benzoates, and the signs of the Cotton effects were characteristic of the chirality of allylic positions, irrespective of the number and the stereochemistry of benzoates in the chain. The magnitude of the DIF CD also indicated that allylic benzoates having a benzoyl 1,3-*syn* to an allylic benzoyl showed a value of $|\Delta\epsilon| > 3.5$ (entries 1, 4, 5, and 7), whereas those having a 1,3-*anti* relationship showed $|\Delta\epsilon| < 3.5$ (entries 2, 3, 6, and 8).[18]

The combination of this DIF CD method with reiterative degradation enabled us to determine the absolute stereochemistry of 1,3-polyols. Thus, the Swern oxidation[19] of (2*S*,4*S*,6*S*)-**3** yielded the unsaturated aldehyde **5**, and its ozonolysis afforded hydroxy benzoate (2*S*,4*S*)-**1** (Scheme II). Dehydration of an aliquot sample of (2*S*,4*S*)-**1** gave allylic benzoate (2*S*,4*R*)-**2**, and the DIF CD measurement at this stage established the absolute configuration of the C-4 allylic position (entry 1). This series can be repeated until the stereochemistry of all chiral centers has been determined.

In summary, the difference CD method presented here has solved complicated conformational problems in acyclic 1,3-polyols. By combinating this method with reiterative degradation, the absolute stereochemistry of 1,3-polyols can be determined, even if the relative stereochemistry is unknown.

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Supplementary Material Available: CD and DIF CD spectra of compounds **1**-**4** listed in Table I (2 pages). Ordering information is given on any current masthead page.

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Efficient Photosensitized Pyrimidine Dimer Splitting by a Reduced Flavin Requires the Deprotonated Flavin

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Pyrimidine dimers in DNA are split by photolyases, novel repair enzymes that bind to dimer-containing DNA in a dark reaction and subsequently split the dimer in a light-dependent step.[1] All known photolyases employ a reduced flavin cofactor ($FADH_2$) and either a deazaflavin or a reduced folate, which apparently function as antennas for the more efficient capture of light by the enzyme.[2] Dimer splitting appears to be a consequence of photoinduced electron transfer to or from the dimer.[3] Although a reduced flavin would be expected to be a source of electrons,[2d,4] the direction of electron transfer in the natural system is unknown.[5] We report herein the finding that, in aqueous solution, a reduced flavin requires deprotonation to the monoanion for efficient photosensitization of *N*(1),*N*(3)-dimethyluracil *cis-syn*-cyclobutane dimer[6] (DMUD) splitting (Figure 1). We also found that dimer splitting occurred by an unprecedented chain reaction, which amplified the splitting efficiency and made possible the determination of the reaction's pH profile. The results strongly imply that in this system, and possibly also the natural system, photoinduced electron transfer occurs *from* deprotonated, reduced flavin *to* dimer, with subsequent splitting by the dimer radical anion.[7]

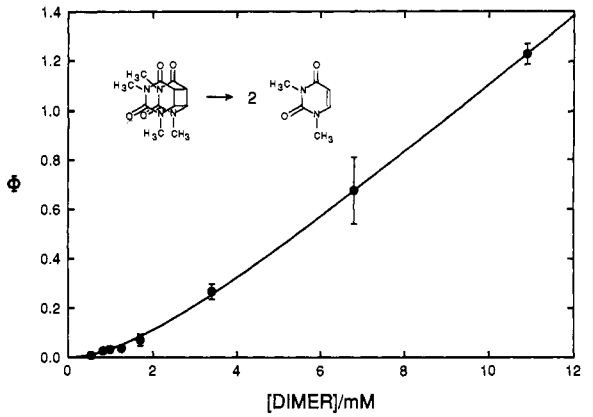

Figure 1. Dependence of DMUD splitting efficiency on dimer concentration at pH 6.3 in argon-purged (Oxiclear filter for trace O_2 removal) aqueous solution. The concentration of ac_4rfH_2 was 7.5 μM. Points are averages of at least two determinations ±SD. The line through the data was generated by curve-fitting an expression [of algebraic form $y = x^2/(ax + b)$] derived for the chain mechanism described in the text.

For these studies 2′,3′,4′,5′-tetraacetylriboflavin[8] (ac_4rf) was reduced to the 1,5-dihydroflavin (ac_4rfH_2) by irradiation at 436 nm in aqueous solutions of oxalate under an argon atmosphere.[9] Reduction appeared to be complete within approximately 30 s, whereupon UV-visible absorption spectroscopy showed the disappearance of maxima at 375 and 445 nm, which are characteristic of oxidized flavins. Irradiation in the presence of DMUD at different concentrations was carried out for a total of 10 min, after which air was admitted, the flavin was returned to the oxidized form, and a final absorbance measurement was made. The quantum yield of splitting (Figure 1) was determined on the basis of the increase in absorbance that accompanies the appearance of dimethyluracil (DMU). Following a prolonged photolysis (110 min), NMR spectroscopy verified the formation of DMU, as did HPLC after short photolyses. A control experiment was carried out with the oxidized flavin, which proved to be ineffective at dimer splitting under these conditions (data not shown).

