Lifetime heterogenity and anisotropic relaxation in excited NADH

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Nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and is an essential biological coenzyme involved in regulation of living cell metabolism that is widely used nowadays as a fluorescent biomarker for monitoring the respiratory chain activity. A high potential of NAD(P)H autouorescence as biomarkers was highlighted by Chance et al. [1]. More recently spectroscopic ways to measure a redox ratios NAD^+/NADH and NADH/FAD were developed for real-time monitoring of the metabolic state of a cell during pathophysiological changes.

The lecture presents the results of experimental and theoretical studies of anisotropic relaxation and energy transfer in excited states of NADH in solutions under excitation with femtosecond laser pulses. Time-resolved transient polarization-modulation [2,3] and fluorescence [4,5] anisotropy signals were recorded and analyzed using the methods developed by the authors.

A novel polarization-modulation transient method has been developed [2,3] for investigation of ultrafast anisotropic relaxation processes occurring in excited states of polyatomic molecules after excitation with femtosecond laser pulses. The method combines an unprecedentedly high sensitivity with a sub-picosecond temporal resolution.

The results obtained suggest that the dynamics of anisotropic relaxation in NADH in water-ethanol solutions under excitation could be described by a vibrational relaxation time τ_v of about 2–15 ps and a rotational diffusion time τ_{rot} of about 100–450 ps, both depended on ethanol concentration. The dependence of the times τ_v and τ_{rot} on solution polarity and viscosity were determined and analyzed by means of a quantum mechanical model [3].

The dynamics of polarized fluorescence in NADH [4,5] under one- and two-photon excitation by ultrashort laser pulses in water-methanol solutions has been studied experimentally and theoretically as a function of methanol concentration. The elucidation of the nature of the heterogeneity of fluorescence decay times in NADH has been suggested based on the influence of the internal electric field in the nicotinamide ring on nonradiative decay rates [4]. Relative concentrations of the folded and unfolded NADH conformations in solutions have been determined by the analysis of rotational diffusion time τ_{rot} as a function of methanol concentration based on the Stokes−Einstein equation.

The dynamics of polarized fluorescence in NADH-alcohol dehydrogenase (ADH) complexes in solution was studied using the TCSPC spectroscopy. The existence of a single and long decay time in NADH−ADH complexes as compared with two decay times in free NADH was referred to a single NADH conformation in the ADH apolar binding site. A fast anisotropic relaxation time of about 1 ns has been observed at the first time and attributed to the rotation of fluorescence transition dipole moment during the rearrangement of NADH nuclear configuration [5]. The studies have brought a new insight on several important aspects of excited states quenching and anisotropic relaxation.

Index Terms: coenzyme, fluorescence, transient monitoring, anisotropy.

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