Tryptophan Radical Formation in DNA Photolyase: Electron-Spin Polarization Arising from Photoexcitation of a Doublet Ground State

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Received July 24, 1992

DNA photolyase photoconverts pyrimidine dimers to pyrimidines in the repair of UV-damaged DNA.[1] The active form of the enzyme contains reduced flavin adenine dinucleotide, probably in its anion form ($FADH^-$),[2,3] as the direct photocatalyst. The repair reaction is initiated by electron transfer between the bound DNA substrate and the $FADH^-$ singlet excited state.[2,4] One-electron oxidation of $FADH^-$ during enzyme isolation produces the neutral flavin semiquinone $FADH^•$, which is inactive in dimer repair but which can be photoactivated to produce functional enzyme.[5] Optical data on this process suggest that photon absorption produces the $FADH^•$ doublet excited state, which intersystem crosses to its quartet state and oxidizes a nearby amino acid.[6] Exogenous donors can reduce the latter species to form the active enzyme. In the absence of reductant, the amino acid radical, which has been implicated as tryptophan-306 from directed mutagenesis work,[7] and the product $FADH^-$ recombine in a few milliseconds. We have now used flash photolysis and time-resolved EPR to generate and characterize the radical intermediate. Isotopic labeling demonstrates the tryptophan origin of the radical and suggests that it occurs in its cation radical form. Our data show that the tryptophan radical is initially spin polarized, which is the first case of spin polarization arising from photoexcitation of a doublet ground-state species, in this case $FADH^•$. We suggest that spin polarization of the doublet tryptophan radical arises as the photoexcited flavin intersystem crosses from its initial doublet state to a quartet state. Subsequent electron transfer from tryptophan forms a doublet–triplet radical pair in which spin polarization develops according to a correlated radical pair mechanism.

The static EPR spectrum of the neutral flavin radical in inactive photolyase[8] is shown in the inset to Figure 1. Photolysis of this form of the enzyme produces an intense transient response that varies with applied magnetic field (Figure 1a,c) and rises and decays with the instrument time constant (35 μs).[9] Replacing

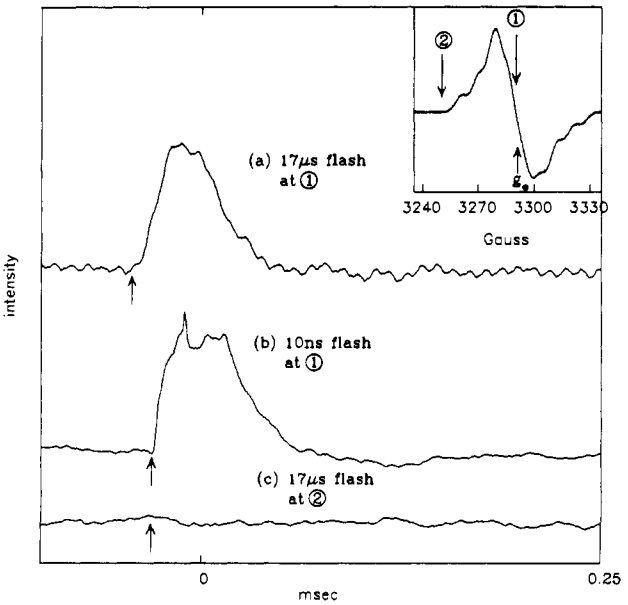

Figure 1. Kinetic traces of the light-induced tryptophan radical in DNA photolyase. In a and c, a Xenon flash lamp with a pulse width of 17 μs was the excitation source; in b, a Nd:YAG laser with 355-nm pulses of 10-ns duration was used. Both a and b were obtained at a magnetic field of 3290 G (field position 1 in inset), and c was obtained at a magnetic field of 3250 G (field position 2 in inset). Instrument conditions: microwave frequency = 9.222 26 GHz, microwave power = 6.3 mW, modulation amplitude = 2.8 G, time constant = 35 μs; for each experiment, 1000 flash-induced transients at a repetition rate of 1 Hz were averaged. The inset shows the dark stable flavin radical and the fields at which the sample was excited to obtain the kinetic traces. The enzyme concentration was approximately 0.1 mM in pH 7.0, 50 mM phosphate buffer that contained 20% glycerol for all experiments.

