13 Energy Transfer in Native Proteins

For several reasons it seems unlikely that the peptide bonds in proteins can act as transmitters of electronic excitation under normal conditions. First, electronic transitions should occur at very high frequencies, i.e. in the UV region at 190 nm, and the fluorescence quantum yield is almost zero. Second, peptide bonds have relatively intense vibrations in the IR region. Therefore, very fast internal conversion should take place. Third, peptide bonds in proteins are surrounded by amino acid residues, which can efficiently deactivate the excited peptide bonds.

Aromatic amino acid residues: tryptophan, tyrosine and phenylalanine as well as special chromophore prosthetic groups and coenzymes (for many redox proteins and enzymes): flavins, hemes, nicotine-amid-adeninenucleotides, and retinals are potential donors and acceptors of electronic excitation energy in protein.

The fluorescence of tryptophan and tyrosine is usually strongly quenched in proteins containing prosthetic chromophore groups or in enzymes bound to substrates or coenzymes containing a chromophore. These are heme proteins (cytochromes, globins, catalases, peroxidases), retinal-containing proteins (rhodopsin, bacteriorhodopsin), copper-proteins (azurin etc.), NADH-dependent dehydrogenases, flavine oxidases, luciferases, etc. Effective energy transfer in these systems usually occurs over distances of about 10 Å [60].

13.1 Tyrosine–Tryptophan Pair

The efficiency of energy transfer from tyrosine to tryptophan residues is very interesting to be studied [60, 67, 15–18, 312]. A theoretical value of the Forster radius for the pair tyrosine - tryptophan is about 10–14 Å [67, 17, 18, 313, 314]. Calculations were based on the quantum yield of fluorescence of tyrosine and the integral of the overlap between the emission spectrum of tyrosine and absorption spectrum of tryptophan in aqueous solutions of these amino acids.

However, spectral parameters are quite different in proteins: the quantum yield of tyrosine residue is substantially lower than that of a free tyrosine; the absorption spectrum of free tryptophan in water is usually wider than that of tryptophan residues; the peak of the absorption band of free tryptophan in water is red-shifted by several nanometers and varies from one protein to another. It should be noted that a low quantum yield of tyrosine fluorescence is also registered in proteins containing no tryptophan residues [17, 313]. The exact estimation of $R_0$ for aromatic residues in proteins seems hardly possible even when the orientations are...
The efficiency of energy transfer from tyrosine to tryptophan residues was estimated in different ways by various authors. Some of them interpret their results for the same proteins as indicative of efficient transfer, others deny any presence of it. The following are some examples.

Kronman and Holmes [164] measured quantum yields of fluorescence from a number of proteins using two excitation wavelengths: 295 nm, where tryptophan residues are preferentially excited, and 280 nm, where both tyrosine and tryptophan residues are excited. They estimated the efficiency of energy transfer from tyrosines to be 100% in trypsin, 50% in papain, 80% in pepsin and >45% in albumin. Lerner et al. registered the efficiency of energy transfer as only 27% in trypsin [315] that was similar to the efficiency of 29% determined in the experiments of Saito et al. [316]. No energy transfer from tyrosine to tryptophan residues in lysozyme, chymotrypsin or chymotrypsinogen was revealed in [315]. Based on the Forster theory, Turoverov et al. [317] predicted 50% efficiency of energy transfer from one of the two tyrosine residues in azurin to the single tryptophan residue in this protein. The authors used X-ray diffraction data for azurin crystals with an exact determination of the distance between the chromophores and their mutual orientations. However, no energy transfer was observed in the experiments [243].

Using Forster theory and X-ray diffraction data for γ-crystalline, Borkman et al. [314] predicted the efficiency of energy transfer of about 83% from 15 tyrosine residues to 4 tryptophan residues. Their experiments yielded the value of 78%. Such good agreement can be explained by various adjustments made by the authors because neither parameters of tyrosine fluorescence in the absence of tryptophan residues nor fluorescence and absorption spectra for individual residues are known for γ-crystalline; reliable data on the overlap of the spectra are completely lacking.

Experiments on ketosteroid isomerase mutants using time-resolved fluorescence and fluorescence anisotropy demonstrated that in a hydrophobic environment Tyr-14 had an unusually long fluorescence lifetime (4.6 ns) as compared to Tyr-55 (2.0 ns) or Tyr-88 (0.8 ns) and to most tyrosine residues in proteins (0.2-2 ns) [318]. Forster radii obtained from the absorption and emission of these tyrosines predict that a total quenching of Tyr-14 fluorescence by Tyr-55, and to a lesser degree by Tyr-88, would take place if their orientations were favorable. The distances calculated using the Forster equation (11.6) are 12.5 Å between Tyr-14 and Tyr-55, 11.1 Å between Tyr-14 and Tyr-88, 10.4 Å between Tyr-55 and Tyr-14, etc. The authors came to the conclusion that the lack of efficient quenching of Tyr-14 by Tyr-55 implies that Tyr-14 and Tyr-55 are oriented unfavorably, and that this orientation is rigid on the time scale of picoseconds to nanoseconds. The rigidity of Tyr-14 and Tyr-55 was confirmed by time-resolved fluorescence anisotropy at 20° and 40°C, where the only correlation time corresponding to the global motion of the protein was resolved. Three factors probably account for the unusually high fluorescence of Tyr-14 [318]: (a) the hydrophobic environment; (b) the absence of quenching groups nearby; (c) the rigidity of Tyr-14 environment.

The 30% efficiency of energy transfer from Tyr to Trp in the peptide hormone...