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-