the 17-μs flash lamp pulses (Figure 1a) with 10-ns, 355-nm laser pulses produces essentially the same kinetic trace (Figure 1b), which indicates that the kinetics of the transient are insensitive to pulse duration under our spectrometer conditions. By using gated integration and ac coupling to discriminate against stable signals, the hatched area in the kinetic trace (inset, Figure 2) was integrated as a function of field to record the spectrum of the transient species (Figure 2a). The resulting spectrum has a mixed emission/absorption line shape, indicating a non-Boltzmann spin distribution, that consists of three components in a 1:2:1 intensity ratio. Partially resolved fine structure is superimposed on the three resolved features. The emission(E)/absorption(A) pattern has E/A/E/A/E/A structure, as indicated by the integral of the first derivative spectrum (Figure 2b); emission and absorption are balanced and the net EPR intensity is close to 0. The spin-polarized nature of this species accounts for the relative ease with which we were able to record kinetic transients.

To test whether the spectrum detected in Figure 2a arises from the indole side chain of a tryptophan residue,[7] time-resolved EPR was used to examine a photolyase sample that contained indole-ring deuterated tryptophan (trp-d_5).[11] A decrease in signal intensity occurred upon deuteration, but increasing the number

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(9) EPR spectra were recorded with a Bruker ER200D spectrometer at a microwave power of 6.3 mW and a modulation of 2.8 G. The temperature was 298 K. Transient EPR spectra were captured by using gated integration techniques described in ref 10. The sample was excited with a xenon flash lamp with 17-μs pulses that were critically damped. The electrical energy supplied to the lamp was 50 J. Some samples were excited by the third harmonic of a Quanta Ray GCR11 Nd:YAG laser, which has a pulse duration of 10 ns and an energy of 20 mJ. The kinetic traces were recorded with a Markenrich WAAG data acquisition board that had been interfaced to the signal output of the spectrometer. The timing was controlled as described in ref 10.

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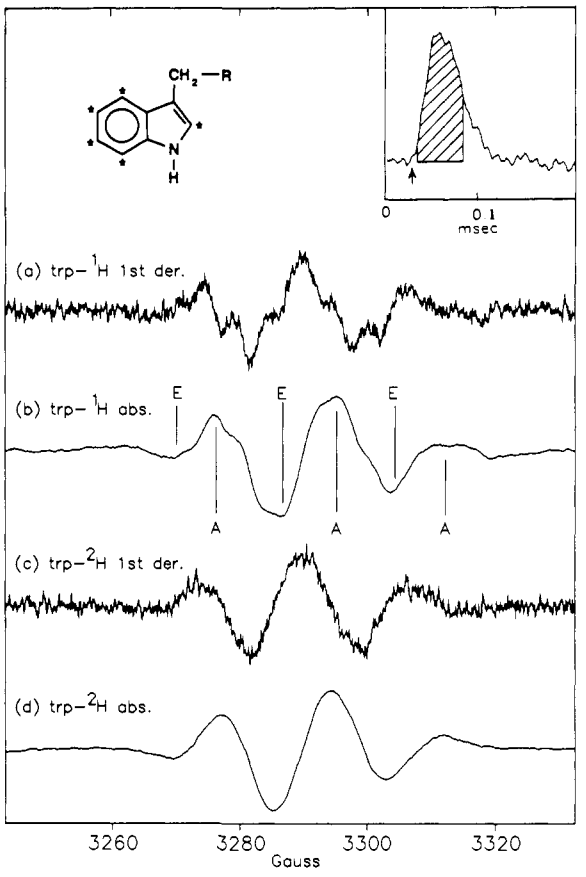

