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LONG-RANGE ELECTRON EXCHANGE REACTIONS OF EXCITED TRIPLET TRYPTOPHAN IN PROTEINS

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Abstract Ten proteins that exhibit long-lived phosphorescence lifetimes at room temperature were examined for sensitivity to quenching by molecules that are external to the protein. The bimolecular quenching rate constant was found to decrease exponentially with the distance of the tryptophan from the protein surface. Theoretical analysis shows that this behavior is expected for an electron-exchange reaction between the buried tryptophan and quenchers in solution in the rapid diffusion limit. The results allow evaluation of the distance parameter, ρ, for electron transfer through the general protein matrix at 1.0 Å. For a unimolecular donor-acceptor pair with $k_{et} = k_o \exp(-r/\rho)$, $k_o = 10^9 sec^{-1}$.

Keywords: Tryptophan, phosphorescence, electron exchange, quenching, parvalbumin, aldolase

INTRODUCTION

A great deal of effort is being made to examine protein structures and their dynamical properties, but ultimately, when all is known about the positions and motions of the nuclei, we will still need to know how these determine the reactivity of electrons. This is because the electrons are what participate in reactions! Optical spectroscopy offers an approach to study the problem of how the protein's many nuclei affect electron transfer reactions, because optical transitions are themselves transitions of electrons and a reactive species in the protein can be formed by light activation. Whether the excited species undergoes chemical reactions depends upon interactions of the excited state species with the polypeptide chain, as well as the excited-state lifetime and the mutual reactivity and accessibility of the excited state molecule with neighboring molecules.

The triplet excited state, as demonstrated by the work of others in this conference, is very long-lived making it especially reactive. In earlier work we showed that tryptophan phosphorescence at room temperature, though previously seen rarely, is in fact to be found in most proteins (1). Therefore, reactions of excited tryptophan in a protein can be monitored by quenching of phosphorescence emission. In screening various quenchers, we have found that for molecules with > three atoms, the quenching depended upon the protein, but not details of the quencher such as charge or shape of the quencher or the viscosity of the medium (2). This suggested to us that the quencher did not diffuse through the protein, and that quenching occurs by long-range interaction. The nature of this interaction is discussed here.

EXPERIMENTAL

The paper by Calhoun et al. (2) gives the sources of supplies, procedures for sample preparation and details of the instruments used. Phosphorescence lifetimes were obtained from decay profiles by analysis for exponential decay. The experimental procedure selects for the longest-lived component of the decay, and its decay could be generally well fit by a single-exponential function. Quenching was monitored by decrease in lifetime, and the quenching rate constant, k_{qe}, was computed from

$$\tau_0/\tau = 1 + k_{qe}\,\tau_0[Q] \qquad (1)$$

as described by Stern and Volmer (3) but modified for lifetimes. Here τ_0 and τ are the lifetimes in the absence and presence of quencher, respectively.

Coordinates for alkaline phosphatase was obtained from Dr. H. W. Wyckoff (New Haven CT); other protein coordinates were obtained from the Protein Data Bank (Brookhaven National Laboratory, Upton L.I., NY). Three-dimensional representations of the structures were plotted using Biograph (BioDesign, Pasadena, CA).

RESULTS

Protein emission

Ten proteins with lifetimes ranging from 5 msec to 1.6 sec were chosen for study. Ribonuclease T1, staphylococcal nuclease, azurin and parvalbumin each have a single tryptophan. The emitting tryptophan of liver alcohol dehydrogenase has been identified as trp 314 and the longest lived emitting species of glyceraldehyde-3-phosphate dehydrogenase appears to be trp 310. On the basis of structure, we made a choice for the probable longest lived tryptophan. Earlier work suggests that long-lived tryptophan phosphorescence is related to local structural rigidity (4). Where long-lived phosphorescence has been assigned to a particular tryptophan, these tryptophans have been found to be in structured regions of the protein (reviewed in 5). The assignments of the emitting species are given in Table 1.

TABLE I. Assignment of phosphorescent tryptophans at room temperature

Protein name	tryptophan	location	distance to surface	lifetime
alkaline phosphatase	109	in α helix	15 A	1.6 sec
azurin	48	in β-barrel	10.2	0.4
aldolase	147	in β-barrel	8-9	0.04
pronase	67	in β-strand	7-8	0.7
GAPDH	310	in β-strand	7	0.75
ADH	314	in β-strand	5.3	0.3
RNase T1	59	in β-strand	2.2	0.014
nuclease	140	in random coil	~2	0.009
parvalbumin	109	not known	~<2	0.005
thermolysin	55	in β-strand	~1	0.01

GAPH: glyceraldehyde phosphate dehydrogenase; ADH: liver alcohol dehydrogenase; RNase T1; ribonucluease T1

Protein quenching

If long range transfer occurs from the protein-buried tryptophan to the quencher in solution, we thought that quenching would be related to the distance of the tryptophan from the surface. This was tested for the ten proteins and various quenchers. The results are shown on Figure 1 where we plotted the second order quenching constant as a function of distance from the surface of the protein.

