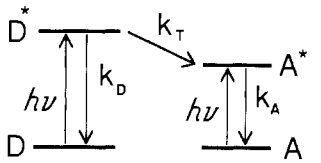

FIGURE 5: General model for energy transfer and decay. Excitation energy is transferred with kinetic rate constant k_T from excited donor group D* to acceptor group A, raising it to excited state A*. In addition, both D and A can be excited by directly absorbed radiation ($h\nu$). Excited-state decay occurs with rate constants k_D and k_A, each combining several different mechanisms of deexcitation. One component of k_A is fluorescence emission from A*, which was measured experimentally.

The results obtained in this study provide an interesting example of a protein that lacks tryptophan residues. This circumstance allowed us to measure the parameters of phenylalanine-to-tyrosine energy transfer. The combination of energy transfer plus the long excited-state lifetime of the phenylalanine residues resulted in two lifetime components in the tyrosine emission. These unusual properties are a chance consequence of the protein's amino acid composition, which is presumed to be a result of adaptation to life at high temperatures.

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REFERENCES

Ambler, R. (1967) *Methods Enzymol. 11*, 155–166.
Berlman, I. (1965) *Handbook of Fluorescence Spectra of Aromatic Molecules*, Academic Press, New York.
Brand, L., & Witholt, B. (1967) *Methods Enzymol. 11*, 776–856.
Burley, S., & Petsko, G. (1985) *Science 229*, 23–28.
Cantor, C., & Schimmel, P. (1980) *Biophysical Chemistry*, Part 2, Chapter 8, W. H. Freeman, San Francisco.
DeLange, R., Williams, L., & Searcy, D. (1981) *J. Biol. Chem. 256*, 905–911.
Drlica, K., & Rouvière-Yaniv, J. (1987) *Microbiol. Rev. 51*, 301–319.
Eisinger, J. (1969) *Biochemistry 8*, 3902–3908.
Eisinger, J., & Flores, J. (1979) *Anal. Biochem. 94*, 15–21.
Eisinger, J., Feuer, B., & Lamola, A. (1969) *Biochemistry 8*, 3908–3915.
Fasman, G. (1976) *Handbook of Biochemistry and Molecular Biology*, 3rd ed., Proteins, Vol. I, CRC Press, Cleveland.
Grinvald, A., & Steinberg, I. Z. (1974) *Anal. Biochem. 59*, 583–596.
Laemmli, U. (1970) *Nature 227*, 680–685.
Longworth, J. (1971) in *Excited States of Proteins and Nucleic Acids* (Steiner, R. F., & Weinryb, I., Eds.) pp 319–484, MacMillan, London.
Pettijohn, D. E. (1988) *J. Biol. Chem. 263*, 12793–12796.
Schauenstein, E., & Bayzer, H. (1955) *J. Polymer Sci. 16*, 45–51.
Searcy, D. (1975) *Biochim. Biophys. Acta 395*, 535–547.
Searcy, D. (1976) *Biochim. Biophys. Acta 451*, 278–286.
Searcy, D., & Stein, D. (1980) *Biochim. Biophys. Acta 609*, 180–195.
Searcy, D., Montenay-Garestier, T., Laston, D., & Hélène, C. (1988) *Biochim. Biophys. Acta 953*, 321–333.
Stein, D., & Searcy, D. (1978) *Science 202*, 219–221.
Tanaka, I., Appelt, K., Dijk, J., White, S., & Wilson, K. (1984) *Nature 310*, 376–381.

APPENDIX: ANALYSIS OF ENERGY TRANSFER AND FLUORESCENCE LIFETIME DATA

The model for energy transfer is diagrammed in Figure 5. The excitation energy is absorbed primarily by donor residue D which then donates energy to acceptor residue A. Experimentally, the concentration of excited A is measured by the intensity of its fluorescent emission. In relation to HTa, D is phenylalanine and A is tyrosine.

Model and Definitions. [D*] is the concentration of excited donor and [A*] the concentration of excited acceptor. The mixture is excited by a brief pulse of radiation and then allowed to decay. Let

$$I_0 = [D^*]_0 + [A^*]_0 \tag{3}$$

where the subscript "0" signifies time $t = 0$ after a brief excitatory pulse. At this moment both A and D have been excited by direct absorption. I_0 is equivalent to the total light absorbed. Also by definition let

$$\gamma = [D^*]_0/[A^*]_0 \tag{4}$$

where γ is equivalent to the ratio of the absorbances of D and A. By rearrangement of eq 3 and 4

$$[D^*]_0 = \gamma I_0/(\gamma + 1) \tag{5}$$

and

$$[A^*]_0 = I_0/(\gamma + 1) \tag{6}$$

By inspection of Figure 5

$$d[D^*]/dt = -(k_T + k_D)[D^*] \tag{7}$$

Following integration and evaluation at $t = 0$

$$[D^*] = \frac{I_0\gamma}{\gamma + 1} e^{-(k_T + k_D)t} \tag{8}$$

For A*

$$d[A^*]/dt = k_T[D^*] - k_A[A^*] \tag{9}$$

$$d^2[A^*]/dt^2 = k_T\, d[D^*]/dt - k_A\, d[A^*]/dt \tag{10}$$

Substituting eq 7 and 9 into eq 10, one obtains

$$\frac{d^2[A^*]}{dt^2} + (k_T + k_D + k_A)\frac{d[A^*]}{dt} + k_A(k_T + k_D)[A^*] = 0 \tag{11}$$

