

Quenching of Zinc-Substituted Cytochrome *c* Excited States by Cytochrome *b*₅

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Determination of the rates of electron transfer at fixed distances in metalloproteins is a very active area of research.²⁻⁷ The present study was undertaken with the goal of observing both energy and electron transfer in a relatively simple protein-protein complex of probable physiological importance, namely, the complex between cytochrome *c* and cytochrome *b*₅ (*c/b*₅).⁸ The approach, which involves elucidation of the reactivity of the singlet and triplet excited states of Zn^{II}-substituted cytochrome *c* (Zn-*c*)⁹ in Zn-*c/b*₅, is similar to that employed successfully for Zn/Fe hybrid hemoglobins.^{6,7} We note at the outset that our experiments are entirely consistent with Salemme's structural model for *c/b*₅.¹⁰

On mixing a 10⁻⁵ M solution of Zn-*c* in 10⁻³ M, pH 7, HEPES buffer with a 2-fold molar excess of ~10⁻³ M Fe^{III}-*b*₅ in the same buffer,¹¹ the integrated fluorescence intensity of Zn-*c* is reproducibly decreased by about 20%. Furthermore, this fluorescence quenching can be reversed by increasing the ionic strength to 0.1 M with the addition of a small volume of a concentrated aqueous NaCl solution. Under these conditions, Zn-*c/b*₅ complex formation is inhibited.⁸ These observations strongly parallel those of Vanderkooi and co-workers on the Zn-*c*/cytochrome *c* oxidase system.^{9c} The most obvious mechanism for such quenching is (Förster) dipole-dipole energy transfer. As shown elsewhere,¹²⁻¹⁴

the distance in centimeters between donor and acceptor in dipolar energy transfer is given by

$$R_0 = \left\{ \frac{(8.8 \times 10^{-28}) \kappa^2 \phi_{em}}{n^4 (I_0/I - 1)} \int F_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda \right\}^{1/6} \quad (1)$$

where κ is the dipole-dipole orientation factor, n is the refractive index of the medium, ϕ_{em} is the fluorescence quantum yield of the donor (Zn-*c*), and I_0/I is the ratio of the emission intensity of Zn-*c* to that of the Zn-*c/b*₅ complex. The integral in eq 1 represents the overlap between the normalized donor emission probability ($F_D(\lambda)$) and the molar extinction spectrum of the acceptor ($\epsilon_A(\lambda)$). Substituting our experimental results along with some estimates¹⁵ of the other parameters into eq 1 leads to a value of 18 Å for R_0 . The uncertainties in the overlap integral and κ suggest that a 20% uncertainty in R_0 is reasonable.

The most striking result is that the triplet emission decay rate of Zn-*c* ($k = 10^2$ s⁻¹) is remarkably accelerated in Zn-*c/b*₅ ($k = 5 \times 10^5$ s⁻¹).¹⁶ There are several possible explanations for this dramatic increase in rate: (1) dipolar energy transfer, (2) electron exchange or magnetic dipole energy transfer, (3) enhanced intramolecular radiationless decay, and (4) electron transfer. Dipole-dipole energy transfer can be excluded by choosing reasonable upper limits for the relevant parameters ($\kappa^2 = 1$, $\tau_r = 10^{-2}$ s, $R_0 = 16$ Å, and complete spectral overlap) and calculating a rate of dipole energy transfer (4×10^3 s⁻¹) that is 2 orders of magnitude smaller than the observed decay rate. Electron exchange energy transfer is, to first order, forbidden and will probably proceed at a slow rate. All energy transfer mechanisms, including magnetic dipole quenching, can be ruled out by two experiments. First, recent studies have demonstrated⁷ that the Zn-porphyrin triplet excited state in a ZnHb/Fe^{III}-*b*₅ complex (ZnHb = $\alpha_2\beta_2$ Fe^{III}CN hemoglobin) is quenched by electron transfer at a rate of 8×10^3 s⁻¹. In this example, the donor-acceptor separation, the triplet state energy, and the overall spin are similar to or identical with those of the Zn-*c/Fe*^{III}-*b*₅ complex. Since energy transfer does not effectively compete with electron transfer in ZnHb/Fe^{III}-*b*₅, it is unlikely to do so in Zn-*c/b*₅. Furthermore, preliminary results from an investigation of Zn-*c/Fe*^{III}(CN)-*b*₅, in which electron transfer is precluded but energy transfer is unaffected, indicate a Zn-*c* triplet decay rate of 10³ s⁻¹.