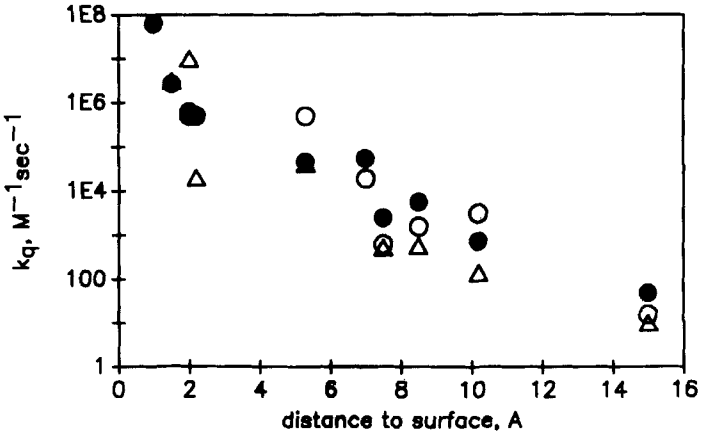

FIGURE 1 Relationship between Stern-Volmer quenching constants and distance to the surface for the proteins in Table I. Quenching by nitrite (●), ethanethiol (Δ), methylvinylketone (○). Original data in Calhoun et al. (2).

DISCUSSION

We have seen a remarkable thing in Figure 1. The log of bimolecular quenching rate constant appears as a linear function of distance of tryptophan from the surface of the protein!

For a donor and acceptor at fixed distance the transfer rate k for an electron exchange reaction *is* expected to be logarithmically dependent on distance r (6,7):

$$k = k_O \exp(-r/\rho) \qquad (2)$$

Here k_O is the first-order quenching rate when donor and quencher are in van der Waals contact, and ρ is the incremental distance between donor and acceptor for a 1/e attenuation of the transfer rate. Therefore, an exponential dependence on distance would indicate that quenching occurs by an electron exchange reaction. But this expression is for a unimolecular reaction in which donor and acceptor are at fixed distance from each other. In our case, the quencher is free in solution. How can we justify the unusual dependence of quenching on the location of the tryptophan-- that appears to follow the prediction for a first order reaction?

We consider the details of the reaction. In many cases where collision between the donor and acceptor is required for reaction, the donor-quencher

interaction is efficient so that all (nearly all) reactions produce a quenching reaction -- the reaction is diffusion-limited.

But, another situation occurs when the quenching reaction is slow in comparison with the encounter rate. In this case the emitter species senses the time-averaged quencher concentration, and the quenching rate becomes independent of diffusion. This is the "rapid diffusion limit" discussed by Thomas et al. (8) and applied specifically to phosphorescence quenching of proteins (9). It was also observed by Calhoun et al. (10) for resonance energy transfer experiments.

In the rapid diffusion limit, the first-order quenching rate is a summation of contributions from all quencher molecules, each weighted according to the distance dependence of the quenching process:

$$\Phi = \int_V Q(\mathbf{r},t)k(\mathbf{r},t)d^3r = 10^{-3}N[Q]\int_{\bar{V}} k(r)d^3r \qquad (3)$$

Here $k(\mathbf{r},t)$ is the properly weighted second-order rate constant for interaction of a single donor-acceptor pair separated by the vector $\mathbf{r}$. The quencher concentration $Q(\mathbf{r},t)$ in molecules/cm^3, is zero within the protein and $10^{-3}N[Q]$, in moles/liter, over the external volume and N is Avogadro's number.

We can substitute Eq 1 into Eq 2 to obtain the second-order rate constant, Φ/Q:

$$k_q = \Phi/Q = 10^{-3}\, k_o \int_{\bar{V}} \exp(-r/\rho)\, d^3r \qquad (4)$$

Fine. Equation 3 states that the second order rate constant is proportional to the quenching constant, provided that the latter is slow relative to the diffusion.

However, there is another reason why the plot of Figure 1 is surprising. As indicated in the introduction, the quencher is in the solvent and not in the protein, and therefore the distance of the tryptophan to the protein surface should be important. But, quenchers colliding any where on the protein surface should contribute to the quenching, and consequently we should integrate the area of the surface as indicated in Equation 2. This we did not do. We simply used the closest distance of tryptophan to the surface and it seems unusual that we would get a straight line when we made such a seemingly drastic assumption! But again, this assumption is not bad, as we can see when we estimate the error by using two extreme models for the protein.

