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Design, Synthesis, and Evaluation of a Biomimetic Artificial Photolyase Model

Olaf Wiest,*,† Christopher B. Harrison,† Nicolas J. Saettel,† Radek Cibulka,‡ Mirjam Sax,‡ and Burkhard König*,‡

Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana 46556-5670, and Institut für Organische Chemie, Universität Regensburg, Universitätsstr. 31, D-93040 Regensburg, Germany

owiest@nd.edu; burkhard.koenig@chemie.uni-regensburg.de

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Two new artificial photolyase models that recognize pyrimidine dimers in protic and aprotic organic solvents as well as in water through a combination of charge and hydrogen-bonding interactions and use a mimic of the flavine to achieve repair through reductive photoinduced electron transfer are presented. Fluorescence and NMR titration studies show that it forms a 1:1 complex with pyrimidine dimers with binding constants of $\sim 10^3$ M^{-1} in acetonitrile or methanol, while binding constants in water at pH 7.2 are slightly lower. Excitation of the complex with visible light leads to clean and rapid cycloreversion of the pyrimidine dimer through photoinduced electron transfer catalysis. The reaction in water is significantly faster than in organic solvents. The reaction slows down at higher conversions due to product inhibition.

Introduction

A major environmental damage to DNA is the formation of photolesions, which compromise the genetic information and can lead to cell death or skin cancer. UV radiation induces a [2 + 2] photocycloaddition between two adjacent thymines on one strand of the DNA to form a *cis-syn* thymine cyclobutane dimer as the major product.[1] DNA photolyase, which is present in many organisms but not in humans, selectively recognizes the thymine dimer in DNA single and double strands and repairs it by photoinduced electron transfer using a noncovalently bound, reduced flavine as the electron donor.[2]

The study of artificial DNA photolyases and enzyme models yielded valuable insights into the mode of action of the enzyme[3] and offers the long-term prospect of artificial DNA repair, which gained additional support by the recent demonstration of xenobiotic repair of thymine dimers in humans.[4] Carell and co-workers presented detailed studies of a series of model systems where a flavine is covalently linked to a pyrimidine dimer model system. Small peptides containing electron-donating groups were also found to induce a cycloreversion of cyclobutane pyrimidine dimers.[5] Several groups have presented small-molecule recognition units that noncovalently bind to derivatives of thymine and uracil dimers in organic solvents.[6] By linking the 2,6-diacetaminopyridine recognition unit to a redox active indole, Rose and co-workers achieved repair of a uracil dimer through reductive photoinduced electron transfer.[7] Although Rose observed binding constants of ~ 200 M^{-1} in a methanol/acetonitrile mixture, protic solvents were found to interfere with the molecular recognition of the donor−acceptor−donor hydrogen-bonding motif. A new strategy is thus needed to achieve molecular recognition and repair in water.

Here, we report a functional photolyase mimic that recognizes pyrimidines in both organic solvents and water and, in analogy to the natural enzyme, uses a flavine to achieve repair through reductive photoinduced electron transfer. Figure 1 shows the function of the

† University of Notre Dame.
‡ Universität Regensburg.

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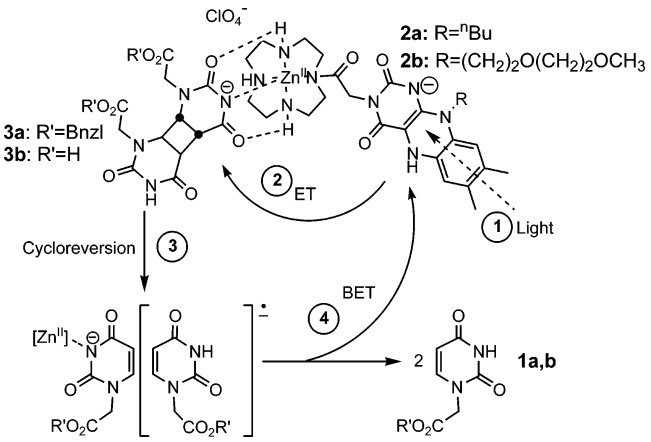


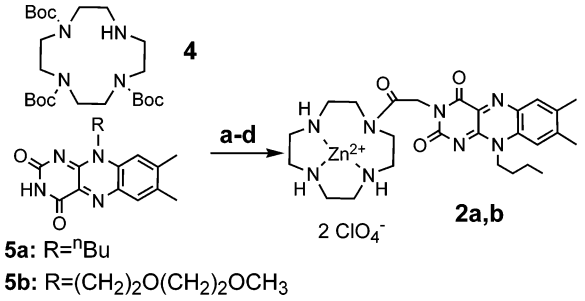
FIGURE 1. Function of artificial photolyases **2**.

reductive artificial photolyases **2**. Binding of **3a**, a model for thymine dimers,[3,8] occurs via a zinc−cyclen moiety covalently linked to a reduced flavine derivative. It was shown that pyrimidines can be selectively recognized in water as well as in the context of a DNA duplex by zinc−cyclen complexes through a combination of hydrogen bonding and complexation of the zinc dication to the deprotonated N(3) nitrogen.[9] Carell and co-workers[3] studied a series of systems where flavine derivatives were covalently linked to thymine dimer models and demonstrated that electron transfer catalyzed (ETC) cycloreversion can be achieved under physiologically relevant conditions, including in a DNA duplex. Excitation of the reduced flavine with visible light then induces electron transfer (ET) to form **3a**$^{\bullet-}$, which undergoes effectively barrierless cycloreversion.[10] Back-electron transfer (BET) to the flavine closes the catalytic cycle and yields 2 equiv of the monomer **1a**.

