Mechanistic Studies on DNA Photolyase. 5. Secondary Deuterium Isotope Effects on the Cleavage of the Uracil Photodimer Radical Cation and Anion

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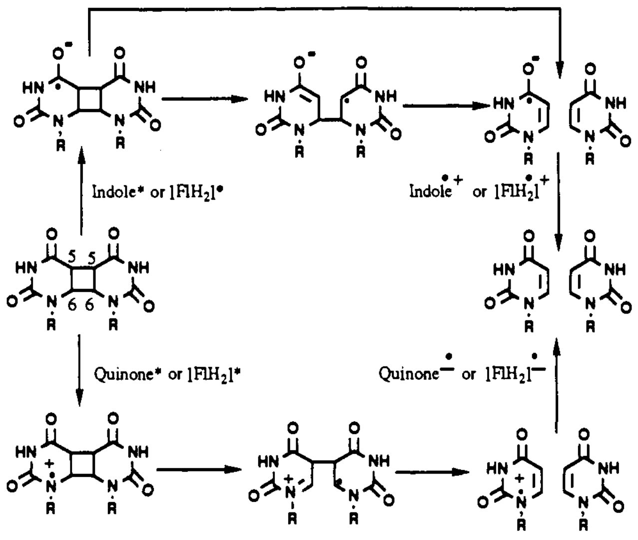
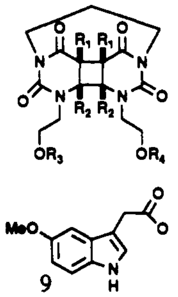
The mechanism for the light-dependent DNA photolyase catalyzed cleavage of cyclobutane pyrimidine photodimers remains an unsolved problem. Various proposals are summarized in Scheme I.[1] Recent transient absorption spectroscopy studies demonstrated the formation of a 400-nm-absorbing intermediate which may be assigned either to the three-electron-reduced flavin or to the deprotonated flavin semiquinone radical.[2] Due to the high reactivity of the photodimer radical cation and anion, it has not been possible to measure reversible redox potentials for the photodimer. This, coupled with lack of information on the stabilization of the photogenerated ion pair at the active site of the enzyme, introduces a serious element of uncertainty into thermodynamic arguments to differentiate between photodimer fragmentation from a radical cation or from a radical anion intermediate.[2] The photodimer cleavage reaction results in the conversion of four sp^3 centers to four sp^2 centers. We therefore expected to observe a normal secondary deuterium isotope effect on the fragmentation reaction.[3] In this communication, we describe the measurement of the isotope effects on the fragmentation of a model photodimer radical anion and cation and use these values to interpret the corresponding *V*/*K* isotope effects previously reported for the enzymatic reaction.[4]

Scheme I



Scheme II



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1: R_1=R_2=H, OR_3=OR_4=(^{3}H)-AcO.
2: R_1=R_2=D, OR_3=OR_4=(^{14}C)-AcO.
3: R_1=D, R_2=H, OR_3=OR_4=(^{14}C)-AcO.
4: R_1=H, R_2=D, OR_3=OR_4=(^{14}C)-AcO.

5: R_1=R_2=H, OR_3=**9**, OR_4=(^{3}H)-AcO.
6: R_1=R_2=D, OR_3=**9**, OR_4=(^{14}C)-AcO.
7: R_1=D, R_2=H, OR_3=**9**, OR_4=(^{14}C)-AcO.
8: R_1=H, R_2=D, OR_3=**9**, OR_4=(^{14}C)-AcO.

Table I. Isotope Effects on Anthraquinone-Mediated Cleavage

substrates	isotope effect
2 + **1**	1.247 ± 0.023^{a-c}
3 + **1**	1.030 ± 0.020^{a,b}
4 + **1**	1.192 ± 0.018^{a,b}

[a] Confidence coefficient = 99%. [b] Based on four determinations. [c] Corrected to 100% deuteriation.

Table II. Isotope Effects on 5-Methoxyindole-Mediated Cleavage

substrates	isotope effect
6 + **5**	1.301 ± 0.021^{a-c}
7 + **5**	1.173 ± 0.010^{a,b}
8 + **5**	1.079 ± 0.008^{a,b}

[a] Confidence coefficient = 99%. [b] Based on six determinations. [c] Corrected to 100% deuteriation.

Table III. Isotope Effects on Enzyme-Mediated Cleavage

substrates	isotope effect
H_4-[U=U] + 6,6′,5,5′-D_4-[U=U]	1.150 ± 0.014^a
H_4-[U=U] + 5,5′-D_2-[U=U]	1.082 ± 0.011^a
H_4-[U=U] + 6,6′-D_2-[U=U]	1.071 ± 0.012^a

[a] Confidence coefficient = 99%.

The model photodimers used in this study are shown in Scheme II.[5,6] The photodimer radical cation was generated using anthraquinone as sensitizer,[7,8] and the photodimer radical anion was generated using covalently linked methoxyindole as sensitizer.[9] The ratios of deuterio to protio starting materials and products were determined by double label scintillation counting as previously described for the enzymatic reaction,[4] and the intermolecular isotope effects were calculated using the integrated rate equation described by Melander and Saunders.[10] Our experimental results are summarized in Tables I and II.

The pattern of isotope effects observed for the fragmentation of the photodimer radical cation is significantly different from the pattern observed for the fragmentation of the photodimer radical anion. For the radical cation fragmentation there is a substantial isotope effect on the cleavage of the first bond (6,6) and a small isotope effect on the cleavage of the second bond (5,5). In contrast, the fragmentation of the photodimer radical anion shows a substantial isotope effect on the fragmentation of both the first (5,5) and the second (6,6) bonds.