A sensitization plot (Φ_{spl}^{-1} vs $[dimer]^{-1}$) is decidedly curved (not shown), in contrast to what is typically observed for photosensitized dimer splitting.[3g] This finding, along with the high quantum yields of splitting and quadratic dependence on [DMUD] (Figure 1), suggested the involvement of a chain reaction. A mechanism consistent with these results is electron transfer from the photoexcited flavin (ac_4rfH^{-*}) to DMUD, followed by splitting[10] of

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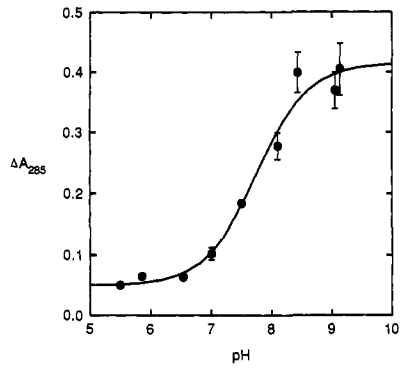

Figure 2. Dependence of ac_4rfH_2-sensitized DMUD splitting efficiency on pH, as measured by the increase in absorbance at 285 nm after a 3-min irradiation at 436 nm in phosphate- or borate-buffered (0.03 M), argon-purged (Oxiclear filter for trace O_2 removal) aqueous solution. The concentration of ac_4rfH_2 was 12 μM. Points are averages of at least three determinations ±SD. The curve was generated by fitting a titration expression $[\Delta A_{min}/(1 + 10^{pH-pK_a}) + \Delta A_{max}/(1 + 10^{pK_a-pH})]$ to the data. The values of ΔA_{min} and ΔA_{max} were thereby found to be 0.049 ± 0.015 and 0.41 ± 0.02, respectively.

$DMUD^{\bullet-}$ to produce DMU and $DMU^{\bullet-}$. Chain propagation occurs by electron transfer from $DMU^{\bullet-}$ to a neutral DMUD. The newly formed $DMUD^{\bullet-}$ can subsequently split. Termination steps that compete with propagation are loss of an electron by either the monomer radical anion or dimer radical anion (presumably to the flavin radical $ac_4rfH^{\bullet}$). This mechanism parallels one we recently proposed for a possible chain reaction in dimer radical cation splitting initiated by a protonated, oxidized flavin.[3k] It differs, however, from a radical cation chain reaction[3l] in which the propagating species was the sensitizer radical cation and sensitization plots were linear in $[dimer]^{-1}$.

The high splitting efficiency of DMUD[11] made possible the determination of a pH profile for the reaction. When photolysis of 1.2×10^{-5} M flavin and 3.4 mM DMUD in solutions ranging from pH 5.5 to 9.1 was carried out for 3 min, a very clear result emerged (Figure 2). Splitting efficiency followed a titration curve centered at pH 7.7. Under these conditions, flavin anion sensitized splitting was approximately 8-fold greater than neutral flavin sensitized splitting (i.e., $\Delta A_{max} = 0.41 \pm 0.02$ and $\Delta A_{min} = 0.049 \pm 0.015$; Figure 2). Possible explanations for this are inefficient electron transfer from ac_4rfH_2 ($pK_a \sim 7$)[12] and/or faster back electron transfer to the protonated radical in the geminate pair $\{dimer^{\bullet-}\ ac_4rfH_2^{\bullet+}\}$. It is also possible that one or more steps subsequent to initiation of the chain reaction are sensitive to pH and this contributes to the observed pH profile (e.g., less efficient chain termination by $ac_4rf^{\bullet-}$; $pK_a \sim 8$[12b] for $ac_4rfH^{\bullet}$).[10]

Previously, photosensitized dimer splitting by oxidized[4,13] and reduced flavins[4] has been carried out in alkaline solution. In the case of oxidized flavins, this was a consequence of the need to form the deprotonated dimer.[14] In the present study, deprotonation of the reduced flavin is apparently necessary. It is implausible that the reduced flavin monoanion would be more efficient at abstracting an electron from the dimer than would the neutral reduced flavin. Thus, the findings reported here virtually rule out electron abstraction from dimers by reduced flavins in aqueous solutions. The active sites of photolyases are hydrophobic,[15] however, and there can be significant solvent polarity effects on dimer radical anion splitting efficiency.[3a] Therefore, whether photolyases employ $FADH_2$ in its deprotonated form ($FADH^-$)[5b] requires further studies of the natural system.

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Redesign of a Type 2 into a Type 1 Copper Protein: Construction and Characterization of Yeast Copper-Zinc Superoxide Dismutase Mutants

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The essential features of type 1 copper proteins appear to be two histidine imidazoles and a cysteine thiolate coordinated to Cu^{2+} in a trigonal planar geometry with one or two additional weak axial ligands.[1] The type 2 copper protein copper-zinc superoxide disumtase, CuZnSOD, contains a copper binding site (four histidines) and a zinc binding site (three histidines and an aspartate) in each of its two identical subunits, and Cu^{2+} can be bound to either or both sites.[2,3] We reasoned that substitution of histidine residues by cysteine in either site would give us new types of metal-binding sites containing histidines and cysteines and that the properties of Cu^{2+} bound to these sites would make an interesting comparison with the properties of the natural type 1 copper proteins.[4-7]

We prepared five single histidine-to-cysteine mutants using oligonucleotide-directed mutagenesis on the cloned CuZnSOD gene from *Saccharomyces cerevisiae*.[8] The mutant genes were expressed in *Escherichia coli* in the T7 RNA polymerase expression system,[9] and the mutant proteins were purified to homogeneity.[10] We describe here our characterization of the most stable copper site mutant, H46C (His 46 to Cys 46), and the most stable zinc site mutant, H80C (His 80 to Cys 80).[11] Apoprotein was prepared using procedures reported previously for the bovine protein,[12] except that 0.25 mM dithiothreitol was added to the

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