Results and Discussion

Compound **2** is easily accessible by coupling of tri-Boc-cyclen **4**[9] to the flavine derivative **5**,[11] synthesized by the classic Kuhn method, via a carboxymethyl linker as shown in Scheme 1. By selecting the appropriate side chain on the flavine, solubility in either organic solvents or water can be ensured. Deprotection and complexation of the zinc ion complete the synthesis. The stoichiometry and binding constant of the complexes of **2** to thymine and **3a** are determined by fluorescence quenching titration of the oxidized flavine.[12] A Job's plot analysis shows a 1:1 stoichiometry of the complex **2a**·thymine and **2a**·**3a**. A binding constant of 6×10^3 M^{-1} for **2a**·thymine in methanol is derived from fluorescence titration. The value is in agreement with the results of Kimura[9] on

SCHEME 1. Synthesis of Artificial Photolyases 2[a]



[a] Reagents and conditions: (a) $BrCH_2CO_2H$, DCC, CH_2Cl_2, 12 h, rt, 70%; (b) K_2CO_3, CH_3CN, rt, 7 d, 75%; (c) TFA/CH_2Cl_2 1:1, rt, 3 h, 94%; (d) $Zn(ClO_4)_2 \cdot 6H_2O$, CH_3OH, reflux, 1 h, 92%.

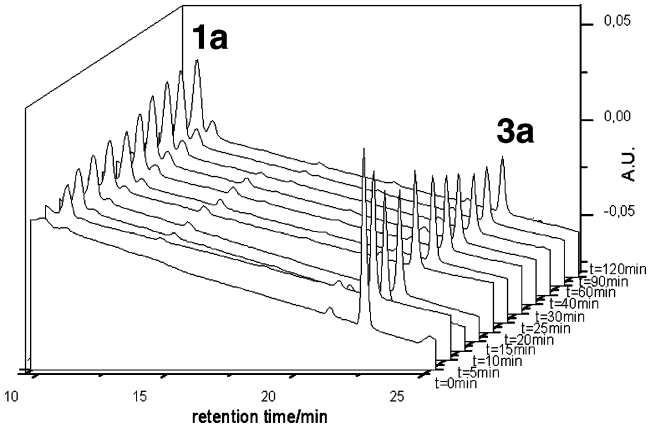


FIGURE 2. HPLC traces for ETC cycloreversion catalyzed by **2a**.

similar systems. Due to the lower solubility of **3a** in methanol, the binding constant for **2a**·**3a** is determined in acetonitrile as 2×10^3 M^{-1} by fluorescence titration. The higher binding constant to thymine can be understood when considering the binding mode of **2**, which binds to the deprotonated N(3) position.[9] Since thymine is approximately 0.6 pK_a units more acidic than **3a**, it is reasonable that the binding to **2a** will be stronger. It is in any case gratifying to note that binding in both protic and nonprotic organic solvents is strong, indicating possible applications to aqueous solutions.

After formation of the reduced and deprotonated flavin using the conditions developed by Carell,[3] the electron-transfer-catalyzed (ETC) cycloreversion of **3a** is induced in a 1:4 mixture of **2a** and **3a** in acetonitrile or methanol by irradiation with a 450 W mercury lamp equipped with a Pyrex filter. HPLC traces (Figure 2) reveal a fast and clean conversion of **3a** to **1a**. Control experiments show that no reaction takes place upon irradiation in the absence of **2a**.

The conversions for the ETC cycloreversion of **3a** catalyzed by **2a** as well as for the control reaction using **5a** in acetonitrile and methanol are summarized in Table 1. It can be noted that the reaction is, with a conversion of more than 25%, catalytic. The reaction in methanol is faster, most likely due to the stabilization of the inter-

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(12) Since the emission of the reduced flavine cannot be observed, binding studies were performed with the oxidized form of the flavine, assuming that the binding constant is independent of the redox state of the flavine.

TABLE 1. Cycloreversion of 3a to 1a Catalyzed by 2a or 5a

		conversion (%)	
solvent	time (min)	**2a**	**5a**
CH_3CN[a]	15	55	38
	30	67	60
	45	75	75
CH_3OH[b]	5	74	59
	10	80	69
	15	84	75

[a] [**3a**] = 4×10^{-4} M^{-1}, [**2a**] = 1×10^{-4} M^{-1}. [b] [**3a**] = 1.8×10^{-4} M^{-1}, [**2a**] = 4.5×10^{-5} M^{-1}.

