(6-4)-Photolyase activity requires a charge shift reaction†‡

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A model compound containing a thymine oxetane moiety linked to a flavin chromophore was investigated regarding (6-4)-photolyase activity. The need for a charge shift reaction was demonstrated by a detailed pH-dependent kinetic analysis.

Photodamage of DNA due to exposure to UV light is one of the major environmental mutagens, which are associated with many diseases like cancer, xeroderma pigmentosum, trichothiodystrophy, and the cockayne syndrome.[1] The two most common forms of photodamage are the *cis, syn* cyclobutane pyrimidine dimer (CPD) and the pyrimidine pyrimidone (6-4) photoproduct. The (6-4) photodamage can be repaired by (6-4)-photolyase enzymes, the mechanism of which has attracted much attention in recent years.[2] Due to the high sequence homology[3] with CPD-photolyases a similar electron transfer repair mechanism involving an oxetane intermediate was suggested by Kim.[4] Falvey exemplified this mechanism by a photolysis study on a thymine oxetane model compound and photosensitisers under reductive conditions.[5] Carell observed the repair of the same thymine oxetane model by covalently linked flavin exclusively under reductive conditions.[6] Although the reductive electron transfer is more plausible[7] for the (6-4)-photolyase, the oxidatively photosensitised cycloreversion (CR) of oxetanes is of high interest by means of artificial DNA repair and electron transfer catalysed pericyclic reactions in general.[8] In this regard, Barton[9] proved the oxidative repair of CPD photodamage and Miranda[10] studied the oxidative CR of oxetanes.

In this study, we compare the strictly light-driven reductive CR of the thymine oxetane described by Falvey[5] with the respective oxidative process using several sensitisers and varying pH conditions. Therefore, model compound **1** containing the thymine oxetane covalently linked to a flavin moiety was synthesised and purified by HPLC.[11] As shown in Fig. 1, the oxetane photocleavage reaction was performed under reductive and oxidative conditions in a fluorescence spectrometer by irradiation at 450 nm§ in methanol and varying buffers¶ in a 1:1 ratio. Reductive conditions were realised by addition of 100 equivalents 0.2 M sodium dithionite solution and controlled by fluorescence depletion at 525 nm. During the irradiation, solutions (1 ml) were bubbled with oxygen-free nitrogen. Oxidative conditions were realised by bubbling with oxygen. The kinetic analysis was performed by HPLC analysis at 450 nm with six to eight samples (25 µl) taken over a period of 75% conversion (Fig. 2). The peak area of starting material **1** was compared with the product signal of thymine derivative **2**. The constitutional integrity of the photoproduct that has been obtained was proven by ESI-MS and co-injection of independently prepared compound **2**. Quantum yields were obtained from the conversion rates after calibration of the fluorescence spectrometer with potassium ferrioxalate actinometry.[12]

For the reductive conversions, all reactions showed first-order kinetics.‖ As shown in Fig. 3 and Table 1, the reductive CR was 4–7% efficient at pH values 5–11. The progression of the pH dependence of the reductive CR showed a maximum at pH 7 with a strong decline to the acidic region and a slight decline to the basic region. This is probably due to a change of the charge injection from charge shift to charge separation. Around pH 7 the reduced flavin is deprotonated (pK = 6.2) and a charge shift reaction takes place.[13] By changing to acidic conditions the flavin is protonated and a charge separation reaction is enforced with a loss in efficiency. With changing to basic conditions the thymine oxetane (pK ~ 10) starts to become deprotonated, inhibiting the negative charge donation from the flavin.[14]

