Short Communications

Intramolecular resonance transfer of energy in proteins

Intramolecular energy transfer in proteins has been interpreted in terms of two contrasting mechanisms: conductance band transfer[1-3] and resonance transfer[4-6]. The quantitative approach[7] to the resonance-transfer mechanism makes it possible to consider critically whether this mechanism can account for the efficient intramolecular energy transfer observed in the u.v.-induced dissociation of CO from CO-myoglobin[8] and CO-mesohemin-IX-poly-DL-(phenylalanine-glutamic acid)[1], and in the sensitized fluorescence observed in phycocyanin[4] and dye conjugates of various proteins[6]. To calculate the critical distance, R_0, for which the efficiency of resonance transfer is 50 % for a specific energy donor-acceptor pair, we use the equation[7]

$$R_0 = \sqrt[6]{\frac{1.69 \cdot 10^{-33}}{n^2} \cdot \frac{\tau J_{\tilde{\nu}}}{\tilde{\nu}_0^2}}$$

where τ is the lifetime of the excited state of the energy donor, $\tilde{\nu}_0$ the mean of the wavenumbers of the fluorescence maximum and longest wavelength maximum of the energy donor, and $J_{\tilde{\nu}}$ the overlap integral, and n the refractive index ($n = 1.6$ for proteins). Table I gives the results of the calculations. We note that the critical distances are of the order of magnitude of the radii of the proteins. With several energy donors and acceptors in a protein molecule, high-efficiency resonance transfer is definitely possible. For example, the efficiency of resonance transfer between two groups separated by a distance $0.8\,R_0$ is over 80 %. It should be noted that the resonance transfer may take place not only from the aromatic amino acids to the heme group or fluorescent dye, but also among the aromatic amino acids[5] and then to a heme group or fluorescent dye that is nearby. Thus the R_0 values set a lower limit on the efficiency of the process.

SHORE AND PARDEE[6] have shown that the efficiency of energy transfer from

TABLE I

CRITICAL DISTANCES (R_0) FOR INTRAMOLECULAR RESONANCE TRANSFER IN PROTEINS

System	*Donor-Acceptor pair*		$\tau \cdot 10^8$	$\nu_0 \cdot 10^{-3}$	$J_\nu \cdot 10^{-10}$	R_0 (Å)
Myoglobin[8]	Tyrosine	CO-heme	0.91	34.3	17	31
	Tryptophane	CO-heme	0.20	32.6	196	37
Mesohemin-IX-poly-DL-phenylalanine glutamic acid[1]	Phenylalanine	CO-heme	1.1	37.1	2.1	22
Phycocyanin[4]	Tyrosine	Heme	0.91	34.4	4.65	25
	Tryptophane	Heme	0.20	32.6	35.6	28
Protein conjugates of 1-dimethylaminonaphthalene-5-sulfonyl chloride[6]	Tyrosine	Dye	0.91	34.4	1.11	20
	Tryptophane	Dye	0.20	32.6	6.75	21

aromatic amino acids to a fluorescent dye group attached covalently to the protein increases as the number of dyes per protein increases. In chymtrypsinogen conjugates of 1.2 dye/protein, they found that the efficiency varies between 9 and 23 %, while for 5.5 dyes/protein, the efficiency was between 46 and 94 %, for λ 260–285 mμ. We have pursued these studies further, using a larger number of conjugates, and refining the method so that the standard error is between 5 and 10 % of the determined efficiency. The results of our experiments are summarized in Fig. 1. The efficiency of transfer appears to be a linear function of the number of dyes/protein, at least up to 5.5 dyes/protein. These findings can be readily interpreted in terms of the resonance-transfer mechanism. As more dyes are attached to a protein, the distance between the

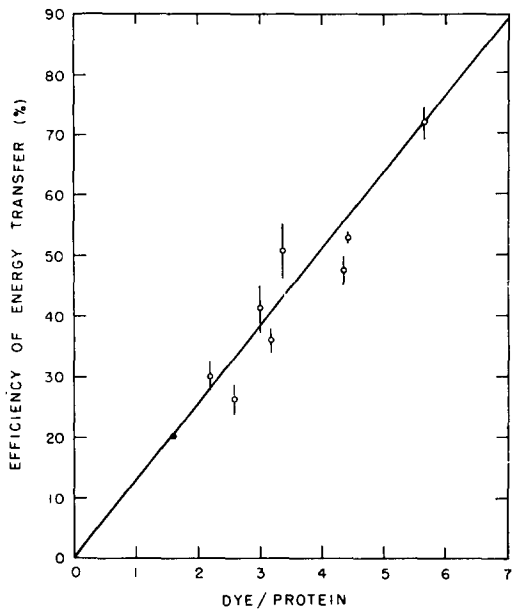

Fig. 1. Efficiency of energy transfer in chymotrypsinogen conjugates as a function of the number of dye molecules (1-dimethylaminonaphthalene-5-sulfonyl chloride) per protein molecule. The solid circle is from the data of SHORE AND PARDEE[6].

