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The Role of Adenine in Fast Excited-State Deactivation of FAD: a Femtosecond Mid-IR Transient Absorption Study

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We present a study of excited-state dynamics of two flavin cofactors: flavin−adenine dinucleotide (FAD) and flavin−mononucleotide (FMN). We used femtosecond mid-R transient absorption spectroscopy to study the effect of FAD conformation on its excited-state behavior. The conformation of FAD was modulated by changing the solvent polarity: in D_2O, FAD is present predominantly in the "stacked" conformation, in which flavin and adenine moieties are in close proximity to each other, whereas the increased amount of DMSO led to an increased amount of the "open" conformer. FMN served as a model system which lacks adenine. We found that the "stacked" conformer undergoes an intramolecular photoinduced electron transfer from adenine to flavin with the forward electron transfer rate of $k_f = 1.9 \cdot 10^{11}$ s^{-1} and the geminate recombination rate of $k_b = 1.1 \cdot 10^{11}$ s^{-1}. In the case of the "open" conformer, no intramolecular electron transfer was observed.

Flavin cofactors are important electron shuttles in living systems. As components of flavoproteins, flavin cofactors catalyze a wide range of one- or two-electron redox reactions.[1] Even though most of the flavoproteins are not light-driven, several flavoprotein photoreceptors have been recently discovered, and the mechanism of their switching behavior, investigated.[2]

To better understand the mechanism of light-driven catalysis by flavoproteins, we[3,4] and others[5,6] have studied excited-state behavior of flavin cofactors in aqueous solution. One of the findings of this research is that flavin−adenine dinucleotide (FAD) in aqueous solution exhibits significantly shorter excited-state lifetime than its analog, flavin−mononucleotide.[4,7] This finding was explained by the presence of a "stacked" FAD conformer, in which isoalloxazine and adenine moieties form a π-complex.[8] The addition of less polar solvents, such as formamide,[6] was shown to break this π-stacked complex and produce predominantly the "open" conformer with a long excited-state lifetime. The reason for fast deactivation of FAD excited state was proposed to be an intramolecular electron transfer from adenine to isoalloxazine. On the basis of the oxidation potential of adenine (E_{ox} = 1.5 V)[9] and a ground-state reduction potential of flavin (E_{red}=−0.24 V),[10] the electron transfer is expected to be thermodynamically favored (ΔG = −1 eV). Even though some experimental evidence suggests the electron transfer mechanism,[5] the unambiguous spectroscopic signature of the radical ions produced in this process has been lacking.

In this paper, we present the first results that demonstrate that the mechanism of fast excited-state deactivation in FAD is, indeed, an intramolecular photoinduced electron transfer from the adenine to the isoalloxazine moiety. We studied the excited-state dynamics of FAD and flavin−mononucleotide (FMN) using femtosecond, time-resolved, mid-IR transient absorption spectroscopy (TRIR). FMN was used as a model system that lacks the adenine moiety and is unable to undergo intramolecular electron transfer. We studied the excited-state dynamics in deuterated water with varying amounts of DMSO as a cosolvent. The details of the sample preparation and the instrumentation are presented in the Supporting Information.

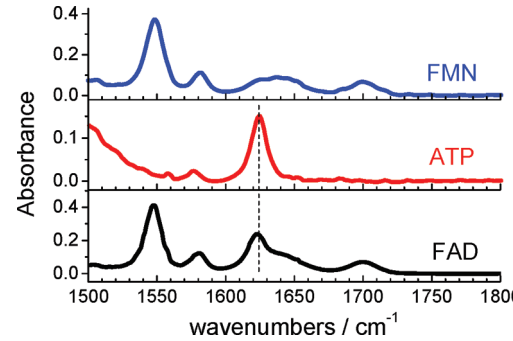

Figure 1. The FTIR absorption spectra of FMN, ATP, and FAD in deuterated water at pH = 7.

