ULTRAFAST TIME RESOLVED SPECTROSCOPY FOR ANALYSIS OF FLAVINS FMN AND FAD, COFACTORS OF CYTOCHROME P450 OXIDOREDUCTASE

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ABSTRACT

In the current research, we study the dynamics of two flavins: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are cofactors of the enzyme P450 oxidoreductase (POR). The POR enzyme contributes to a wide variety of biological systems, thus it is important to understand the mechanism of this enzyme. The study of these flavins and in particular the photophysical and photochemical processes in which these cofactors participate leads to acquiring useful information about the biological mechanism of the POR enzyme.

Ultrafast time-resolved laser spectroscopy is used to conduct the current study. The main characteristic of this technique is the use of an ultrafast pulse to excite the flavin's molecules (30×10^{-15} s). The limited duration of the system's perturbation allows the detection of the ultrafast processes by observing changes in the absorption spectrum, which may involve spectral characteristics (new transitions appearing under perturbation) and/or absorbance characteristics (increase or decrease of the optical density). The wavelength of the pump pulse is λ = 400 nm and excites the flavins in the excited states S_1 and S_2.

The transient absorption spectra of the FMN, which are recorded after the excitation at many different time delays, consist of four characteristic bands. The transitions that induce bands' formation are $S_0 \rightarrow S_1$ (450 nm), stimulated emission $S_1 \rightarrow S_0$ (560 nm) and excited-state absorptions $S_1 \rightarrow Sn$ (505 nm and 600 nm). Decay dynamics arise from the transient absorption spectra, which indicate the kinetics of FMN's states S_0 and S_1. The current results are in agreement with previously published data, in which a slow 5.4×10^{-9} s lifetime process is presented. Moreover, the analysis of the decay dynamics reveals an ultrafast dynamic with lifetime $1\text{-}2\times10^{-12}$ s, which results from the internal conversion of the higher excited state S_2 to S_1 [1].

Concerning FAD spectra, we observe the same transitions as FMN and the same spectral characteristics. The data analysis reveals two dynamics, one ultrafast with $5\text{-}12\times10^{-12}$ s lifetime and one slow, which cannot be calculated because the time range is limited. This dynamic attributes to the transition $S_1 \rightarrow S_0$. These results are also in agreement with previously reported data, in which the lifetime of the ultrafast dynamics is $5\text{-}10\times10^{-12}$ s. This dynamic is associated with the conformation of FAD, in which the isoalloxazine ring is in close proximity to the adenine ring. This fast quenching of the flavin's excited state is proposed to be attributed to the photoinduced intramolecular electron transfer from the isoalloxazine moiety to the adenine ring of FAD [1,2].

The aforementioned results demonstrate the capabilities of the ultrafast time-resolved laser spectroscopy in the analysis of the dynamics of biological molecules. Thus it gives the opportunity to apply the current method in further studies in order to understand the photophysical processes of flavins and several other complex molecules such as the POR enzyme.

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