† Dedicated to Professor Albert Eschenmoser on the occasion of his 80th birthday.
‡ Electronic supplementary information (ESI) experimental section, actinometry. See http://www.rsc.org/suppdata/cc/b5/b503699b/
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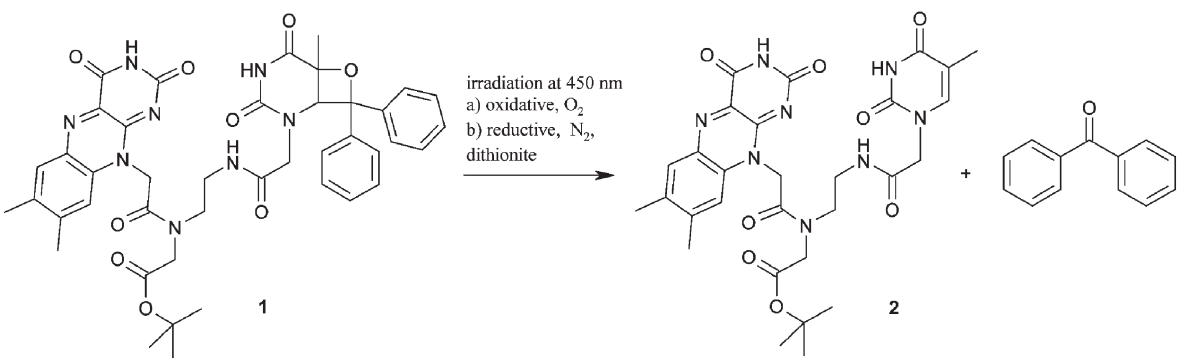

Fig. 1 Model compound **1** for simulation of (6-4)-photolyase activity by cycloreversion of the oxetane moiety forming **2**.

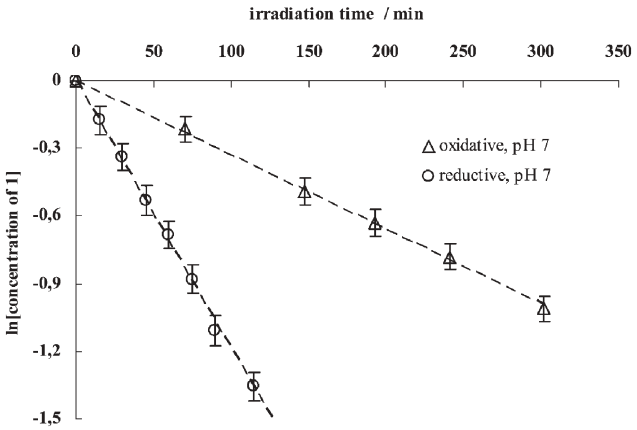

Fig. 2 Kinetic analysis of the photoconversion of model compound **1**.

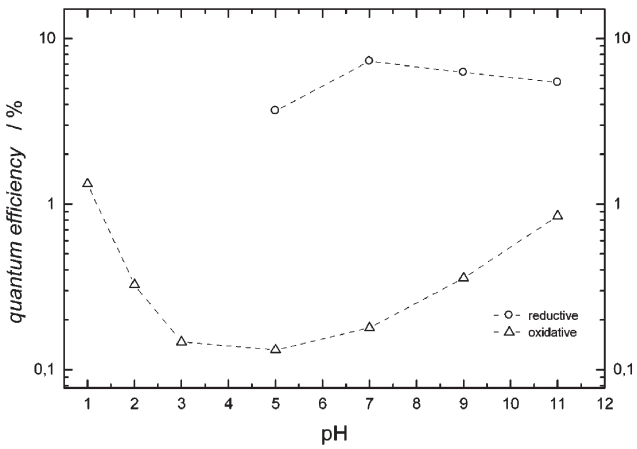

Fig. 3 Quantum yields of the photoconversion of model compound **1** depending on pH.

The oxidative CR showed a clean conversion to compound **2** obeying first-order kinetics with minimum efficiency at pH 5. Interestingly, the oxidative CR was accelerated by one order of magnitude on changing to acidic medium and by a factor of 6 by changing to basic conditions (Fig. 3 and Table 1). This is again consistent with a change in the charge injection mechanism. The oxidised flavin is hardly basic (pK ~ 0), but the excited state is much more basic (pK* ~ 2).[13] Therefore, the excited state of the flavin could be protonated and react with the oxetane in a charge shift reaction, which is in accordance to oxidative CR of oxetanes observed by Miranda with positively charged pyrylium salts.[8,10] By changing from neutral to basic conditions, the deprotonation of the thymine oxetane would change the mechanism again towards charge shift.

