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DIFFUSION-ENHANCED LANTHANIDE ENERGY TRANSFER STUDIES OF PROTEIN PROSTHETIC GROUPS

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A long-lived luminescent solute in aqueous solution (e.g., $^5D_4$ terbium, $\tau \simeq 10^{-3}$ s) can donate its excitation energy to a chromophore such as a protein prosthetic group by, e.g., the radiationless dipolar mechanism of Förster. However, in contrast to the usual energy-transfer experiment, a donor with a 10^{-3} s lifetime can diffuse extensively through the solution and, in a time scale short compared to its excited lifetime, sample all permitted locations with respect to chromophoric acceptors. As recently indicated by Thomas et al. (1) energy transfer in this rapid-diffusion limit can permit direct measurement of the allowed distance of closest approach of small solute molecules to chromophores which may be buried within proteins or membranes.

The rate of radiationless energy transfer by the dipolar mechanism from an excited donor to a stationary acceptor a distance, r, away is

$$k_T = \frac{1}{\tau_0}\left(\frac{R_0}{r}\right)^6 \text{s}^{-1}, \tag{1}$$

where τ_0 is the excited donor's lifetime in the absence of acceptor, and R_0 is the distance at which the rate of energy transfer equals the rate of donor de-excitation in the absence of acceptor (2). If the donor and/or acceptor diffuse significantly during the donor lifetime, Eq. 1 no longer holds (3). If diffusive motion is rapid enough so that $\sqrt{6D\tau} >> s$ (where the diffusion coefficient $D = D_d + D_a$, τ is the experimental lifetime of the donor, and s is the average separation between acceptors), then the energy acceptors are effectively uniformly distributed in space (1). In this rapid-diffusion limit, the rate of energy transfer by the dipolar mechanism from an excited donor to the surrounding acceptors is found by integrating Eq. 1 over all space which the acceptors are allowed to occupy with respect to a given donor. As

shown by Thomas et al. (1) energy transfer in the rapid-diffusion limit is particularly sensitive to the distance of closest approach of donor to acceptor.

We have prepared a series of metal-chelating reagents which bind terbium and europium very strongly and greatly enhance their luminescence intensity. An electrically neutral Tb(III) chelate has been used to probe the environment of the antibiotic rifamycin bound to RNA polymerase; the results complement those obtained by Stender and Scheit (4), who used antibodies to study the topography of the rifamycin binding site on this multisubunit enzyme. Measurement of the lifetime of chelated terbium in the presence of free rifamycin yields rate constant for energy transfer $k_f = 2.5 \times 10^7\ M^{-1}s^{-1}$, which is more than an order of magnitude greater than expected from the dipolar mechanism alone. This indicates that energy transfer takes place predominantly by the collisional "exchange" mechanism in this case (5). Measurements of energy transfer from chelated terbium to the rifamycin-RNA polymerase complex yield a rate constant $k_b = 1.1 \times 10^7 M^{-1}s^{-1}$. Since $(k_b/k_f) \simeq 1/2$, this implies that enzyme-bound rifamycin is still directly accessible to chelated terbium. Quantitative bonding of rifamycin ($K_s = 3 \times 10^9\ M^{-1}$) was assured by using a twofold excess of enzyme and confirmed by enzyme assay.

On the other hand, the RNA polymerase inhibitor cibacron blue changes its energy-transfer rate constant from $k_f = 9.9 \times 10^7\ M^{-1}s^{-1}$ to $k_b = 0.9 \times 10^7\ M^{-1}s^{-1}$ upon binding to the enzyme. Again, the magnitudes of these rate constants indicate that the exchange mechanism predominates. The result that $(k_b/k_f) = 1/11$ implies that enzyme-bound cibacron is only partially accessible to chelated terbium.

Studies of each monoferric species of the two-site iron binding protein transferrin indicate that each bound iron is several Ångstroms below the surface of the protein. Model calculations suggest that the distance of each metal ion below the surface of the protein is in the 5–10 Å range. Substituting oxalate ion for the "normal" anion bicarbonate in the ternary transferrin-iron-anion complex significantly increases the apparent distance of closest approach of the excited donor to bound Fe(III). Substituting Mn(III) for Fe(III) in the transferrin-bicarbonate-metal complex has little effect on the apparent distance of closest approach of donor to bound metal.

It is reasonable to expect that electrostatic effects will be very important in energy transfer measurements in the rapid-diffusion limit, and we have recently confirmed this with experiments involving terbium chelates with -1 and 0 charges and acceptors having -2 or -1 charges. Energy transfer in the rapid-diffusion limit should provide a measure of the radial distribution function of ions about the average terbium chelate. Our initial results, obtained by varying the ionic strength of the medium, are in good quantitative agreement with calculations using the Debye-Hückel radial distribution function. We hope that future studies may provide information about, e.g., the net electrostatic charge near a protein-bound chromophore.

We are grateful to Professor Lubert Stryer for his help and encouragement, and for allowing us to use his instrumentation in making the lifetime measurements. This work was supported by NIH research grants GM-25909 and CA-16861 and by research career development award CA-00462 to C. F. Meares.

Received for publication 17 December 1979.

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POSSIBLE DISTORTION OF ANTIBODY BINDING SITE OF THE Mcg BENCE-JONES PROTEIN BY LATTICE FORCES

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The Mcg Bence-Jones protein dimer closely resembles an antigen binding fragment of a functional antibody molecule both in its structure and function (1, 2). It consists of two chemically identical light chains which have different conformations: monomer 1 resembles the heavy chain, and monomer 2 the light chain of an Fab fragment. Each monomer consists of a variable and a constant domain connected by a switch peptide. In the crystal, the two variable domains, and also the two constant domains, have very similar structures related by local twofold axes. However, because the symmetry is only a local one, the surface of the domains is influenced by their different environments in the crystal.

The refinement of the Mcg Bence-Jones protein is in progress, using 12,500 reflections with $I > 5\ \sigma(I)$ from 6.5 to 2.3 Å. The refinement process started with the constrained crystallographic refinement of Deisenhofer and Steigemann (3). The positions of the atoms were improved by real-space refinement, followed by the calculation of structure factors and a new improved electron density map. This process was repeated cyclically, to the point of diminishing returns. At this stage, "manual" corrections were performed with the GRIP 75 molecular graphics system[1] at the University of North Carolina, using calculated Fourier and difference Fourier maps. At present, restrained least-squares refinement (4) is under way. The R-factor with overall temperature factor has now been decreased from the initial 43% to 26%.

As the refinement progressed, core segments of domains, which are expected to be identical, became increasingly similar. Upon superposition of α-carbon positions of identical regular segments, the average deviation decreased from 0.95 to 0.4 Å. Fine points of the structure become evident; for example, the electron density distribution indicates that Pro 145 is a *cis* proline in both monomers. It is located in the third position of a reverse turn, as are *cis* prolines previously identified in other protein structures, including the variable domain of a κ light chain (5). Proline 145 must be a structurally important residue, as the equivalent of Pro 145 is conserved in almost all constant region domains.

Initially, 360 atoms out of a total of 3,222 in the structure had no associated electron

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[1]GRIP-75 developers included E. G. Britton, F. P. Brooks, Jr., J. Hermans, J. S. Lipscomb, J. E. McQueen, M. E. Pique, and W. V. Wright. GRIP-75 development has been supported by the National Institutes of Health Division of Research Resources, the National Science Foundation, the Atomic Energy Commission, and the International Business Machines Corporation.