For example, for a spherical protein of radius a with tryptophan at its exact center the dependence of quenching on a is:

$$k_q^{sph} = 4\pi\, 10^{-3}Nk_o \int_r^{\infty} r^2\exp(-r/\rho)dr$$

$$= 4\pi\, 10^{-3}Nk_o\,[a^2\rho + 2a\rho^2 + 2\rho^3]\exp(-a/\rho) \qquad (5).$$

A protein in which the tryptophan is at distance a near the surface can be considered as an effective planar surface. Then

$$k_q^{pl} = 2\pi\, 10^{-3}Nk_o \int_a^{\infty} dz \int_z^{\infty} \exp\,[-(r^2+z^2)^{1/2}/\rho]rdr$$

$$= 2\pi\, 10^{-3}Nk_o\,[a\rho^2 + 2\rho^3]\exp(-a/\rho) \qquad (6).$$

Figure 2 shows the dependence on a of these equations, with the natural logarithm of the "attenuation factor" plotted against a, the distance of closest approach. In both cases, the exponential part of the curve dominates at distances > 4A. This simulation argues for the case that the geometry of the system does not greatly matter -- the most important factor is the distance of closest approach. This assumption will only hold when the rate is very sharply dependent upon distance, as in our case.

So we conclude that the bimolecular rate constant for the phosphorescence quenching is proportional to the unimolecular transfer rate and is a function of the distance of tryptophan burial. This is a useful insight, since it permits the study of electron exchange/transfer reactions in proteins without the use of covalent modifications. Theory predicts that the electron transfer rate is a function of the redox potential (7). In our case, because tryptophan is always the donor and is buried, we expect that its redox potential does not change from protein to protein. This assumption is supported by the observation that the phosphorescence spectrum of tryptophan is relatively independent of protein. Further, the acceptor is always in the aqueous phase and therefore its potential does not change from reaction to reaction.

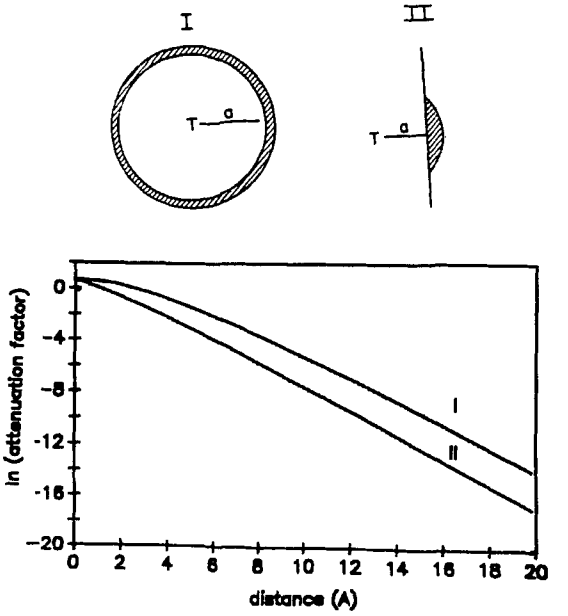

FIGURE 2 Top: Protein models with simple geometry. T. represents tryptophan, and a is the distance of closest approach to the aqueous surface. Scheme I. Tryptophan at the center of a spherical protein; II. tryptophan in a protein with planar surface. Bottom: Quenching probability (attenuation factor) for the spherical (I) and planar (II) cases plotted against the distance of closest approach. For case I, this factor is defined as $[a^2\rho + 2a\rho^2 + 2\rho^3]\exp(-a/\rho)$ and for case II it is $[a\rho^2 + 2\rho^3]\exp(-a/\rho)$.

The slope of the curve (Fig 1) gives us the attenuation factor for the electron exchange. We found for the quenchers used, that ρ is 1 A, i.e., the rate decreases one natural log unit/ angstrom. In electron transfer theory, the distance parameter is a sensitive function of the height of the energy barrier associated with tunneling through the protein (11). Other experimental data available for electron transfer from excited states in proteins gives values for ρ between 1.1 and 1.4 A (reviewed in 12,13) and therefore the results are in reasonable agreement. Because our measurements use many proteins to determine ρ, our value refers to electron exchange when the intervening medium is represented by averaged protein matrix.

There are several points which are of further interest in this work. We would like to know what the determinants are for the quencher and what the products of the reaction are. At question is the meaning of the value of k_Q, the rate of quenching at contact. We obtain a value of about 10^9/sec, and how this value is related to relaxation of the protein and intersystem crossing rates is being determined.

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