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Flavin- and Deazaflavin-Containing Model Compounds Mimic the Energy Transfer Step in Type-II DNA-Photolyases

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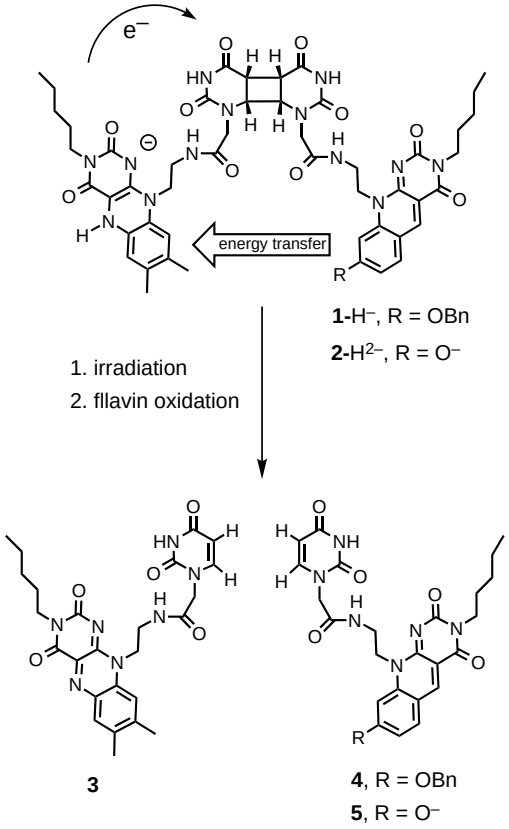
DNA-photolyases are DNA-repair enzymes, which remove pyrimidine dimer lesions (which are cyclobutane derivatives) from the genome. These lesions are formed upon irradiation of cells with UV light.[1, 2] The basis of the repair reaction is a light-driven electron transfer from the enzyme to the dimer to form the radical anion, which subsequently monomerizes.[3] For the repair reaction DNA-photolyases need a reduced and deprotonated riboflavin cofactor as the electron donor, and they require a methenyltetrahydrofolate (MTHF) or an 8-hydroxy-5-deazaflavin as the second cofactor.[4] Model investigations show that deazaflavins are able to initiate the electron-transfer-driven repair as well, but with a very low quantum yield.[4, 5] Detailed enzymatic studies indicate, however, that the deazaflavin functions within the enzyme entirely as a photoantenna.[6] It transfers excitation energy to the reduced flavin. This energy transfer was shown to accelerate the repair rate and to shift the wavelength of maximal activity from 370 nm to 430 nm.[7]

Although such studies indicate that an efficient interaction of the reduced, deprotonated flavin and the oxidized deazaflavin, and therefore their close juxtaposition, would be desirable, time-resolved fluorescence data[8] and the X-ray crystal structures[9] of the *E. coli* photolyase and the *A. nidulans* photolyase revealed a surprisingly large cofactor separation of 16.8 Å and 17.5 Å (center-to-center distance), respectively. This unexpected finding raised speculation that the energy transfer is not rate-limiting and has never been optimized during evolution.[9, 10] In the photosynthetic apparatus, however, the large distance between the final antenna pigment and the electron donor is explained by the need to suppress a competing electron transfer from the electron donor to the antenna.[11] In order to study energy transfer processes between deazaflavins and flavins, to clarify the influence of the protonation state of the deazaflavin on the energy transfer, and to investigate electron transfer possibilities between both cofactors, we prepared a series of flavin- and deazaflavin-containing model compounds like **1** and **2** (Scheme 1).[12] The analysis of these model compounds provides insight into the interactions of both cofactors depending on their redox and protonation states.



Scheme 1. The flavin- and deazaflavin-containing model compounds **1**-H$^-$ and **2**-H^{2-} and its investigated photoinduced cleavage reaction to **3** – **5**.