Figure 2. Time-resolved, first-derivative EPR spectra and the corresponding integrated spectra of the light-induced tryptophan radical in DNA photolyase. Spectrum a is the first-derivative spectrum of a protiated tryptophan sample. The field positions of emissive (E) and absorptive (A) peaks in the corresponding absorption spectrum were obtained by integrating the first-derivative spectrum in a to obtain the absorption spectrum b. Spectrum c is of a sample that contained tryptophan deuterated at the indole ring positions indicated by asterisks in the structure; d is the first integral of c. Instrument conditions were as in Figure 1. A 4-μs delay and a 48-μs aperture were used for the integration window. For a, five scans were averaged; this was increased to 20 scans for c. The inset shows the kinetic trace of the transient radical; the hatched area was integrated as a function of field to obtain the time-resolved spectrum.

of scans 4-fold produced a good-quality transient spectrum of the labeled species (Figure 2c). Comparison of the first-derivative spectra (Figure 2a,c) or of the first integrals (Figure 2b,d) shows that the partially resolved fine structure in the protiated enzyme is lost in the trp-d_5 sample. We conclude that the transient species arises from a tryptophan radical formed in the FADH$^\bullet$ reduction process.

The rapid decay of the spin-polarized signal is followed by less intense kinetic components. The amplitudes of these slower phases are field dependent and persist into the millisecond time regime.[6]

These results provide important insight into the mechanism of photoactivation of photolyase. Tryptophan involvement in this process[7] is confirmed here. Moreover, semiempirical MO calculations have indicated that significant hyperfine coupling to α-protons on the indole ring occurs for the cation radical but not for the neutral paramagnet.[12] These calculations, in conjunction with the labeling results in Figure 2, indicate that the trp radical occurs in its cation form, that is, the initial photoprocess involves electron transfer, not H atom transfer, from the indole side chain to the excited-state flavin. The implications of this conclusion for the photolyase thymine dimer repair mechanism will be presented in detail elsewhere.[3]

The observation of spin polarization in the photoactivation reaction is, at first glance, surprising, Previous work on this phenomenon has focused almost exclusively on either triplet precursors or doublet–doublet radical pairs.[13] The development of spin polarization through triplet–doublet splitting has been reported,[14] however, and studied recently in some detail.[15] In these cases, net emission or absorption is observed, and mechanisms that adapt aspects of the conventional triplet and radical pair mechanisms have been invoked to explain the observed polarization. The photolyase data here differ from these solution results as neither net emission nor absorption occurs; moreover, the extent of polarization is dependent upon hyperfine coupling, as shown by the data in Figure 2. These characteristics, as well as the polarization pattern observed in Figure 2b,d, are similar to the interacting CIDEP-correlated radical pair polarization (CRPP) case considered by Norris et al. for doublet–doublet spin polarization.[13] The fact that both the flavin triplet and tryptophan doublet are protein bound and are likely to remain interacting throughout the lifetime of the spin polarization suggests that an analogous CRPP mechanism[16] can be adapted to the triplet–doublet case that occurs in photolyase.[17]

Acknowledgment. This work was supported by NIH grants to A.S. (GM31082) and G.T.B. (GM37300). We thank Dr. Brian Hoffman for bringing the molecular orbital calculations on tryptophan radicals to our attention and Dr. Marion Thurnauer for discussions of doublet–triplet radical pair systems.

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A Novel Strategy for Synthesis of Ganglioside GM3 Using an Enzymatically Produced Sialoside Glycosyl Donor

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Received September 11, 1992

Synthesis of biologically important glycoconjugates by combined chemical and enzymatic methodologies is recognized as a promising practical approach.[1] The strategy of the combined synthesis has, to date, been limited to enzymatic glycosylation following chemical synthesis and deblocking of oligosaccharide precursors.[2] We report here an efficient synthesis of ganglioside GM3 using

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