This is a second-order linear homogeneous differential equation. It can be integrated to an expression of the form

$$[A^*] = \beta_1 e^{t/\tau_1} + \beta_2 e^{t/\tau_2} \tag{12}$$

From the algorithm for this integral

$$\tau_1 = -1/(k_T + k_D) \quad \text{and} \quad \tau_2 = -1/k_A \tag{13}$$

τ_1 and τ_2 are the inverse of the kinetic decay constants, which by definition are the excited-state lifetimes of D and A. Expressions for β_1 and β_2 can be obtained as follows. Equation 12 is differentiated and evaluated at $t = 0$ to obtain

$$d[A^*]/dt|_0 = \beta_1/\tau_1 + \beta_2/\tau_2 \tag{14}$$

Separately, when expressions 8 and 12 are substituted into eq 9 and evaluated at $t = 0$, one finds

$$d[A^*]/dt|_0 = k_T I_0\gamma/(\gamma + 1) - k_A(\beta_1 + \beta_2) \tag{15}$$

Substituting expressions 13, eq 14 and 15 can be solved simultaneously for β_1 and β_2:

$$\beta_1 = \frac{\gamma k_T I_0}{(\gamma + 1)(k_A - k_D - k_T)} \quad (16)$$

$$\beta_2 = \frac{I_0}{\gamma + 1}\left(1 - \frac{\gamma k_T}{k_A - k_D - k_T}\right) \quad (17)$$

The final expression is

$$[A^*] = \frac{\gamma k_T I_0}{(\gamma + 1)(k_A - k_D - k_T)} e^{-(k_T + k_D)t} + \frac{I_0}{\gamma + 1}\left(1 - \frac{\gamma k_T}{k_A - k_D - k_T}\right) e^{-k_A t} \quad (18)$$

All parameters in expression 18 are amenable to measurement.

One might expect that expressions 12 and 18 represent simply the sum of two different types of emission with different lifetimes. Such is not the case; β_1 and β_2 have no physical correlates, and β_2 might even be negative. For example, when the fluorescence emission from A* is due entirely to energy transfer from D*, then $\beta_2 = -\beta_1$. In such a case the expression reduces to one similar to that given by Brand and Witholt (1967).

Experimental Validation. Using the above expressions, it is possible to relate β_1 and β_2 to the ultraviolet absorbances of D and A. Dividing expression 16 by expression 17:

$$\frac{\beta_1}{\beta_2} = \frac{\gamma k_T}{k_A - k_D - (\gamma + 1)k_T} \quad (19)$$

The above expression can be solved for γ by using values obtained from the fluorescence lifetime measurements: $k_A = 250 \times 10^6\ s^{-1}$, $k_D = 44.4 \times 10^6\ s^{-1}$, $k_T = 38.9 \times 10^6\ s^{-1}$, $\beta_1 = 0.1401$, and $\beta_2 = 0.238$. Thus, from the fluorescence decay data, $\gamma = 1.59$.

γ is equivalent to the absorbance ratio of the donor and acceptor residues at the excitation wavelength (252 nm), with adjustment for the hypochromicity of the phenylalanine, and including only the fluorescently active residues (77% of the tyrosine and 53% of the phenylalanine; see Discussion). Then, from the ratio of the absorbances, $\gamma = 1.54$. This agrees well with the value above that was calculated from the fluorescence decay kinetics.

Fraction of Emission from A Attributable to Energy Transfer.* After pulse excitation the total fluorescent emission by A*, including both direct excitation and energy transfer, can be obtained by integrating the kinetic equation over infinite time. Expression 12 becomes

$$\int_{t=0}^{\infty} [A^*]_{total} = -\beta_1\tau_1 - \beta_2\tau_2 \quad (20)$$

Next consider only that part of the fluorescence from A that is a result of energy transfer from D. For this component the excitatory radiation is absorbed entirely by D. Thus, $1/\gamma = 0$. Inserting this value for γ into eq 16 and 17, we obtain $\beta_1 = -\beta_2$. Therefore

$$\int_{t=0}^{\infty} [A^*]_{energy\ transfer} = -\beta_1(\tau_1 - \tau_2) \quad (21)$$

Combining eq 20 and 21, the fraction R of the emission that can be attributed to energy transfer is

$$R = \frac{\int_{t=0}^{\infty} [A^*]_{energy\ transfer}}{\int_{t=0}^{\infty} [A^*]_{total}} = \frac{\beta_1(\tau_1 - \tau_2)}{\beta_1\tau_1 + \beta_2\tau_2} \quad (22)$$

Experimentally, the values for β and τ were obtained from the fluorescence lifetime data. Expression 22 was used to calculate the percent contributions in Tables I and II.