Control experiments designed to investigate possible mechanisms of complex-enhanced radiationless decay involved determination of the effects of apocytochrome *b*₅ and Zn^{II}-substituted cytochrome *b*₅ (Zn-*b*₅) on the Zn-*c* triplet lifetime.¹⁷ In the former case, the Zn-*c* lifetime actually increased to 12 ms. In the latter case, the measured triplet decay rate was 10³ s⁻¹,¹⁸ which is entirely consistent with long-distance triplet-triplet dipolar energy transfer quenching.¹⁴ Using eq 1, we find $R_0 = 18$ Å for the triplet-triplet quenching, in excellent agreement with the singlet quenching results.¹⁹ Thus, while the Zn*/Zn* quenching can be well described by Förster theory, the Zn*/Fe quenching cannot. It also should be emphasized that the 18-Å center-to-center distance estimated from singlet and triplet-triplet quenching accords closely with the value (17 Å from the coordinates)¹⁰ predicted by Salemme's *c/b*₅ model.

Electron transfer is the most likely mechanism for the rapid quenching of ³Zn-*c* by ²Fe^{III}-*b*₅, though electron transfer products are not directly detected (<10%) following laser excitation. In-

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(11) Porphyrin-cytochrome *c* and its Zn^{II}-substituted derivative were prepared according to published procedures,^{9a,b} purified by cation exchange chromatography, and characterized by electronic absorption spectroscopy. All manipulations of these proteins were performed with the exclusion of room light. The tryptic fragment of beef liver cytochrome *b*₅ was purified by the method described previously (Reid, L. S.; Mauk, A. G. *J. Am. Chem. Soc.* **1982**, *104*, 841). Fluorescence spectra of protein samples at room temperature were recorded on an instrument constructed at Caltech (Rice, S. F.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 4571).

(12) Equation 1 is a rearrangement of Förster's expression for the rate of dipolar energy transfer.^{13,14} The factor $\phi_{em}/(I_0/I - 1)$ in eq 1 is equal to $1/k_T\tau_r$, where k_T is the rate constant of dipolar energy transfer and τ_r is the reciprocal of the pure radiative decay rate.

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(15) In evaluating R_0 we have set κ^2 and $\phi_{em} = 0.1$ (Vanderkooi, J. M.; Glatz, P.; Casadei, J.; Woodrow, G. V., III. *Eur. J. Biochem.* **1980**, *100*, 189).

(16) Triplet decay rates of protein samples at room temperature were determined by transient absorption spectroscopy using a Quanta Ray laser system described in ref 2b.

(17) Apocytochrome *b*₅ was prepared as described previously (Reid, L. S.; Mauk, M. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 2182). Zn-*b*₅ was prepared by slow addition of Zn protoporphyrin (in 0.1 M NaOH) to a solution of apocytochrome *b*₅ (pH 7) at 4 °C. The Zn-*b*₅ was separated from excess porphyrin by gel filtration chromatography.

(18) The lifetime of Zn-*b*₅ was found to be $\tau_0 = 9 \times 10^{-3}$ s.

(19) The parameters used in this calculation were those used in ref 14 for a calculation of triplet-triplet energy transfer rates.

voking an electron transfer quenching mechanism, therefore, requires that the rate of recombination (k_b) greatly exceed that of forward electron transfer. It is noteworthy that in the two analogous studies with ZnHb/Fe^{III}-b₅⁷ and α_2 Fe^{III} β_2 Zn^{II}-Hb,⁶ in which electron transfer products have been detected, the low yields of formation of Fe^{II} are consistent with $k_b > 10k_f$.

Our results suggest that electron transfer within a protein-protein complex can be quite efficient, even over a large distance (18-Å center-center, 8-Å edge-edge). And they underscore the

value of *energy* transfer as a tool for testing protein-protein structural models.

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Registry No. Cytochrome b₅, 9035-39-6.

