

Ultrafast Absorption Kinetics of NADH in Folded and Unfolded Conformations

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Abstract. The non-radiative energy transfer is shown to occur on a ~3ps time scale for NADH in the folded form in H₂O. Addition of methanol thermodynamically favours the open form, for which energy transfer does not occur.

1 Introduction

Nicotinamide adenine dinucleotide (NAD⁺) and its phosphorylated and reduced forms, NADP⁺, NADH and NADPH, have central roles in cellular metabolism and energy production as hydride-acceptor and hydride-donor coenzymes. Intracellular NADH exists in two forms with different fluorescence spectra and lifetimes: one is free and the other is bound to various proteins. The ratio of the two forms depends on the microenvironment and can indicate the metabolic state of a cell, providing a route for cancer diagnostic purpose (or neurodegenerative diseases, diabetes, and aging.) [1]. Time- and wavelength-resolved fluorescence spectroscopy combined with imaging (e.g. FLIM, FRET) has been widely used to extract free and protein bound NADH signals from cellular fluorescence.

NADH contains two chromophores: adenine (A) and dihydronicotinamide (NA) with absorption maxima at 260 and 340 nm, respectively [2]. Solvents, temperature, etc. have a strong influence on the conformational structure of NADH [3,4]. Efficient fluorescence resonance energy transfer (FRET) between the two chromophores after excitation occurs only in the folded conformational state [2]. The presence of methanol (MeOH) results in an unfolding of the NADH molecule and a decreased FRET between the chromophores.

In contrast to the detailed studies on NADH and its derivatives by picosecond fluorescence spectroscopy, only very little is known about the time scale of FRET in NADH and how it is modulated by the solvent. Here we characterize the transient absorption of NADH in H₂O/MeOH mixtures, as a function of MeOH content, and following the excitation of the adenine chromophore with 120 fs time resolution.

2 Materials and methods

A 3.0×10⁻⁴ M NADH solution was prepared freshly in 0.1 M PIPES buffer and was kept at 24°C during the measurements. This buffer was proven optimal in avoiding thermal NADH degradation [4]. The change in the ground state absorption spectra was found negligible in the course of the absorption kinetic measurements.

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The fraction of NADH in the folded and unfolded conformations was characterized by excitation energy transfer measurements. Fluorescence excitation spectra were taken between 240 and 440 nm, and the energy transfer efficiency was determined by the ratio of the fluorescence intensities at 260 and 340 nm. The relative probability of energy transfer is maximal at low (0-30%) MeOH concentration and minimal at high MeOH concentration [2].

Femtosecond absorption kinetics measurements were carried out by the standard pump-probe method. For the excitation of the adenine group pump pulses of 266 nm were generated by the third harmonic of an amplified Ti:Sapphire system (5 kHz, 40 fs, 500 μ J). It was found that at an average power of 650 μ W the pump did not cause any degradation of the sample (OD=0.25) circulating through a 0.5-mm square-aperture quartz cell. The 330-630-nm portion of a white light continuum was used as probe, passing through a monochromator and detected by a cooled CCD camera. The time resolution of the measurement was \sim 120 fs. Data are corrected for the solvent response, composed of pump-probe two-photon absorption and solvated electron signals. Noise reduction involved filtering using SVD followed by a sliding window averaging.

3 Results and Discussion

The time evolution of differential absorption spectra upon excitation of the A group in the absence and presence of methanol is shown in Figure 1. In both cases the signal is dominated by an increased absorption at around 350-450 nm. This excited state absorption (ESA), falls in the range of the absorption peak of the NA group, and overcomes the related ground state bleach. As shown below, this band is due to ESA from both A and NA, with the early A-related ESA spectrum being slightly larger. An additional ESA is observed for $\lambda > 500$ nm, attributed to the A group [5]. It decays very

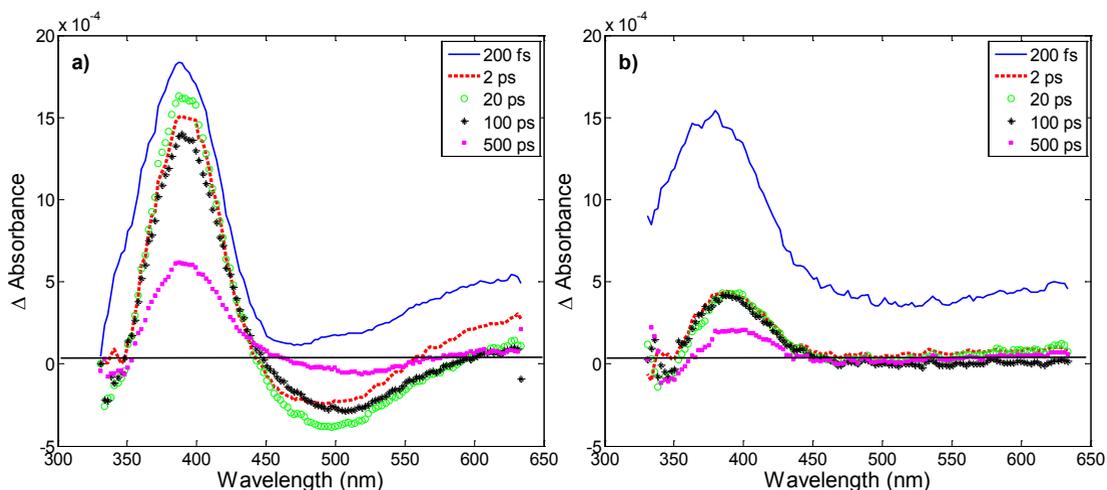


Fig. 1. The temporal changes of differential absorption spectra of NADH in 0% (a) and 80% (b) methanol.

