Ultrafast photochemistry of flavoenzymes: spectroscopic demonstration of protonated tyrosine radical TyrOH°$^+$

Lipsa Nag[1], Pierre Sournia[1], Ursula Liebl[1], Marten H. Vos[1]

[1]LOB, Ecole Polytechnique, CNRS, INSERM, Université Paris-Saclay, 91128 Palaiseau, France

E-mail: marten.vos@polytechnique.edu

Flavoproteins are ubiquitous and involved in many biological functions that exploit the ability of flavins to act as electron- and/or proton-transfer intermediates. Most flavoproteins are not naturally photoactive, yet some are involved in photobiological processes. Flavin chromophores are highly fluorescent in solution, but this fluorescence can be strongly quenched in a protein environment, in particular by photoreduction or photooxidation processes. Flavin photoreduction can be achieved by electron transfer from nearby tryptophan (TrpH) or tyrosine (TyrOH) amino acids. Thus, the TrpH°$^+$ and Trp° radical forms play an important role in the functional flavin photoreduction in the DNA photolyase/cryptochrome photoreceptor family. By time-resolved absorption spectroscopy, and comparison with radiolysis-derived TrpH°$^+$ and Trp° spectra, it was demonstrated that the electron and proton transfer processes are not coupled in this case.[1] The situation is different for tyrosine electron donors. It is known that tyrosine can strongly decrease the lifetime of flavin fluorescence in non-naturally photoactivatable flavoproteins[2, 3] and this property has been used as a probe for active site dynamics in flexible flavoenzymes.[2] However, the photoproduct state remains uncharacterized to date. Indeed, the putative TyrOH°$^+$ state is probably highly unstable (pK ca -2) and cannot be generated by pulsed radiolysis, unlike TyrO°.

In this context, we have studied, by ultrafast fluorescence and absorption spectroscopy, the photochemistry of variants of the bacterial RNA methyltransferase TrmFO.[4] In these mutants, the excited state FAD* decays in ~ 1 ps, and the product state is sufficiently long-lived, ~ 3 ps, to be spectroscopically characterized. The results indicate that this state is the FAD°$^-$TyrOH°$^+$ state, which decreases predominantly by charge recombination. This implies that the oxidation of TyrOH does not necessarily induce its concerted deprotonation. The TyrOH°$^+$ spectrum was deduced from our data for the first time, and is consistent with predictions based on model compounds. This result crucially allows disentanglement of product states following photoexcitation of flavoproteins in often-encountered complex situations.[2] More generally, our results are important for understanding a large range of biochemical redox chains that rely on tyrosyl intermediates, but where such intermediates cannot be kinetically resolved.

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