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HETEROGENEITY AND SOLUTE QUENCHING OF PROTEIN FLUORESCENCE

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The early studies on lysozyme (1) provided clear-cut examples of spectral heterogeneity of tryptophyl fluorescence in a protein. This heterogeneity was observed in difference fluorescence spectra produced by certain mild perturbations that did not alter the conformation of the protein. The results were interpreted as due to selective quenching of the fluorescence of one (or more) tryptophyl(s) whose Stokes shift depended upon the polarity of its immediate environment. Increasing the pH from 7.5 to 11.5 resulted in the selective quenching of the fluorescence of "blue" tryptophyls for both the free enzyme and the lysozyme—tri-*N*-acetyl-D-glucosamine (triNAG) complex due to energy transfer to ionized tyrosyl. Quenching of a "reddish" tryptophyl and of a blue tryptophyl was also observed as the pH was decreased in the pH 7.5–5.5 region and the 5.5–2.0 region, respectively, only for the lysozyme-triNAG complex. Selective quenching of the fluorescence of red tryptophyls in the free enzyme resulted from the addition of iodide (2, 3). Similar selective quenching effects were obtained due to energy transfer to solute (4). These studies suggested that protein fluorophors in general can be grouped into classes.

Solute quenching studies can help to separate fluorescence contributions of classes of fluorophors with differing accessibility. In these cases the resultant heterogeneity of quenching can be noted by the shifting spectra to the blue for tryptophyl or, more generally, by downward-curving Stern-Volmer plots. By replotting these data as fluorescence quenched rather than fluorescence remaining, information on those residues whose fluorescence is quenched most readily is obtained. Thus, the limiting straight

line of a reciprocal Stern-Volmer plot ($F_0/\Delta F$ vs. $1/[X]$) provides an effective quenching constant, $K_e = \Sigma f_i K_i^2 / \Sigma f_i K_i$, and an effective fractional accessible fluorescence, $f_e = (\Sigma f_i K_i)^2 / \Sigma f_i K_i^2$. These parameters provide a better index of the properties of the most accessible fluorophors than the corresponding parameters obtained from the limiting slopes of simple Stern-Volmer plots, $K_e = \Sigma f_i K_i$; $f_e = (\Sigma f_i K_i^{-1})^2 / \Sigma f_i K_i^{-2}$ (5). With the use of sensitive differential fluorometers these parameters may reveal subtle changes of accessibility accompanying a variety of protein interactions.

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IS OPTICAL DETECTION OF MAGNETIC RESONANCE USEFUL IN DETECTING HETEROGENEITY IN PROTEIN PHOSPHORESCENCE?

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Optical methods have been used for some time in detecting heterogeneity in the tryptophan sites of proteins. Some of these methods rely on the sensitivity of the Stokes shift of the tryptophan phosphorescence origin to the nature of the environment, particularly its electrical polarizability.

A good example of the sensitivity of the Stokes shift of the indole chromophore phosphorescence to its environment is found in indole itself dissolved in the crystalline Shpol'skii matrix, indane. Four well-resolved O—O bands with 12 cm^{-1} widths are observed at 1.25 K spread over a range of about 300 cm^{-1}. These discrete origins probably result from distinct guest sites in the matrix which have differing host polarizabilities along the direction of dipole moment change in the $S_0 - T_1$ process.

Possible heterogeneity within these optically resolved bands was investigated by utilizing another property of the excited state that also is expected to vary with the environment–the zero field splittings (ZFS). The splitting pattern of the magnetic sublevels is determined principally by the magnetic dipole-dipole coupling of the spin-parallel electrons and is characterized by the ZF parameters D and E. In zero magnetic field, magnetic dipole-allowed transitions can be induced between the sublevels at the frequencies $(|D| + |E|)c$, $(|D| - |E|)c$, and $2|E|c$, where c is the speed of