Secondary intermolecular isotope effects reflect hybridization changes occurring during or before the first irreversible step of

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(5) Compounds **2** and **6** had ≥98% deuterium incorporation at the 6,6 positions and 89 ± 2% deuterium incorporation at the 5,5 positions as determined by ^{1}H NMR and mass spectrometric analyses.

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(8) No isotope effect was observed on the fragmentation reaction when DDQ was used as the sensitizer. This presumably means that back electron transfer from the weakly reducing DDQ semiquinone radical cannot compete with photodimer cleavage, thus making the isotopically insensitive initial electron transfer step irreversible with this sensitizer.

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a nonbranching reaction sequence.[3] Therefore, if we consider the first bond cleavage reaction to be irreversible for both the indole- and quinone-sensitized cleavage reactions, we would predict all of the isotope effect to be associated with cleavage of the 5,5 bond in the indole case and with cleavage of the 6,6 bond in the quinone case. Observation of an isotope effect associated with cleavage of the second bond in each case would be indicative of one or more of the following: (a) a β-secondary isotope effect associated with the first bond cleavage, (b) reversible first bond cleavage, and (c) concerted cleavage of both bonds.

On the basis of previous mechanistic studies on the fragmentation of the photodimer radical cation, we propose that the isotope effects measured for the anthraquinone-sensitized cleavage support a stepwise mechanism where the first bond cleavage is effectively irreversible.[7,11-14] The small effect on the 5,5 bond cleavage may thus be due to a hyperconjugative effect[15] or due to the small hybridization change occurring at C5 due to elongation of the 6,6 bond in the transition state.[16]

For the radical anion fragmentation, the relatively large isotope effect observed with the 6,6-dideuterio photodimer may be indicative of an intrinsically different transition state for the first bond cleavage of the radical anion compared to the radical cation leading to a larger secondary β-isotope effect. Alternatively, since the fragmentation of the photodimer radical anion is estimated to be $\sim 10^3$ times slower than the radical cation fragmentation,[17] the large 6,6 isotope effect may be due to reversibility of the 5,5 bond cleavage. Thirdly, the pattern of effects observed for the indole-sensitized cleavage is also consistent with a concerted fragmentation with an asymmetric transition state.

The previously published isotope effect studies on the DNA photolyase catalyzed cleavage of deoxyuridine dinucleotide photodimers are shown in Table III.[4] While these isotope effects are clearly not identical to either of the model systems due to the different forward commitment factors for the models and the enzyme, the pattern (i.e., substantial isotope effects for both the cleavage of the 5,5 and the 6,6 bonds) is similar to that observed for the indole-sensitized cleavage. Thus, we propose that the enzymatic reaction proceeds via the fragmentation of a photodimer radical anion. Studies utilizing photodimers substituted with an iodomethyl radical trap[12] are now in progress to examine the timing of the bond cleavage steps.

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Registry No. 1, 138572-10-8; **5**, 138572-11-9; DNA photolyase, 37290-70-3; deuterium, 7782-39-0.

Supplementary Material Available: Synthetic scheme for the preparation of the deuteriated photodimers (2 pages). Ordering information is given on any current masthead page.

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[11] Previous studies on the reaction of the photodimer radical cation in related model systems have suggested that the fragmentation is a stepwise process[7,12] and that it undergoes ring cleavage at a rate $\geq 10^9$ s^{-1}.[13] Therefore the rate constant for the second bond cleavage has a lower limit of ca. 10^9 s^{-1}. Newcomb[14] has recently measured the cyclization of the 2,2-dimethyl-5-cyano-4-pentenyl radical as proceeding with a rate of 1.9×10^4 s^{-1}. Hence, although reclosure of the one-bond-cleaved photodimer radical cation may be faster than the 2,2-dimethyl-5-cyano-4-pentenyl radical closure, it is unlikely that it will compete with the second bond cleavage.

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(15) Hyperconjugation is a major contributor to β-isotope effects on carbocations and radicals (Shiner, V. J.; Humphrey, J. S. *J. Am. Chem. Soc.* **1963**, *85*, 2416). The degree of hyperconjugation is dependent on the dihedral angle between the β-CH bond and the bond undergoing cleavage. For the first bond cleavage of the photodimer radical cation or anion we expect the hyperconjugation contribution to the β-isotope effect to be small since the bond undergoing cleavage is almost orthogonal to the β-CH bond.

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Tuning the Intermolecular Dative Interactions in Vanadium-Oxo Linear Chain Compounds: Formation of a New Type of Liquid Crystalline Polymer

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Liquid crystals with transition-metal core groups (metallomesogens) are increasingly a topic of investigation since metals can impart useful shapes and properties which are not easily produced in totally organic liquid crystals.[1] A major distinction between metallomesogens and most organic mesogens is their greater tendency to exhibit intermolecular dative coordination in the solid state.[1] These interactions can result in phase behavior with kinetic, structural, and thermodynamic complexities, but they also provide an opportunity to create useful supermolecular organizations. We have been exploring the use of intermolecular dative coordination in liquid crystalline materials to create noncentrosymmetric assemblies with second-order nonlinear optical[2] and ferroelectric[3,4] properties. Toward this end we have been investigating high oxidation state early metal monooxo complexes which have a propensity to exhibit either trigonal-pyramidal or square-pyramidal structures[5] and in some cases assemble to give polymeric linear chain structures (e.g., $(\text{—M=O}\cdots\text{M=O—})_n$) in the solid state. We have been focusing initially on the vanadyl Schiff-base complexes *n*(salen)VO, *n*(salpn)VO, and *n*-(Me$_2$salpn)VO shown[6,7] and report herein that *n*(Me$_2$salpn)VO species form a new type of unidirectional (head-to-tail) liquid crystalline linear chain polymer.

The *n*(salen)VO complexes are green polymorphic materials which all display smectic mesomorphic behavior. A high-tem-

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