Figure 1 presents the ground-state infrared absorption spectra for FMN, FAD, and adenosine triphosphate (ATP). The spectrum of FMN is characterized by four absorption bands: (i) a band at 1700 cm^{-1} due to C_4═O_4 stretching vibration, (ii) a band at 1637 cm^{-1} due to C_2═O_2 stretching, (iii) a band at 1581 cm^{-1} due to in-phase C_{4a}═N_5 and C_{10a}═N_1 stretching, and (iv) a band at 1547 cm^{-1} that is a combination of C_{4a}═N_5 and C_{10a}═N_1 stretching with C═C vibrations of the phenyl ring.[11] The absorption spectrum of ATP in the same spectral range contains two absorption bands at 1623 and 1577 cm^{-1} that arise from C═C and C═N modes of the adenine ring.[12] The 1623 cm^{-1} band will serve as a vibrational marker to study the role of the adenine moiety in the excited-state dynamics of FAD. The excited-state behavior of FMN and FAD was studied in two solvents: deuterated water (D_2O) and dimethyl sulfoxide (DMSO). Although FAD adopts a predominantly "stacked"

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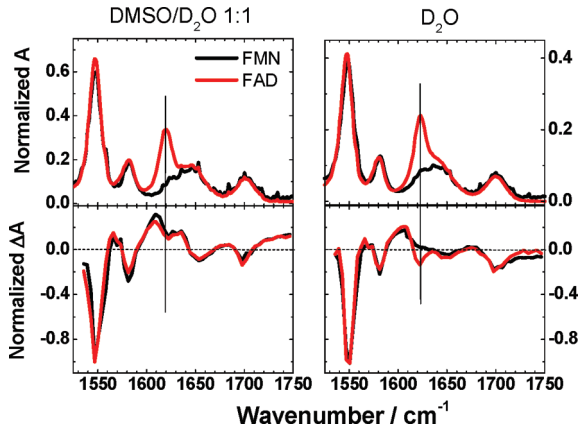


Figure 2. Ground-state FTIR (upper panels) and transient mid-IR (lower panels) absorption spectra of FMN (black line) and FAD (red line). Left panels, DMSO/D_2O 1:1 as a solvent; right panels, D_2O as a solvent. TRIR spectra were collected 2 ps after a 400 nm excitation pulse.

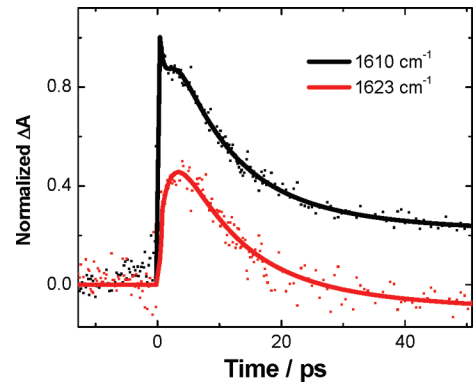


Figure 3. Decay dynamics of FAD in D_2O collected at 1610 cm^{-1} (black dots) and 1623 cm^{-1} (red dots). The solid lines represent fits obtained using sum of exponentials (see text for more details).

conformation in D_2O, the increasing amount of DMSO reduces the dielectric constant of the solvent and gives rise to a higher concentration of the "open" conformer. This effect was observed in the decay dynamics of FMN and FAD at 1548 cm^{-1} using several DMSO/D_2O solvent mixtures (Supporting Information).

