Time-resolved polarized fluorescence of NADH and FAD in solutions: picosecond relaxation dynamics

O.S.Vasyutinskii[1]
[1]Ioffe Institute, Russian Academy of Sciences, St. Petersburg, Russia

Abstract— The lecture presents the results of experimental and theoretical studies of anisotropic relaxation and energy transfer in excited states of NADH and FAD in solutions under excitation with pico- and femtosecond laser pulses.

Keywords— femtosecond laser spectroscopy; polarized fluorescence; anisotropic relaxation; biological coenzymes

I. Introduction

Nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and flavin adenine dinucleotide (FAD) are essential biological coenzymes involved in regulation of living cell metabolism that are widely used nowadays as fluorescent biomarkers for monitoring the respiratory chain activity. A high potential of NAD(P)H and FAD 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 and FAD in solutions under excitation with pico- and femtosecond laser pulses. Time-resolved transient polarization-modulation [2,3] and fluorescence [4-6] anisotropy signals were recorded and analyzed by means of the methods developed by the authors.

II. Transient polarization-modulation and polarized fluorescence spectroscopy

A novel polarization-modulation transient method has been developed [1] 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 solution under excitation with femtosecond laser pulses could be described by a vibrational relaxation time τ_v of about 2–15 ps and a rotational diffusion time τ_r of about 100–450 ps, both depended on ethanol concentration. The dependence of the times τ_v and τ_r on solution polarity and viscosity were determined and analyzed by means of a quantum mechanical model [2].

The dynamics of polarized fluorescence in NADH [4,6] and FAD [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. An explanation of the heterogeneity in the fluorescence decay times in NADH has been suggested based on the influence of the internal molecular electric field in the nicotinamide ring on nonradiative decay rates. Relative concentrations of the folded and unfolded NADH conformations in solutions have been determined using a new method of analysis of the rotational diffusion time τr as a function of methanol concentration using the Stokes−Einstein equation [4].

The dynamics of polarized fluorescence in NADH-enzyme alcohol dehydrogenase (ADH) complexes in solution was studied using the TCSPC spectroscopy. The existence of a single decay time in the ADH−NADH complex in comparison with two decay times in free NADH was referred to a single NADH unfolded conformation in the ADH binding site. An anisotropic relaxation time of about 1 ns has been observed at the first time and was attributed to the rotation of fluorescence transition dipole moment due to the rearrangement of NADH nuclear configuration [6]. The studies have brought a new insight on several important aspects of excited states quenching and anisotropic relaxation.

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