Table 1 Kinetic data for light-driven conversion of **1**

pH	Φ_{ox}/%	Φ_{red}/%[a]	τ_{ox}/min	τ_{red}/min[a]
1	1.32	—	27 ± 1.5	—
2	0.33	—	112 ± 6	—
3	0.15	—	248 ± 14	—
5	0.13	3.7	277 ± 15	115 ± 6
7	0.18	7.3	207 ± 11	58 ± 3
9	0.36	6.2	102 ± 6	68 ± 4
11	0.85	5.4	43 ± 2	79 ± 4

[a] No measurement for pH < 5 due to instability of dithionite.

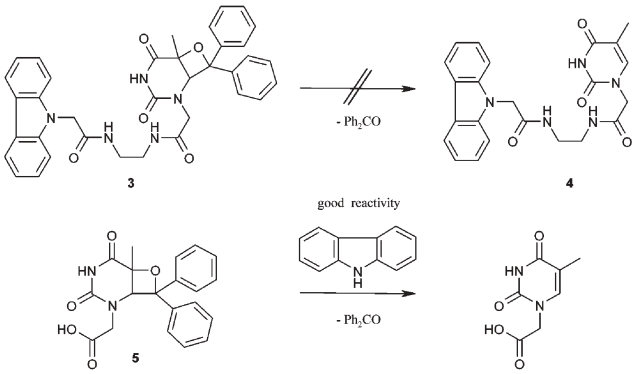

Fig. 4 Photolyase activity of carbazole containing model compound **3**. The reaction conditions were the same as for model compound **1** (buffer 10 mM phosphate (pH 7)/methanol 1:1, reductive conversion).

Compared to the reductive conversion, the oxidative CR showed the opposite progression with maximum efficiency strictly at extreme pH values. This was reasoned by a maximum efficiency only *via* charge shift reaction. In this context, Falvey observed the reductive CR of thymine oxetanes with photosensitisers *via* charge separation, but only in diffusion controlled contact pairs.[5] We synthesised model compound **3**, which contained carbazole as reductive sensitiser covalently linked to the thymine oxetane (Fig. 4). This model compound showed no photolyase activity. Obviously, CR cannot compete against charge back transfer. This is in agreement with our observation that in a diffusion controlled reaction, photolyase activity occurs when the sensitiser and the thymine oxetane **5** are not covalently linked.

In conclusion, the CR of oxetanes in covalently linked systems requires a charge shift reaction, since a charge separation reaction is impeded by a fast charge back transfer. This was shown with covalently linked flavin/thymine oxetanes and carbazole/thymine oxetanes as model systems for (6-4)-photolyase activity.

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Notes and references

§ Use of sodium dithionite as reducing agent in combination with irradiation at 365 nm should be avoided due to the strong absorbance at 350 nm (lg(ε) > 3) which leads to substantial errors in the kinetic analysis.[15]
¶ The following 10 mM buffer solutions containing 0.10 M NaCl were used: citric acid (pH 2–5), sodium hydrogenphosphate (pH 7), borax (pH 9), and glycine (pH 11); pH 1 was realised with 1.0% $HClO_4$ in water/methanol 1:1.[16]
‖ A strictly light-driven side reaction was observed and a side product with two mass units higher than starting material **1** was formed in an overall yield of 25%.

1 D. L. Mitchell, in *CRC Handbook of Organic Photochemistry and Photobiology*, editor W. M. Horspool, CRC press, 2004, 2nd edition, chapter 140, 1–7; O. D. Schärer, *Angew. Chem.*, 2003, **115**, 3052–3082; *Angew. Chem. Int. Ed.*, 2003, **41**, 2946–2974.

