Processes of reversible proton transfer in solutions of polymethine dyes have been investigated in the work just described. The dyes investigated can serve as convenient model objects for studying the processes of proton migration, since their absorption and luminescence spectra are shifted hundreds of nanometers upon protonation. The probabilities of the radiative and radiationless processes are also altered.

The investigations of the lasing properties of the protolytic nonequilibrium forms of polymethine dyes have shown that a lasing spectrum with a large Stokes shift relative to the pumping frequency which is tunable over a broad range is of practical value. In order to increase the efficiency of lasers based on these dyes it is necessary to increase the quantum yield and rate of the proton-removal reaction in the excited state.

LITERATURE CITED


POSSIBILITY OF THE INVESTIGATION OF CONFORMATIONAL CHANGES IN PROTEIN MOLECULES WITH FLUORESCENT PROBES IN THE PICOSECOND TIME RANGE

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Fluorescence methods are widely used for studying the properties of biological macromolecules, including proteins. The modern techniques make it possible to measure excitation and emission spectra, the kinetics of fluorescence decay, and the depolarization of the emitted light. In the case of proteins with an unknown structure, fluorescence methods make it possible to obtain unique information on the conformational state of the protein macromolecule, the topography of the chromophoric groups, and the dynamic properties of the systems studied. The application of fluorescent probes, whose relaxation times lie in the picosecond time range, to the investigation of the conformational changes in protein molecules will make it possible to significantly expand the possible set of fluorescent probes.

In the present work we investigated molecules of transport proteins in human blood, viz., human serum albumin (HSA) with a molecular weight of 60,000. The fluorescent probe selected was a dye which satisfies

Fig. 1. Schematic representation of the apparatus for measuring fluorescence lifetimes.

Fig. 2. Absorption spectra (1, 3) and fluorescence spectra (2, 4) of the free dye (1, 2) and a dye-protein mixture (3, 4).

| TABLE 1. Fluorescence Lifetimes (psec) of Rose Bengal in Various Solvents |
|-----------------|-----------------|-----------------|
| Solvent        | Expt. values    | Values from [5] |
| Water          | 120±10          | 118±5           |
| Ethanol        | 780±19          | 739±3           |
| Acetone        | 2790±63         | 2370±64         |

three main requirements: first, the relaxation time of the dye must be in the picosecond time range; second, the dye must absorb radiation in a spectral range where the protein molecules do not absorb in order to protect the protein from degradation, this being especially important in the case of ultrashort pulses having a power on the order of 10^9 W, as in the case of emission with the wavelength \( \lambda = 0.53 \mu \text{m} \) (the second harmonic of the YAG:Nd laser used in the present work; and, third, the relaxation time of the dye must vary as a function of the polarity of the dye solution.

A dye which satisfies these requirements is Rose Bengal. The molecular weight of the dye is 902.5 [1].

The performance of the investigations described below was based on the following assumption. When the dye molecules are in a solution together with the protein molecules, binding of the dye molecules by molecules of HSA takes place, and the relaxation time of the dye is altered from the relaxation of the free dye as a result. When the temperature and pH of the solution are varied, the conformation of the protein molecule, i.e., the polarity of the medium surrounding a dye molecule bound by the protein, changes. Thus, by measuring the relaxation time of the dye serving as a fluorescent probe with different parameters of the solution we can obtain information on the conformational changes in the protein molecule.

The apparatus used to measure the relaxation times of the dye (Rose Bengal) molecule is shown in Fig. 1.

A yttrium-aluminum garnet laser with passive mode synchronization 1, which was described in detail in [2], emits a train of picosecond pulses, from which a single picosecond pulse is singled out with the aid of Pockel's-effect cell 2. This pulse is amplified by YAG:Nd laser 3, and is transformed into the second harmonic (\( \lambda = 0.53 \mu \text{m} \)) with the aid of lithium niobate crystal 4. Glass filter 5, which is of the SZS-21 type, cuts off the untransformed part of the radiation with the fundamental wavelength \( \lambda = 1.06 \mu \text{m} \).

The relaxation times of the dye are determined from the induced absorption by the method of excitation and probing in [3].

For the work according to this method, a probing beam is formed from the laser radiation reflected by glass plate 11, i.e., low-intensity radiation. The bulk of the laser radiation is transmitted by the glass plate and acts as the exciting beam, i.e., high-intensity radiation. The probing and exciting beams are directed with the aid of reflecting prisms and mirrors into lens 12, which focuses them onto cuvette 13, in which the solution of the substance under investigation is located. Lens 14 together with lens 12 creates a collimator. Two diaphragms (15 and 16) were used to protect the light detector from parasitic illumination. Interference filter 17 with a transmission maximum corresponding to the wavelength of the probing beam does not transmit