The synthesis of the deazaflavin- and flavin/deazaflavin-containing model compounds **1**, **2**, and **6** – **8** is depicted in Scheme 2. For the preparation of the aminoethyl-substituted deazaflavin **9**, 6-chlorouracil **10** was treated with the Boc-protected ethylene diamine **11**.[13] Subsequent treatment of the product **12** with the bis-benzyl-protected 2,4-dihydroxybenzaldehyde **13**[14] afforded the deazaflavin **14**, which was alkylated to **15** with pentyl bromide to increase the solubility of the model compounds. Cleavage of the Boc group to yield **9** and reaction with the bis-carboxymethyl-substituted cyclobutane derivative, uracil dimer **16**,[12] afforded the bis-deazaflavin model compound **7**. Reaction of **16** with one equivalent of **9** and of the aminoethyl flavin **17**[12b,c] furnished the mixed flavin/deazaflavin-containing model compound **1**. In the model compounds **1** and **7** the 8-OH group of the deazaflavin moiety is benzylated, which prevents its deprotonation and therefore maintains the deazaflavin chromophore in the protonated "OH-form". Hydrogenolytic cleavage of the

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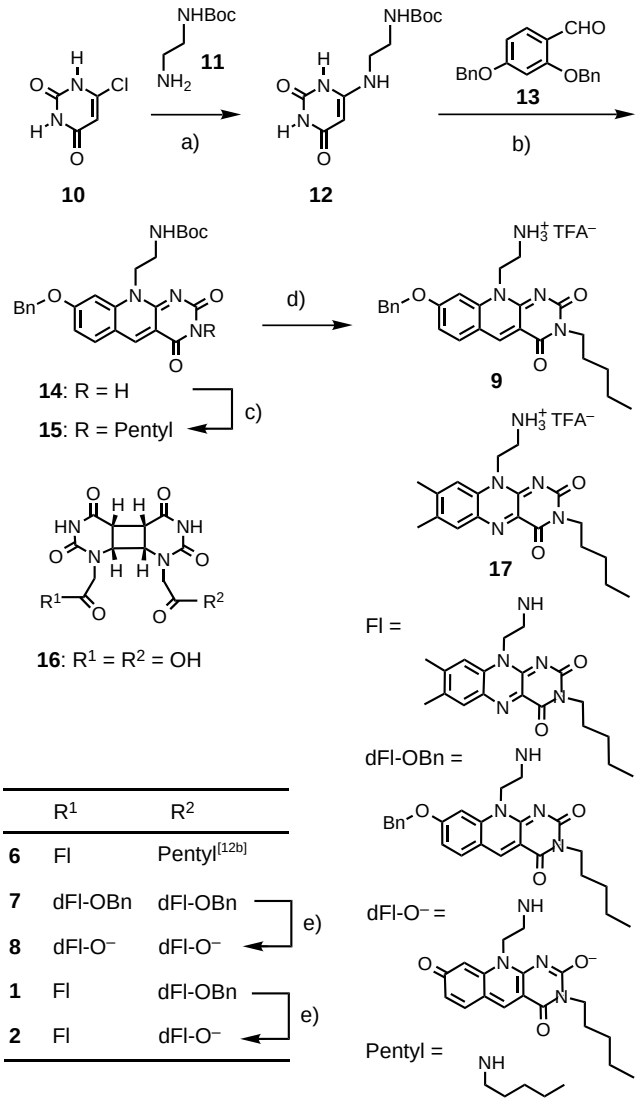

Scheme 2. Synthesis of the model compounds **1**, **2**, **6**–**8**. a) BnOH, 120°C, 4 h, 70%; b) DMF, 120°C, 12 h, 65%; c) pentyl bromide, DMF, $CsCO_3$, 1 d, 80%; d) conc. trifluoroacetic acid (TFA), room temperature, 1 h, quant.; e) AcOH, Pd/$BaSO_4$, H_2, 1 h, 90%. TFA^- = trifluoroacetate.

benzyl group in **1** and **7** with Pd/$BaSO_4$ catalyst in acetic acid and the adjustment of the pH to above 7 ($pKa_{8\text{-}OH} = 6$) yielded the model compounds **2** and **8**, which contain the deazaflavin cofactor in the deprotonated "8-(O^-) form".[4]

