*ON RESONANCE TRANSFER OF EXCITATION ENERGY BETWEEN AROMATIC AMINOACIDS IN PROTEINS**

By George Karreman, Richard H. Steele,† and Albert Szent-Györgyi

INSTITUTE FOR MUSCLE RESEARCH, MARINE BIOLOGICAL LABORATORY, WOODS HOLE, MASSACHUSETTS

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Teale and Weber,[1] from studies of absorption and singlet emission spectra for the aromatic amino acids phenylalanine, tyrosine, and tryptophane and from the number of amino acid residues and the dimensions of ordinary globular proteins, point out that internal transfer of excitation energy must occur in proteins. They postulate a transfer between phenylalanine, tyrosine, tryptophane, and heme, the energy being passed from earlier to later members of this series The calculations reported in this paper give not only quantitative corroboration to this concept but emphasize that such a flow of energy should be essentially unidirectional.

In Figure 1 we present the absorption and fluorescent spectra for the aromatic amino acids phenylalanine, tyrosine, and tryptophane and the absorption spectra for reduced diphosphopyridine nucleotide and oxidized riboflavin. An examination of these spectra reveals a considerable overlap of the amino acid emission spectra with their own absorption spectra and with the absorption spectra of reduced diphosphopyridine nucleotide and oxidized riboflavin. The possibility that these spectral overlaps may provide a mechanism for the resonance transfer of excitation energy prompted us to evaluate this possibility quantitatively.

Using the theory of Förster[2] as applied by Karreman and Steele,[3] we have calculated critical distances, R_0 (that distance, in Å, between an energy donor and an acceptor molecule over which excitation energy may be transferred by resonance with the same probability that it may be emitted as fluorescence), for the several pair systems between which resonance transfer of energy may occur. Critical distances were calculated with the formula (Förster;[4] Karreman and Steele[3]),

$$R_0 = \sqrt[6]{0.95 \times 10^{-33} \frac{\tau J_{\tilde{\nu}}}{\tilde{\nu}_0^2}},$$

in which τ is the lifetime of the lowest excited singlet state (calculated from absorption spectra and using the quantum yields reported by Teale and Weber,[1] see Förster[5]), J is the overlap integral and $\tilde{\nu}_0$ is the average wave number between the fluorescent and the absorption maxima of the energy donor molecule. The calculated critical distances, R_0 (Å), together with the pertinent parameters, are tabulated in Table 1.

The question arises whether the critical distances calculated will allow a resonance transfer of excitation energy within a protein molecule. The ratio of the three aromatic amino acids within various proteins varies within rather wide limits, but the sum of the three acids is surprisingly constant. In Table 2 we present the ratio of the total number of amino acids to the number of aromatic amino acids in several proteins. Within narrow limits this ratio is close to 12. If the specific weight of

the protein is taken as 1.2 and the average molecular weight of an amino acid as 120, then the protein contains amino acids in a concentration of 10 molar. If every twelfth amino acid is aromatic, then the concentration of aromatic amino acids is approximately 0.8 molar. At this concentration the statistical distance between the aromatic amino acids is of the order of 10 Å. This distance is smaller than the critical distances for the hetero-amino acid pairs, and we might thus anticipate a considerably efficient resonance transfer of excitation energy within protein molecules.