To understand the role of adenine in the fast excited-state deactivation of the "stacked" FAD conformer, we obtained TRIR spectra of FMN and FAD in two solvent mixtures (Figure 2). The TRIR spectra of FMN in both solvents are very similar. The spectra consist of four bleach signals (1547, 1581, 1637, and 1700 cm^{-1}) arising from the ground-state C═O and C═N vibrations of the isoalloxazine moiety. The positive peaks in the DMSO/D_2O mixture arise from the FMN excited state and can be assigned as follows:[11] (i) a 1570 cm^{-1} band arising from stretching of C═C bonds of the phenyl group, (ii) a 1607 cm^{-1} band arising from C_2═O_2 stretching, (iii) a 1631 cm^{-1} band arising from C_4═O_4 stretching, and (iv) a 1730 cm^{-1} band arising from C_2═O_2 stretching. The TRIR spectrum of FAD in the DMSO/D_2O mixture is almost identical to that of FMN, with all vibrations arising from the isoalloxazine moiety. Even though the ground-state FTIR spectrum of FAD exhibits an absorption band at 1623 cm^{-1} due to vibrational modes of adenine group, the bleach at this wavenumber is absent in the TRIR spectrum of FAD. This result suggests that the adenine moiety is not involved in the excited-state dynamics of the FAD "open" conformer. The situation is different in D_2O, where FAD adopts a "stacked" conformer. In this case, the TRIR spectrum of FAD exhibits a pronounced bleach at 1623 cm^{-1} arising from the adenine C═C and C═N vibrations. Further confirmation that the signal comes from the adenine moiety is the fact that the bleach at 1623 cm^{-1} is not present in the TRIR spectrum of FMN in D_2O.

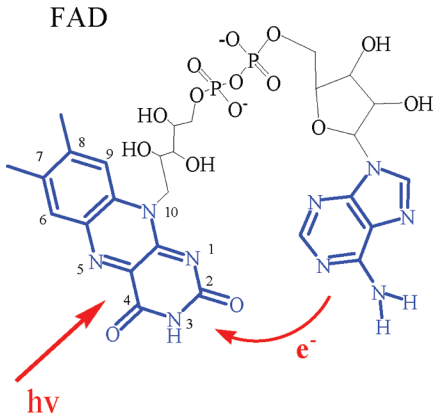
The appearance of the adenine bleach signal in the aqueous FAD solution suggests that adenine does play a role in the fast excited-state dynamics of FAD. However, three possible mechanisms can be used to interpret the presence of adenine bleach: (i) the formation of the ground-state complex between flavin and adenine, (ii) the formation of the excited-state charge transfer complex (exciplex), and (iii) the photoinduced intramolecular electron transfer process. If the ground-state complex is produced in FAD, the UV/vis and FTIR spectra of FMN and FAD would be significantly different. However, this is not the case. For example, the FTIR spectrum of FAD presented in Figure 1 is basically the sum of FMN and ATP spectra. Mechanisms ii and iii are fairly similar: both of them imply a transfer of electron from adenine to flavin. The difference between the two mechanisms is the level of electronic coupling between donor and acceptor. The strong electronic coupling related to the exciplex formation is expected to produce large spectral changes in the TRIR spectra. Since we did not observe such drastic changes, we believe that the fast excited-state deactivation in FAD is due to the intramolecular electron transfer.

As the forward electron transfer takes place, some of the ground-state adenine molecules convert into adenine radical cations, creating a bleach signal at 1623 cm^{-1} which further decays due to the geminate recombination. Thus, the dynamics at 1623 cm^{-1} give us information on the rates of forward and back electron transfer (red curve in Figure 3). The rise time is best fit by a 1.1 ps lifetime, whereas the decay corresponds to a 9 ps component. We assign these two components to the forward ($k_f = 1.9 \cdot 10^{11}$ s^{-1}) and back ($k_b = 1.1 \cdot 10^{11}$ s^{-1}) electron transfer rates of the stacked FAD conformer. The large values of these rates suggest a contact distance and a strong electronic coupling between the two aromatic moieties of the "stacked" conformer. The geminate recombination rate is slower than k_f, consistent with the geminate recombination's being in the Marcus inverted region. It is interesting to note that the 1.1 ps lifetime we obtained for the radical formation process is faster than the excited-state decay of 5−10 ps obtained from time-resolved stimulated emission measurement[4,6] and fluorescence up-conversion technique.[13] We explain this discrepancy by the higher sample concentrations required for TRIR measurements, which produce a tighter "stacked" FAD conformer due to stronger intramolecular interactions. Due to the low sensitivity of the TRIR experiment, we were unable to detect the signal at a concentration below 2 mM. Thus, we could not perform the measurements at low FAD concentrations.

