G. 1966. Polarization of the fluorescence of solutions. *In* Fluorescence and Phosphorescence Analysis. D. M. Hercules, editor. John Wiley & Sons, Inc., New York. 217–240.

YEAGERS, E., F. R. BISHAI, and L. AUGENSTEIN. 1966. On the nature of tyrosine phosphorescence from proteins. *Biochem. Biophys. Res. Commun.* **23**:570.