2 A. Sancar, *Science*, 1996, **272**, 48–49; Y. Wang, P. P. Gaspar and J.-S. Taylor, *J. Am. Chem. Soc.*, 2000, **122**, 5510–19; X. Zhao, J. Liu, D. S. Hsu, S. Zhao, J. Taylor and A. Sancar, *J. Biol. Chem.*, 1997, **272**, 32580–32590; K. Hitomi, H. Nakamura, S. Kim, T. Mizukoshi, T. Ishikawa, S. Iwai and T. Todo, *J. Biol. Chem.*, 2001, **276**, 10103–10109; T. Mizukoshi, K. Hitomi, T. Todo and S. Iwai, *J. Am. Chem. Soc.*, 1998, **120**, 10634–42; M. G. Friedel, M. K. Cichon and T. Carell, in *CRC Handbook of Organic Photochemistry and Photobiology*, editor W. M. Horspool, CRC press, 2004, 2nd edition, chapter 141, 1–15.

3 T. Todo, H. Takemori, H. Ryo, M. Ihara, T. Matsunaga, O. Nikaido, K. Sato and T. Nomura, *Nature*, 1993, **361**, 372–374; T. Todo, H. Ryo, K. Yamamoto, H. Toh, T. Unui, H. Ayaki, T. Nomura and M. Ikenaga, *Science*, 1996, **272**, 109–112.

4 S. Kim, K. Malhotra, C. A. Smith, J. Taylor and A. Sancar, *J. Biol. Chem.*, 1994, **269**, 8535–8540.

5 A. Joseph, G. Prakash and D. E. Falvey, *J. Am. Chem. Soc.*, 2000, **112**, 11219–11225; A. Joseph and D. E. Falvey, *Photochem. Photobiol. Sci.*, 2002, **1**, 632–635; additionally, there is a high relevance to apply the thymine oxetane as charge trap for monitoring electron transfer in DNA: P. Kaden, E. Mayer-Enthart, A. Trifonov, T. Fiebig and H.-A. Wagenknecht, *Angew. Chem. Int. Ed.*, 2005, **44**, 1636–1639.

6 M. K. Cichon, S. Arnold and T. Carell, *Angew. Chem.*, 2002, **114**, 793–796; *Angew. Chem. Int. Ed.*, 2002, **41**, 1763–1764.

7 P. F. Heelis, R. E. Hartman and S. D. Rose, *J. Photochem. Photobiol. A*, 1996, **95**, 89–98.

8 M. A. Miranda and M. A. Izquierdo, *J. Am. Chem.Soc.*, 2002, **124**, 6532–33.

9 P. J. Dandliker, R. E. Holmlin and J. K. Barton, *Science*, 1997, **275**, 1465–68.

10 M. A. Miranda, M. A. Izquierdo and F. Galindo, *J. Org. Chem.*, 2002, **67**, 4138–4142; M. A. Miranda and M. A. Izquierdo, *Chem. Commun.*, 2003, 364–365.

11 I. v. Wilucki, H. Matthäus and C. H. Krauch, *Photochem. Photobiol.*, 1967, **6**, 497–500; R. Kuhn and F. Weygand, *Ber. Dtsch. Chem. Ges.*, 1935, **68**, 1282–1288.

12 C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London. Ser. A.*, 1956, **235**, 518–536; W. D. Bowman and J. N. Demas, *J. Phys. Chem.*, 1976, **80**, 2434–2435.

13 W. R. Weimar and A. H. Neims, in *Riboflavin*, Plenum Press, editor R. S. Rivlin, New York, 1975, 1–47.

14 R. Epple, E.-U. Wallenborn and T. Carell, *J. Am. Chem. Soc.*, 1997, **119**, 7440–7451.

15 R. J. Meyer, E. H. E. Pietsch and A. Kotowski, *Gmelin Handbuch der Anorganischen Chemie, Schwefel Teil B Lieferung 2*, VCH, Weinheim, 1960, 8th edition, 9S[B]387–396; L. Lorenz and R. Samuel, *Z. Phys. Chem.*, 1931, **B14**, 219–31.

16 P. F. Heelis, R. F. Rosemarie and S. D. Rose, *Photochem. Photobiol.*, 1993, **57**, 442–446.