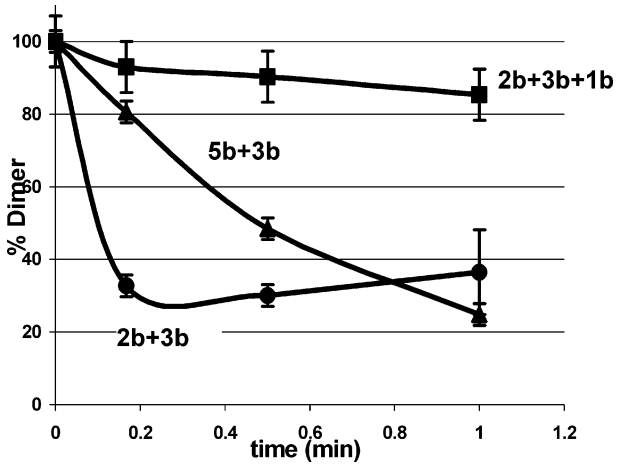

FIGURE 3. ETC repair of **3b**.

mediate radical anion by hydrogen bonding in the protic solvent, which was found previously to be important for this reaction.[10] The reaction can be run essentially to completion by irradiation for 120 min in acetonitrile and 60 min in methanol (data not shown). It is interesting to note that the repair of **3a**, complexed to **2a**, starts out faster than the bimolecular reaction with (flavine) **5a** but then slows down at longer reaction times. This is consistent with a product inhibition caused by the higher binding constant of **2a** to **1a** formed over the course of the reaction. Thus, the reaction is after short reaction times (at larger than ~15% conversion) effectively a bimolecular reaction between **2a·1a** and **3a**. The overall acceleration observed at our first datapoint is therefore only 2-fold, even though the rate constant in **2a·3a** at very low conversions is expected to be significantly higher than the bimolecular rate constant of **3a** and **5a**.

As the next step toward the biologically relevant system, we investigated the repair of the thymine dimer model **3b** in aqueous solution using the water-soluble artificial photolyase **2b**. The binding of pyrimidines by zinc-cyclen complexes in aqueous solution has been studied extensively by Kimura et al., who found apparent binding constants on the order of ~10^3 M^{-1}.[9] Our own results, obtained by NMR titrations in aqueous borate buffer at pH 7.2, are consistent with these results and the lower acidity of the pyrimidine dimers (see the Supporting Information).

Figure 3 summarizes the results for the repair of **3b** in aqueous solution. It is noteworthy that the reaction in water is much faster than in organic solvents. Even at the shortest time that is practical with our current experimental setup, irradiation of the complex of **2b·3b** leads to 78% conversion after 10 s, compared to 20% for the bimolecular background reaction catalyzed by **5b**. The very high rate compared to the reaction in methanol cannot be rationalized only by the higher polarity and hydrogen bonding ability of water, but might also be affected by a hydrophobic effect that biases the conformational equilibria toward a stacking of the flavine toward **3b**, which will facilitate the electron transfer. At this conversion, the reaction is already subject to product inhibition in analogy to the reaction of **2a** discussed above. This was confirmed by addition of one equivalent of the product **1b**, which significantly slows down the reaction as shown in Figure 3. This is most likely due to the slower diffusion of the complex **2b·1b**, which makes it less efficient in the bimolecular repair reaction. Nevertheless, the 4-fold acceleration at 10 s indicates that the initial rate for repair in the complex is much higher than for the bimolecular background reaction.

The product inhibition observed for these simple model systems prevents the determination of the quantum yield in our current experimental setup. However, the very short reaction times in water qualitatively indicate that the reaction is as efficient as the reaction of the covalently linked model systems,[3] but shows catalytic turnover. Product inhibition will not be a problem in real DNA since, similar to the case of DNA photolyase,[13] the product is removed from the equilibrium by reconstitution of the Watson−Crick pairing. Since the application of our artificial photolyase to a DNA duplex is a long-term goal of the project, the problem of product inhibition was not further pursued at this point.

In summary, we present for the first time a small-molecule photolyase model that binds to the model dimers **3** with millimolar affinity in both protic and nonprotic solvents by reversible coordination. Upon irradiation with visible light, **2** cleanly and efficiently cycloreverts the cyclobutane pyrimidine dimers **3** to the monomers **1**. The reaction proceeds, analogous to the natural DNA photolyase, through photoinduced reductive electron transfer using a reduced flavine as the redox active cofactor. Based on the work by Carell[3] and Kimura,[9] it can be anticipated that the water soluble derivative **2b** could be used to repair thymine dimers in a DNA duplex under physiological conditions. These studies are currently underway and will be reported in due course.

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Supporting Information Available: Experimental procedures for the synthesis and spectral characterization of new compounds, details of the determination of the binding constants, and a procedure for the repair assay used. This material is available free of charge via the Internet at http://pubs.acs.org.

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