Initial measurements were performed to clarify whether or not deazaflavins are able to initiate the electron-transfer-based repair in the absence of a flavin chromophore. To this end, the bis-deazaflavin model compounds **7** and **8** were dissolved in ethylene glycol, acetonitrile, or DMF, and triethylamine was added to ensure basic conditions. The cleavage rate of the model compounds **7** and **8** to **4** and **5** (Scheme 1) was investigated with a recently described assay:[12] The solutions were purged with N_2 and irradiated at a given wavelength. After certain time intervals, small aliquots were removed and analyzed by reverse phase HPLC. For the determination of the quantum yields ϕ (ϕ = number of reacted molecules/number of absorbed photons), the photon flux of the monochromatic light beam was measured with ferrioxalate actinometry.[15] In the case of the model compounds **7** and **8**, however, even intense irradiation for more than 60 min yielded no photoconversion. In particular, none of the expected photoproducts **4** and **5** ($\phi < 10^{-4}$) were detected. Reduction of the deazaflavin in **7** to **7**-H_4 and subsequent irradiation ($\lambda > 350$ nm)[16] also gave no photoproduct **4**. In contrast, the reduced flavin-containing model compound **6**-H^-[12b] cleaved under comparable conditions within minutes ($\tau_{1/2} \approx 15$ min) to the expected photoproduct **3**. These experiments show that oxidized and reduced deazaflavins are unable to initiate the cleavage reaction with a substantial quantum yield.[4, 5]

In order to clarify the effect of the protonation status of the deazaflavin on the energy transfer, a variety of methods were explored to reduce the flavin-moiety to **1**-H^- or **2**-H^{2-} selectively in the presence of the protonated (benzylated) and the deprotonated deazaflavin species. In model compound **1** photoreduction finally proved to be successful. Anaerobic solutions of the model compound **1** were irradiated at $\lambda = 480$ nm, and a small amount of triethylamine was added as the $H^\bullet$ donor and to ensure deprotonation of the reduced flavin. Reduction of the flavin unit in the model compound **2** to **2**-H^{2-} was achieved with sodium dithionite/triethylamine.[17] UV spectra of the semireduced model compounds **1**-H^- and **2**-H^{2-} (Figure 1) prove that the flavin moiety was

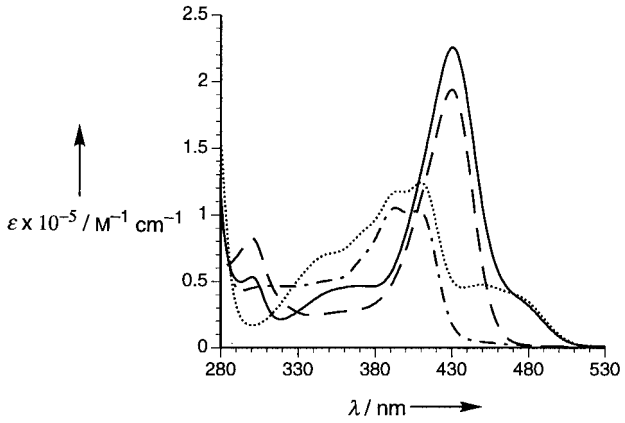

Figure 1. UV spectra of the model compounds **1** and **2** in their oxidized and semireduced forms (10^{-5} M in ethylene glycol). —— **1** (oxidized), – – – – **1**-H^- (semireduced), ···· **2** (oxidized), ·–·–·–· **2**-H^{2-} (semireduced).

successfully reduced. They show the disappearance of the oxidized flavin absorption at 340–360 nm and 450–520 nm and the intact absorption of the oxidized deazaflavin moieties at 390–410 nm (**1**-H^-) and 430 nm (**2**-H^{2-}).

The fluorescence spectra of bis-deazaflavin model compounds **7** and **8** showed strong fluorescence at 430 nm (**7**) and 470 nm (**8**) (Figure 2) corresponding to the protonated (benzylated) and the deprotonated oxidized deazaflavin, respectively.[18] Within the mixed cofactor model compounds **1** and **2**, however, the fluorescence of both deazaflavin moieties is quenched and stays significantly quenched even upon reduction of the flavins to **1**-H^- and **2**-H^{2-}. The experiments indicate the presence of an efficient energy transfer from both types of deazaflavins to the oxidized and the reduced flavin species in our model compounds. In