Additions and Corrections

The Relaxational Behavior of Self-Associated 6-Methylpurine [*J. Am. Chem. Soc.* 1984, 106, 2239]. HEINZ STERK* and HERMANN GRUBER

Page 2240: The formula of the AK model should read

$$a_0 = a_1 + \frac{2K(2)}{K} a_1 [e^{Ka_1} - 1]$$

Book Reviews *

Electrical Properties of Polymers. Edited by Donald A. Seanor (Xerox Corporation). Academic Press: New York. 1982. xi + 379 pp. \$52.00.

This is a comprehensive work edited by a chemist who has worked for many years on this complex interdisciplinary subject. Chapter 1 is an excellent overview of electrical conduction in polymers, written by the editor. It can be easily understood by readers with diverse scientific backgrounds. It assumes no previous knowledge of the subject but goes into some depth in the topics covered. It is rich in references but is much more than a simple listing of the literature, since it effectively merges much past work into understandable summaries and conclusions.

Chapters 2-8 are written in the same mode as Chapter 1, although they are by different authors. These chapters are much more detailed descriptions of the topics summarized in Chapter 1, such as photoconductivity, electrets, contact electrification, thermally stimulated discharge currents, and dielectric breakdown in polymers. The chapters overlap to some extent, but the book avoids the discontinuities present in many books written by multiple authors. A more detailed description of the interaction between electrical properties and polymer molecular structure and morphology would be useful.

Overall the book is well-written and a very valuable reference source on the electrical properties of polymers.

Perry L. Grady, *North Carolina State University*

Building Scientific Apparatus: A Practical Guide to Design and Construction. By J. H. Moore, C. C. Davis, and M. A. Coplan (University of Maryland). Addison-Wesley: Reading, MA. 1982. xiii + 483 pp. \$54.95.

The authors' stated intention is to provide a practical volume to serve as "an introductory text for the beginning researcher and as a shelf reference for the experienced scientist". Given the breadth of their topic, they have done an excellent job. The material is divided into six chapters of varying length with greatest coverage given to optics (including charged particle optics) and electronics, each of which receives about one third of the volume's total coverage. The other third provides brief coverage of glassworking, mechanical design, and vacuum technology. The authors realize that the modern scientist's greatest need when approaching the construction of scientific apparatus will be to decide what

he himself can do and what is best left to others. In the latter case, an ability to define and communicate needs is all important. Sufficient information is provided on each of the topics to permit the reader to converse intelligently without having to master excessive detail. End of chapter reference lists are provided to allow further study, as needed. It is worth noting that these lists are well categorized and include both basic and more advanced works.

The book does have some flaws. Most importantly, a more detailed index would be expected in a book planned as a general shelf reference. Also, the listings of manufactures and suppliers given with each chapter, while not a bad idea, will date very rapidly. Particularly in a field such as electronics, a check with someone having current knowledge of suppliers is advisable. Finally, cost is likely to keep this volume from taking its place as a common reference in the libraries of many young scientists. If so, much of its great worth will be lost.

Donald Bath, *Western Illinois University*

Aggregation Processes in Solution. Edited by E. Wyn-Jones & J. Gormally (University of Salford). Elsevier Science Publishing Company: Amsterdam and New York. 1983. x + 632 pp. \$138.50.

This book is comprised of 20 chapters, each written by different authors, with an overall broad coverage of the field from a generally fundamental, physical-chemical point of view. Topics include micellar solutions, liquid crystals, bilayer membranes, colloidal properties of drugs, aggregation of dyes and of polymers, drug/protein binding, and even ferrofluids. A number of experimental techniques are also covered in separate chapters, including ultrasonic absorption, ultrasonic relaxation spectrometry, and stopped-flow measurements. The authors are from all over the world, mostly from England, many from Europe, with Americans in the minority.

The chapters generally stand alone as reviews of recent developments in the theoretical and physical chemistry of the systems discussed; all are appropriately subsumed under the general topic of aggregation in solution. As the editors point out, these areas traditionally have been pursued in isolation, and it is the express intention of this volume to bring such work and workers together. There are a number of common threads which run through several chapters each, such that the book may well succeed in its purpose. All chapters are authoritatively written, with generally good coverage of the literature, usually with emphasis on the

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