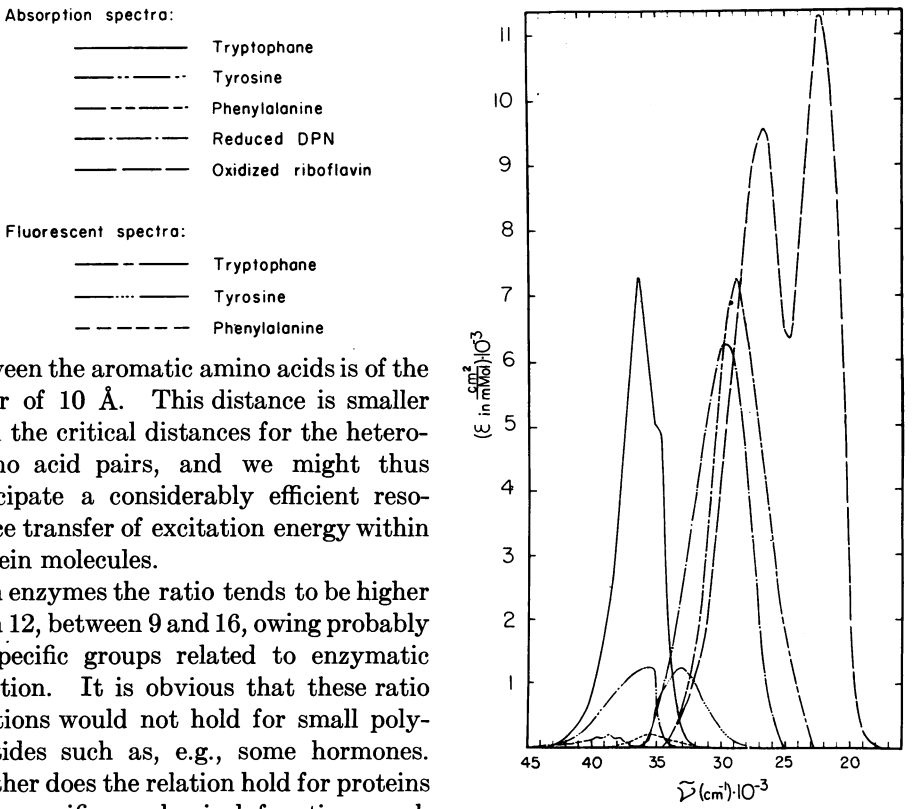

FIG. 1

In enzymes the ratio tends to be higher than 12, between 9 and 16, owing probably to specific groups related to enzymatic function. It is obvious that these ratio relations would not hold for small polypeptides such as, e.g., some hormones. Neither does the relation hold for proteins with specific mechanical functions such as L-meromyosin (ratio = 39).

A significant feature revealed by a study of the spectral data in Figure 1 is that, for any heteromolecular pair combination, an excitation generated in the donor molecule must flow unidirectionally in the direction of lower-excitation states. It is interesting to note that unidirectional flow of excitation energy could occur in two ways: (1) linearly, in the direction of the protein axis, in which case the amino acid sequence and interamino acid residue distances within the protein molecule must be within certain critical values or (2) "three-dimensionally" (as discussed above), in which case the acceptor molecule to which energy is transferred by resonance would be that molecule lying only within a certain critical distance. In both instances coiled or helical structures would impose additional directional specificity to energy flow by constraining the donor and acceptor electronic oscillators in proper alignment for maximum transfer efficiency. From the few published data on proteins with known amino acid sequence, it would be premature to suggest in which way energy might be transferred by resonance within the protein molecule. It is possible that both mechanisms may operate. Estimating

the radii of globular proteins to range between 15 and 30 Å, it can be seen from the reported R_0 values that an energy donor molecule can transfer its energy to an acceptor molecule located at almost any point in the molecule with considerable efficiency. In such proteins a certain amino acid sequence would not be a prerequisite for intramolecular energy flow, but in fibrous proteins, where asymmetry is all-important, a specific sequential arrangement of the participating amino acids would be a necessity for a unidirectional resonance transfer of energy along the fiber axis.

TABLE 1

CRITICAL DISTANCES, R_0, FOR RESONANCE TRANSFER OF EXCITATION ENERGY BETWEEN GIVEN DONOR AND ACCEPTOR MOLECULAR PAIRS

Donor-Acceptor Pair	$\tau \times 10^8$	$\bar{\nu}_0 \times 10^{-3}$	$J\bar{\nu} \times 10^{-8}$	R_0 (Å)
Phenylalanine ⇆ phenylalanine	1.1	37.1	0.0404	5.6*
Phenylalanine → tyrosine	1.1	37.1	4.1	12.0
Phenylalanine → tryptophan	1.1	37.1	21.1	16.0
Tyrosine ⇄ tyrosine	0.91	34.4	0.458	8.3*
Tyrosine → tryptophane	0.91	34.4	13.0	15.0
Tyrosine → reduced DPN	0.91	34.4	341.0	25.0
Tyrosine → oxidized riboflavin	0.91	34.4	124.0	21.0
Tryptophane ⇄ tryptophane	0.20	32.6	0.326	6.3*
Tryptophane → reduced DPN	0.20	32.6	1210	25.0
Tryptophane → oxidized riboflavin	0.20	32.6	1670	26.0

* See text for explanation of the parameters (in absolute units). We realize that the assumptions made by Förster (*Ann. d. Phys.*, **2**, 55–75, 1948) may impose considerable uncertainty in the R_0 values when R_0 is not several times larger than the radii of the interacting molecular species.