rapidly, although somewhat slower in pure H₂O. Concomitant to this red-ESA decay, a negative band rises in the 450-570 nm region, best seen in pure H₂O. The maximum of the negative peak coincides well with the emission maximum of NADH (480 nm), hence it is interpreted as stimulated emission. At 200 fs after the excitation the shape of the spectra are fairly similar in the two solutions. In contrast to this between 2 and 100 ps the methanol-free sample shows a pronounced change, especially in its negative peak.

The kinetics at 380 and 520 nm (Figure 2a and 2b) are markedly different in the two cases, in 80% methanol it decays in a few ps, while in water a second increasing phase occurs in the 10 ps

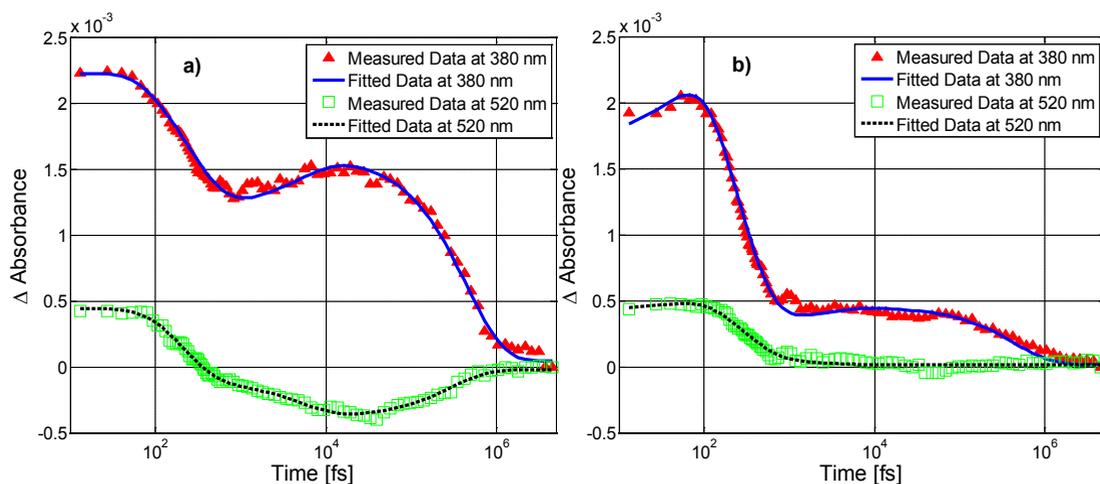


Fig. 2. Time evolution of the transient absorption of NADH in 0 % (a) and 80 % (b) methanol.

range. This difference is well manifested also in the time evolution of the spectra (Figure 1). It was found that the kinetics at every wavelength and methanol concentrations can be fitted by three exponentials. The fastest one with time constant of ~ 250 fs appears both in the presence and in the absence of methanol and can be attributed to the excited-state absorption of the A group, as we are directly exciting A. It characterizes the A-related red-ESA decay when probed at >600 nm (not shown). A second and a third component of ~ 3 ps and ~ 350 ps lifetimes take place with high weight at both 380 nm and 520 nm in the methanol-free sample. The ~ 3 ps component is also in the red-ESA decay. These observations indicate that in pure water an energy transfer takes place from A to NA with the 3 ps time constant, followed by the decay of the fluorescent excited-state of NA within 350 ps. In the presence of MeOH, the NA related long-lived ESA and SE rise with the same 3 ps time constant, but with much lower amplitudes, in line with the energy transfer being reduced. In summary, the decay of the excited state of A is biphasic, and only the slower (3 ps) component leads to the excitation of NA. This is possibly only if at least two ground state conformations, the open and closed forms of the NADH molecules, coexist in pure H_2O . Upon addition of MeOH, the open form is thermodynamically favored, leading to the reduction of FRET.

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References

1. J. McGinty, N.P. Galletly, C. Dunsby, I. Munro, D.S. Elson, J. Requejo-Isidro, P. Cohen, R. Ahmad, A. Forsyth, A.V. Thillainayagam, M.A.A. Neil, P.M.W. French, G.W. Stamp, *Biomed.Opt.Exp.* **1**, 627 (2010)
2. R.V. Hull, P.S. Conger, R.J. Hoobler, *Biophys. Chem.* **90**, 9 (2001)
3. A.S. Ladokhin, L. Brand, *J. Fluorescence*, **5**, 99 (1995)
4. L.R. Julio jr., C.B. Fernandes, G. de Oliveira Neto, L.T. Kubota, E. Katekawa, S. H. P. Serrano, *Anal. Biochem.* **260**, 50 (1998)
5. B. Cohen, P.M. Hare, B. Kohler, *J. Am. Chem. Soc.* **125**, 13594-13601 (2003).