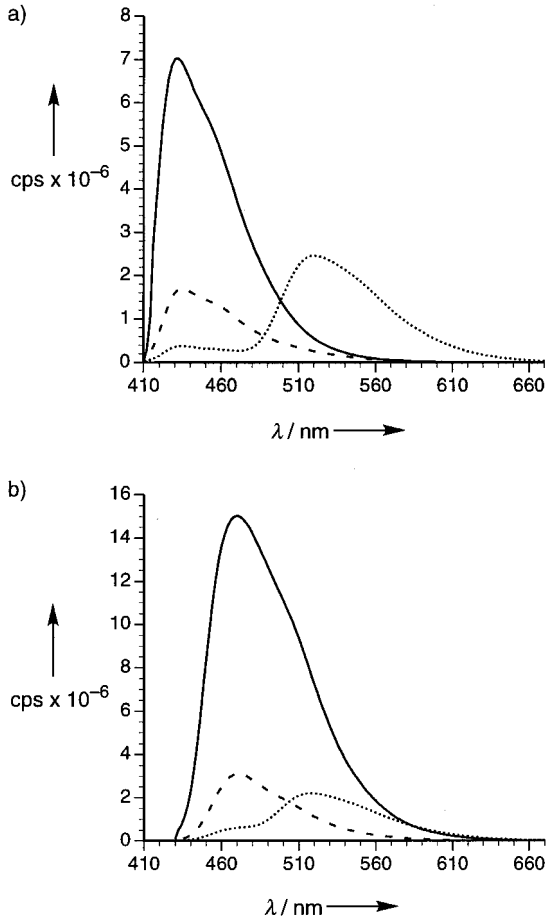

Figure 2. Comparison of the fluorescence spectra of the model compounds **1** and **2** in their oxidized and semireduced states and the bis-deazaflavin model compounds **7** and **8** (cps = counts per second). a) Protonated (benzylated) deazaflavin species (10^{-6} M in ethylene glycol, excitation wavelength 410 nm,) – **7** (5×10^{-7} M), – – – – **1**-H$^-$ (semireduced), ···· **1** (oxidized). b) Deprotonated deazaflavin species (10^{-6} M, excitation wavelength 430 nm, ethylene glycol) —— **8** (5×10^{-7} M), – – – – **2**-H^{2-} (semireduced), ···· **2** (oxidized). Flavin fluorescence is observed at 520 nm.

agreement with enzymatic studies, reduction of the flavin caused an increase of the fluorescence intensity. This can be explained by the lowered extinction coefficient of the reduced flavin, which results in a smaller spectral overlap between the deazaflavin fluorescence and the flavin absorption and consequently yields a less efficient energy transfer.[19]

Finally, action spectra were measured for both semireduced model compounds **1**-H$^-$ and **2**-H^{2-} for comparison with the reduced flavin-only model compound **6**-H$^-$ to quantify the effect of the deazaflavin–flavin interaction in both model compounds **1**-H$^-$ and **2**-H^{2-} on the photoinduced cyclobutane ring cleavage (Figure 3). The reference compound **6**-H$^-$ shows, as expected, maximal cleavage at 370 nm. In the presence of an oxidized and protonated (benzylated) deazaflavin (model compound **1**-H$^-$), this maximal cleavage wavelength is shifted from 370 nm to 390/410 nm. These are the wavelengths at which the benzylated deazaflavin features maximal absorption. Investigation of the model compound **2**-H^{2-} reveals maximal cleavage at 430 nm (a 60 nm shift!), where the deprotonated deazaflavin species possesses its absorption maximum. The latter result (430 nm) is in full

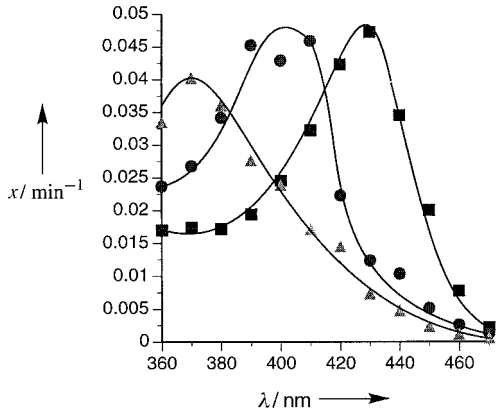

Figure 3. Action spectra of **1**, **2**, and **6** measured in ethylene glycol (5×10^{-5} M; 400 W xenon lamp, double grating monochromator, 3.4 nm bandwith, photon flux 5×10^{-7} Einstein min^{-1} ± 20%. x = relative conversion; ■ = **2**-H^{2-}, ● = **1**-H$^-$, ▲ = **6**-H$^-$.

agreement with studies at the *A. nidulans* photolyase[6] and confirms the presence of the deprotonated deazaflavin cofactor in these enzymes. These results, together with the finding that oxidized deazaflavins alone are unable to initiate the cleavage, support the view that the deazaflavin cofactor functions entirely as a photoantenna. The experiments reveal that both types of deazaflavins (protonated and deprotonated) would be able to perform the antenna function, and further measurements are now required to clarify why photolyases prefer to deprotonate the deazaflavin.