The dynamics of electron transfer process can also be observed at 1610 cm^{-1} (the black line in Figure 3). At initial time, we observe a 1.1 ps decay that corresponds to a decay of the flavin S_1 state of the "stacked" FAD conformer. This decay overlaps with a 1.1 ps growth that we assign to the absorption of the C_2═O_2 vibrational mode of the flavin radical produced during the electron transfer. The radical signal decays with a lifetime of 9 ps, as in the case of adenine bleach. At a longer time scale, both 1623 and 1610 cm^{-1} signals exhibit two more decay lifetimes (96 ps and 5 ns) that are assigned to the decay of "open" FAD conformers (Supporting Information).

In summary, we used TRIR spectroscopy to study excited-state dynamics of FAD. We find that the fast excited-state

SCHEME 1: Schematic Representation of a Photoinduced Electron Transfer in Flavin-Adenine Dinucleotide



deactivation of FAD occurs due to the photoinduced intramolecular electron transfer from adenine to isoalloxazine. We expect this finding to be useful for mechanistic studies of flavoproteins, such as DNA photolyase and cryptochromes.

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Supporting Information Available: Description of our TRIR setup, sample preparation details, and excited-state decays of FAD in D_2O solutions with varying concentrations of DMSO. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Ghisla, S.; Kroneck, P.; Macheroux, P.; Sund, H. *Flavins and Flavoproteins*; Rudolf Weber: Berlin, Germany, 1999.
(2) Van der Horst, M. A.; Hellingwerf, K. J. *Acc. Chem. Res.* **2004**, *37*, 13.
(3) Li, G.; Sichula, V.; Glusac, K. D. *J. Phys. Chem. B* **2008**, *112*, 10758.
(4) Li, G.; Glusac, K. D. *J. Phys. Chem. A* **2008**, *112*, 4573.
(5) Kao, Y. T.; Saxena, C.; He, T. F.; Guo, L. J.; Wang, L. J.; Sancar, A.; Zhong, D. P. *J. Am. Chem. Soc.* **2008**, *130*, 13132.
(6) Stanley, R. J.; MacFarlane, A. W. I. V. *J. Phys. Chem. A* **2000**, *104*, 6899.
(7) Weber, G.; Tanaka, F.; Okamoto, B. Y.; Drickamer, H. G. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71* (4), 1264.
(8) Van den Berg, P. A W.; Feenstra, K. A.; Mark, A. E.; Berendsen, H. J. C.; Visser, A. J. W. G. *J. Phys Chem. B* **2002**, *106*, 8858.
(9) Seidel, C. A. M.; Schulz, A.; Sauer, M. H. M. *J. Phys. Chem.* **1996**, *100*, 5541.
(10) Draper, R. D.; Ingraham, L. L. *Arch. Biochem. Biophys.* **1968**, *125*, 802.
(11) Wolf, M. M. N.; Schumann, C.; Gross, R.; Domratcheva, T.; Diller, R. *J. Phys. Chem. B* **2008**, *112*, 13424. Kondo, H.; Nappa, J.; Ronayne, K. L.; Stelling, A. L.; Tonge, P. J.; Meech, S. R. *J. Phys. Chem. B* **2006**, *110*, 20107.
(12) Herny, H. Mantsch, D. C. *Infrared Spectroscopy of Biomolecules*; Wiley-Liss: New York, 1996.
(13) Chosrowjan, H.; Taniguchi, S.; Mataga, N.; Tanaka, F.; Visser, A. J. W. G. *Chem. Phys. Lett.* **2003**, *378*, 354.

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