aromatic amino acids and the dyes attached to chymotrypsinogen diminishes. Since the efficiency of resonance transfer varies with r, the distance between a specific energy donor-acceptor pair, in the following manner:

$$E = \frac{1}{(r/R_0)^6 + 1}$$

it is evident that changes in the concentration of the dye group will alter the efficiency of resonance transfer.

SHORE AND PARDEE also found that the efficiency of energy transfer decreased 30–50 % in concentrated urea, while it increased about 100 % in conc. LiBr. These findings can readily be interpreted by the resonance mechanism, since the changes in efficiency are due to alterations in intramolecular distances, causing the energy donor-acceptor pair to be either closer or further apart. Urea increases this distance by disrupting intramolecular hydrogen bonding, while LiBr maximizes intramolecular hydrogen bonding[9] and thus brings the energy donor and acceptor closer.

GOLDFARB *et al.*[2] have recently interpreted the experiments of SHORE AND PARDEE[6] as evidence for energy transfer in proteins by means of an excited-peptide-bond mechanism, similar to conductance-band transfer. These authors have failed to recognize that the findings of Shore and Pardee are reaily interpreted by the resonance mechanism as is shown in this paper. Their most significant error in assuming that an excited-peptide-bond transfer mechanism can account for the energy transfer observed in the protein conjugates is that the peptide bond requires much higher energies of excitation than is provided in a quantum absorbed at 280 mμ by an aromatic amino acid[10].

The author wishes to thank Dr. ELKAN BLOUT, Dr. DOUGLAS E. SMITH, and Dr. DAVID A. YPHANTIS for encouragement and advice. The experimental work described in this paper was started at the Children's Medical Center, Boston, Mass. while the author held a research fellowship. The dye was kindly supplied by the Polaroid Corporation.

This work was performed under the auspices of the U.S. Atomic Energy Commission.

Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill. (U.S.A.)

LUBERT STRYER*

1 W. BROSER AND W. LAUTSCH, *Z. Naturforsch.*, 11b (1956) 453.
2 A. R. GOLDFARB, E. HOFFMAN AND N. GUTSTEIN, *Arch. Biochem. Biophys.*, 76 (1958) 161.
3 I. ISENBERG AND A. SZENT-GYÖRGYI, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 857.
4 T. T. BANNISTER, *Arch. Biochem. Biophys.*, 49 (1954) 222.
5 G. KARREMAN, R. H. STEELE AND A. SZENT-GYÖRGYI, *Proc. Natl. Acad. Sci. U. S.*, 44 (1958) 140.
6 V. G. SHORE AND A. B. PARDEE, *Arch. Biochem. Biophys.*, 62 (1956) 355.
7 G. KARREMAN AND R. H. STEELE, *Biochim. Biophys. Acta*, 25 (1957) 280.
8 T. BUCHER AND J. KASPERS, *Biochim. Biophys. Acta*, 1 (1947) 21.
9 W. F. HARRINGTON AND J. A. SCHELLMAN, *Compt. Rend. Ser. Chim., (Carlsberg)*, 30 (1957) 167.
10 G. H. BEAVEN, *Disc. Faraday Soc.*, 9 (1950) 406.

Received January 2nd, 1959

* Resident Associate. Present address: Harvard Medical School, Boston 15, Mass.

Zone-electrophoretic studies on soluble RNA from rat-liver cytoplasm

The soluble polynucleotides present in rat-liver cytoplasm, until recently almost unexplored, aroused the interest of many investigators in the field of protein biosynthesis for several reasons. Following activation of amino acids by ATP and specific enzymes, the amino acids are transferred to soluble RNA of low molecular weight. Incubation of this sRNA-amino acid complex with liver microsomes in the presence of GTP results in the incorporation of amino acid into microsomal ribonucleoprotein[1]. Isotope experiments *in vivo* revealed that sRNA becomes more highly labelled than microsomal RNA[2-4], while evidence has been presented for a metabolic transfer *in vitro* of the sRNA to the microsomal RNA [5]. Finally it was shown that CTP, ATP

Abbreviations: ATP, adenosine triphosphate; GTP, guanosine triphosphate; CTP, cytosine triphosphate; UTP, uridine triphosphate; RNA, ribonucleic acid; sRNA, soluble RNA.