Photo-Induced Conformational Motility of Proteins

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Abstract—The dynamics of proteins, detected by fluorescence, consists of three components: spontaneous dynamics, dipole-dipole photo-induced dynamics, thermal photo-induced dynamics. The photo-induced dynamics can lead to activation as well as inactivation of enzymes.

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INTRODUCTION

Photoexcitation of proteins (in particular, enzymes) in biological structures may lead to most diverse consequences (biologically useful or, conversely, negative): intensification of internal dynamics, acceleration of rotation of separate groups and the very globule in a whole, emergence of photoconformers, denaturation and aggregation, acceleration of desorption of substrate or product from the active center of enzymes, synthesis of ATP in mitochondria, etc. [1, 2].

EXPERIMENTAL

In the work use was made of a block phase-modulation spectrophotofluorimeter SLM-4800 (USA), permitting executing registration of spectra of excitation, radiation, measuring the degree of polarization and the life time of excited state, and also actualizing upon necessity powerful irradiation in the visible and UV region of the spectrum. At a very small optical density and weak intensity of fluorescence the spectral measurements and photochemical reactions were conducted in mirror cuvettes and microcuvettes [2]. The indicated cuvettes permit raising the intensity of irradiation and light-harvesting of fluorescence several-fold. At this expense it becomes possible also to raise the precision of measuring the degree of polarization and the life time. The heating of solution in the course of powerful irradiation (in conditions of thermostatting) did not exceed 0.5°C.

RESULTS AND DISCUSSION

Spectral nonuniformity of tryptophanic radiation. Upon absorption of a photon by a chromophore molecule therein takes place a redistribution of electronic density and an increase of the dipole moment. In an excited state the molecule interacts with the microenvironment more strongly. An excited molecule wastes a part of energy on vibrational modes of the microenvironment and its dielectric relaxation. There arises an additional (as compared with vacuum or nonpolar solvent) Stokes shift of the radiated spectrum.

Especially interesting is the case when there is one type of absorption center (spectral uniformity of absorbing centers), but after photoexcitation there arise two or more centers of radiation (spectral nonuniformity of radiating centers). This is often observed for flexible or polar molecules in viscous or polar solvents. For example, for tryptophan in glycerol there takes place spectral uniformity with respect to the excitation center, but disclosed is spectral nonuniformity with respect to the radiation center, with that the life time growing from the «blue» region of the spectrum to the «red» one [1, 2]. Excited tryptophan forms with glycerol two types of complex: weak low-polar complex with CH groups and strong polar complex with OH groups. The former has radiation prevalently in the short-wavelength part of the spectrum, the latter—in the long-wavelength one. Radiation of tryptophan in alcohols in essence appears exciplex. For example, upon addition of alcohols to a solution of indole in hexane one observes quenching of monomeric indolic fluorescence at 308 nm and in the range of 340 nm (red shift — about 3000 cm⁻¹) there arises a broadened structureless band belonging to a solvate exciplex indole-alcohol. Radiation of exciplexes of tryptophan with OH groups of glycerol or ethanol is formed at the expense of changing a part of energy of
the electronic excitation of the indolic chromophore into OH-vibrations of solvate.

A spectral nonuniformity of radiation of biopolymers takes place not only upon availability of different chromophores (with overlapping radiation spectra, for example, tryptophan and tyrosine in protein) and conformers (for example, as an extreme case, native protein and denatured), but also photocounters (for example, monomeric and exciplex radiation of tryptophan in protein), characterized, in honesty, by different life time ($\tau$). Nonuniformity does not appear continuous, but bears a discrete character: upon transition from the «blue» region of radiation to the «red» quite often there change only the amplitude of short- and long-lived $\tau$-components, but the $\tau$-components themselves remain constant. The mean $\tau$ upon transition from the «blue» region to the «red» noticeably grows.

The distribution of the life time of tryptophan in 90% glycerol at 330 nm has three components: $\tau_1 = 0.54$ ns, $\tau_2 = 2.3$ ns, $\tau_3 = 5.4$ ns. In the «red» region at 390 nm, detected is only one of these components — long-lived, at that there appears a negative short-lived component (with negative amplitude) $\tau_{\text{neg}} = -0.3$ ns. This signifies that in the given case there is not only fading but also ignition of radiation, i.e. simultaneously there pass two processes: decline in the quantity of monomeric radiating centers and accumulation of exciplex ones. The negative component $\tau_{\text{neg}} = 0.3$ ns in the «red» region corresponds to the time necessary for formation of an exciplex between the photoexcited tryptophan and OH groups of glycerol [1, 2]. An exciplex band of radiation is forming as the photoexcited chromophore is spending some part of energy onto vibrational perturbation of the solvate shell. A negative component of tryptophanic exciplexes is also found in proteins.

Taking into account the amplitudes, the mean life time of tryptophan in glycerol grows from 2.4 ns at 320 nm to 5.4 ns at 390 nm. In Fig. 1, shown is a decomposition of the spectrum of radiation of N-acetyltryptophanamide (NATA) into components, which correspond to three centers of radiation. In the «blue» region they give commensurate contributions, while in the «red» there is only a long component, belonging to the exciplex of tryptophan-glycerol. The «red shift» of radiation of tryptophan exciplexes as compared with tryptophan in vacuum constitutes about 3300 cm$^{-1}$. This magnitude is close to intense frequencies of vibrational transitions of OH and CH groups.

The majority of proteins have internal, hidden from water, tryptophans, because tryptophan is the most hydrophobic amino acid. The mobility of tryptophan inside the protein is sharply limited, which is equivalent to a viscous environment, the time of relaxation of which is close to the life time: $\tau_{\text{rel}} \sim \tau$. The tryptophanic radiation of proteins usually appears nonuniform, because in the time $\tau_{\text{rel}}$ there occurs relaxation of the microenvironment.

**Photoinduced conformational mobility.** Many investigators using tryptophanic fluorescence in the quality of a method of indication of conformational dynamics of the protein imply the availability of only the sole spontaneous (thermal) dynamics. This assumption is valid only when tryptophans are completely submerged in the water phase, i.e. when $\tau_{\text{rel}} \ll \tau$. In the opposite case (for proteins with hidden tryptophans), fluorescent analysis carries information also about the forced dynamics caused by photoexcitation. That is why theoretical estimates give low values of spontaneous rotational mobility of tryptophans, while experimental data on fluorescence depolarization, high ones.

In glycerol, ethanol, water and other polar solvents, photoexcitation of a chromophore leads to emergence of forced mobility of the solvate. Such mobility emerges, firstly, in consequence of a sharp increase in the dipole moment of the chromophore upon excitation and, secondly, at the expense of instant «heating» of solvated molecules of solvent by the energy released in the course of vibrational relaxation of the chromophore. A similar situation takes place in proteins and peptides; therein there emerges photoinduced mobility. The spectral nonuniformity of the tryptophanic radiation of proteins and peptides in many cases appears as a result of formation of exciplex photocounters.

In Table 1, presented is the life time and its amplitudes for three one-tryptophan proteins (with known spatial structure). The decay of fluorescence of ribonuclease T1 at pH 5.5 is strictly exponential ($\tau = 4$ ns) and does not change with respect to the radiation...