HETEROGENEITY OF FLUORESCENCE

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Some of the factors that influence the character of tryptophan fluorescence in proteins have been identified and studied. The techniques of chemical modification, steady-state fluorescence, transient fluorescence, and fluorescence quenching have been used to isolate the contribution of individual tryptophans to the total tryptophan fluorescence. Lysozymes from various sources, lysozyme derivatives, and bovine α-lactalbumin have been examined. In hen lysozyme only three tryptophans appear to emit significantly. The intensity of emission and spectral characteristics of individual tryptophans depend not only on exposure to solvent, but also on tryptophan contact with other amino acids in the protein. The vicinity of disulfide residues appears particularly important and leads to extensive quenching of tryptophan emission. Energy transfer between tryptophans is also an important factor, and both steady-state and transient results support the existence of energy transfer from tryptophan 108 to tryptophan 62 in hen lysozyme. Tryptophan emission appears to be very sensitive to small changes in its environment. The modification of one residue in hen lysozyme leads to observable differences in tryptophan emission. Under certain conditions, turkey lysozyme, which differs from hen lysozyme by only seven residues, appears to have one more emitting tryptophan than hen lysozyme. In addition, the fractional static quenching of some tryptophan residues suggests that in the native state some proteins exist in multiple conformations, which interconvert slowly relative to the fluorescence lifetime.

SITE HETEROGENEITY OF TRYPTOPHYL RESIDUES IN PROTEINS DETERMINED BY FLUORESCENCE QUENCHING

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Acrylamide is an efficient neutral quencher of tryptophyl fluorescence, which we report to be very discriminating in sensing the exposure of this residue in proteins. Lifetime measurements confirm that the quenching reaction can be kinetically described in terms of a collisional and a static component. The rate constant for the collisional component has values ranging from $4 \times 10^9\ M^{-1}s^{-1}$ for the fully exposed tryptophyl in corticotropin to $<5 \times 10^8\ M^{-1}s^{-1}$ for the buried residue in azurin. A static component is detected in some of the single-tryptophyl-containing proteins and in all denatured proteins. Quenching patterns for multi-tryptophyl-containing proteins can be analyzed only qualitatively, but may be used to monitor the direction of protein con-

formational changes. In contrast to ionic quenchers, acrylamide not only senses the existence of an indole ring on the surface of a protein, but can report how deeply a residue is buried within a protein as well. The quenching rate constants for RNase T_1, human serum albumin (HSA), and aldolase are found to be independent of the solvent viscosity. Thus, the reaction is limited by penetration of the quencher through the protein matrix. Temperature dependence studies indicate that the tryptophyl residues in aldolase and RNase T_1 are shielded by a large activation energy barrier, while the single residue in HSA is shielded by a large activation entropy barrier. These parameters characterize the nature of the protein matrix enveloping the fluorophores.

A less polar neutral quencher, 2,2,2-trichloroethanol (TCE), was examined to investigate whether it would preferentially interact with apolar regions of proteins. For most proteins studied, the degree of quenching by TCE is found to be about the same as with acrylamide. Thus, either the fluorophores are not located in large hydrophobic regions or the oily regions surrounding them are not capable of expanding to accomodate the probe. However, for HSA and bovine serum albumin hydrophobic interactions between TCE and these proteins occur, leading to an exalted quenching. The fluorescence quencher thus senses the presence of a hydrophobic domain in the vicinity of indole side chains in these proteins.

TCE is shown to be a potentially useful quencher for proteins having predominantly tyrosyl emission. The excitation wavelength dependence of the quenching constant, K, can be measured since TCE is transparent in the ultraviolet ($\epsilon_M^{240nm} = 0.29$). For example, the K for TCE quenching of *N*-acetyl-L-tryptophanamide (NATA) has a constant value of $11.7 \pm 0.7\ M^{-1}$ from 240 to 295 nm. This argues that the lifetime of NATA is the same whether it is excited into its first or second absorption band.

In summary, fluorescence quenching studies can provide information about the nature of the microenvironment and accessibility of fluorescent residues of proteins.

HETEROGENEITY AND DYNAMICS OF PROTEIN CONFORMATION REVEALED BY FLUORESCENCE DECAY KINETICS OF TRYPTOPHAN RESIDUES

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The lifetime of the excited state of proteins is in the range of 0–8 ns; thus fluorescence is unique as a tool to characterize dynamic events in the nanosecond time range. Study of the fluorescence characteristics of the aromatic amino acids in proteins offers the advantages of intrinsic probes and with the correct choice of experimental conditions one

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