TABLE 2*

RATIO OF TOTAL NUMBER OF AMINO ACIDS TO NUMBER OF AROMATIC AMINO ACIDS IN SEVERAL PROTEINS

Protein	Ratio
Horse hemoglobin†	11
Bovine serum albumin	13
Human fibrinogen	11
Human serum albumin	10
Human globulin	12
Whole casein	11
Ovalbumin	12
Edestin	13
Conalbumin	12
Silk fibroin	12
Gliadin	13
Zein	11
Actin	12
Heavy meromyosin	12.5

* In the construction of this table we calculated for randomly selected proteins the ratio of the total number of amino acids to the total number of aromatic amino acids, based on protein analyses given by G. R. Tristram in *The Proteins*, ed. Hans Neurath and Kenneth Bailey (New York: Academic Press, 1953), **1**, Part A, 181–233. Analyzed data of actin and H. meromyosin were taken from Andrew Szent-Györgyi's review in *Advances in Enzymol.*, **16**, 313, 1955.

† Corresponding to the 4 hemes of the molecule, with a M. W. of 2,400, 22 were subtracted from the total number of amino acid residues in the molecule.

We have included critical distances for the resonance transfer of energy from the amino acids tyrosine and tryptophane to the coenzymes reduced diphosphopyridine nucleotide and oxidized riboflavin because of the potential significance that this interaction may be found to have in energy transformations (e.g., oxidative phosphorylations) and/or energy transfer (conduction). The high R_0 values

calculated for these interactions would provide an efficient intermolecular transfer of excitation energy. As regards energy transfer, it might be noted that the results of this paper and the paper by Karreman and Steele[3] provide a "path" for resonance transfer of excitation energy from phenylalanine → tyrosine → tryptophane → reduced diphosphopyridine nucleotide → oxidized riboflavin → cytochromes. Further, this flow should be unidirectional and, within the respiratory components, should proceed in the same direction that electrons move during mitochondrial respiration.

A feature of resonance transfer of excitation energy of obvious importance is that the donor and acceptor molecules may be separated by considerable distances. This fact would eliminate the dilemma of "collisional-orientation" of iron-iron residues for intercytochrome energy transfer and, further, make unnecessary surface-surface interaction specificity other than perhaps electronic oscillator alignment. In this regard, e.g., the iron-heme moiety of cytochrome c, considered by Ehrenberg and Theorell[6] to be localized subsurfacely in the protein matrix, would be unavailable for collisional contact but readily accessible by resonance transfer. Mitochondrial rigidity would maintain the proper donor-acceptor sequence and electronic oscillator alignment. This mechanism, however, still leaves unanswered the details of electron transfer per se, with its probable interaction specificity.

In conclusion it should be pointed out that the observations reported in this paper refer to singlet excitation states of reacting molecules, while the research of this institute suggests that triplet excitation states may play the major role in energy exchanges and transformations. Though we have obtained the triplet emission spectra for the aromatic amino acids, the as yet unavailable triplet absorption data for these amino acids preclude our calculating resonance transfer data for the triplet excitation states. Nonetheless, it should be emphasized that Terenin and Ermolaev[7] have presented experimental evidence for a resonance transfer of triplet state energy.

Summary.—Critical distances, R_0, have been calculated for the resonance transfer of singlet excitation energy between the aromatic amino acids phenylalanine, tyrosine, and tryptophane and between these amino acids and the coenzymes reduced diphosphopyridine nucleotide and oxidized riboflavin. The significance of the mechanism of resonance transfer of excitation energy is briefly discussed.

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[3] George Karreman and Richard H. Steele, *Biochem. Biophys. Acta* **25**, 280, 1957.

[4] Th. Förster, *Fluoreszenz organischer Verbindungen* (Göttingen: Van den Hoeck & Ruprecht, 1951), p. 226.

[5] Th. Förster, *ibid.*, p. 158.

[6] Anders Ehrenberg and Hugo Theorell, *Acta Chem. Scand.*, **9**, 1193–1205, 1955.

[7] A. Terenin and V. Ermolaev, *Trans. Faraday Soc.*, **52**, 1042–1052, 1956.