Table 1 summarizes the quantum yields for the cleavage reactions measured under our conditions. The best yield (ϕ = 0.031) was obtained for the flavin-containing compound **6**-H$^-$;

Table 1. Wavelength of maximal activity (λ_{max}) and averaged quantum yield ϕ (±25%, 5×10^{-5} M solutions) of the cleavage reaction, measured in ethylene glycol.[23]

Cmpd.	R^1	R^2	$\lambda_{max.}$ [nm]	ϕ
1-H$^-$	FlH$^-$	dFl-OBn	400	0.014
1-H$_3^-$	FlH$^-$	dFlH$_2$-OBn	370	0.030
2-H^{2-}	FlH$^-$	dFl-O$^-$	430	0.012
6-H$^-$	FlH$^-$	Pentyl	370	0.031
7	dFl-OBn	dFl-OBn	–	$< 10^{-4}$
8	dFl-O$^-$	dFl-O$^-$	–	$< 10^{-4}$

in this system absorbed photons are most efficiently used for the dimer cleavage. Despite the observation that in deazaflavin-dependent photolyases the quantum yield remains high in the presence of the deazaflavin cofactor, the quantum yields of the deazaflavin-containing models compound **1**-H$^-$ and **2**-H^{2-} are significantly decreased to ϕ = 0.012 (**2**-H^{2-}) and ϕ = 0.014 (**1**-H$^-$). Particularly interesting is the finding that the maximal cleavage rates are very similar for all three model compounds **1**-H$^-$, **2**-H^{2-} and **6**-H$^-$, which shows that the deazaflavins are unable to improve the reaction rates in our model compounds. In order to clarify how the oxidation state of the deazaflavin influences the cleavage rate, we reduced the deazaflavin unit in **1**-H$^-$ to **1**-H$_3^-$ during an irradiation experiment at 370 nm by addition of a dithionite solution. This reduction yielded an immediate improvement of the

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cleavage rate ($\phi = 0.014$ jumps to $\phi = 0.03$), which shows that the nonabsorbing, reduced deazaflavin does not influence the flavin-driven cleavage rate. We currently have no explanation why the oxidized deazaflavins cause no cleavage rate increase. One reason clearly is that in the presence of a light-absorbing deazaflavin fewer photons are directly absorbed by the reduced flavin unit. Based on our cleavage results at 400 nm and 430 nm, however, we suspect that we also have to consider an alternative intercofactor electron transfer process from the reduced, photoexcited flavin to the oxidized deazaflavin, which would lower the cleavage efficiency. Such an electron transfer would be not entirely unexpected. The redox potentials show that an electron transfer from the photoexcited reduced flavin ($E^{*}_{ox} = -2.8$ V)[20] at least to the deazaflavin unit ($E_{red} \approx -0.9$ V)[21, 22] is more favorable then to the dimer unit ($E_{red} = -2.2$ V).[20]

With the help of the first flavin- and deazaflavin-containing model compounds and with selective reduction procedures for the corresponding flavin units in these compounds, it was possible to show that deazaflavins alone are unable to initiate the cleavage of cyclobutane-linked pyrimidine dimers at wavelengths above $\lambda = 350$ nm. Both the protonated (benzylated) and the deprotonated deazaflavins are able to transfer excitation energy to an oxidized and to a reduced flavin unit. The energy transfer process causes a shift of the maximal repair wavelength from 370 nm to 400 nm and 430 nm for the protonated and deprotonated deazaflavins, respectively. The observation that the presence of the deazaflavins does not improve the cleavage rate could mean that an alternative electron transfer between the reduced flavin and the oxidized deazaflavin interferes with the cleavage reaction in our model compounds. Within the enzyme such an electron transfer would jeopardize the DNA-repair function, and maybe, DNA-photolyases prefer a large separation between cofactors to suppress this process.

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[23] The presented quantum yields are preliminary due to the rather high concentrations (5×10^{-5} M), which were required for the measurements. All quantum yield measurements were performed under identical conditions within a short period of time to ensure maximal comparability of the data. We observed a very strong solvent dependence of the cleavage reaction in deazaflavin-containing model compounds. Best data were obtained in ethylene glycol (5×10^{-5} M)).

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