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Supplementary Material Available: ORTEP structure, crystallographic data, and bond lengths and angles for adduct **5** (3 pages). Ordering information is given on any current masthead page.

2′-Azido-2′-deoxynucleotide Interaction with *E. coli* Ribonucleotide Reductase: Generation of a New Radical Species

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Ribonucleotide reductase (RDPR)[1] from *E. coli* catalyzes the conversion of nucleoside diphosphates to deoxynucleoside diphosphates.[2] This enzyme is composed of two subunits: B_1 (α, α' M_r 160 000) binds NDP[1] substrates and contains redox active thiols and binding sites for the allosteric effectors; B_2 (β,β M_r 78 000) contains an unusual cofactor composed of two Fe^{+3} and one tyrosine radical, which is an integral part of the B_2 polypeptide chain. The active site is thought to be at the interface between the two subunits.[2] Evidence from our laboratory is consistent with a proposed radical cation mechanism for this reduction reaction.[3] However, until recently, no direct evidence in support of any substrate radical intermediates was available.[4] Sjöberg et al. observed that incubation of RDPR with suicide inhibitor 2′-azido-2′-deoxycytidine 5′-diphosphate (N_3CDP)[1] resulted in the formation of a new radical species.[4] Furthermore in the presence of ^{15}N- or ^{2}H-labeled RDPR and H_2O or D_2O the new radical generated showed no change in its hyperfine splitting pattern. They interpreted this data as evidence for formation of a "substrate analogue" radical. These studies prompted us to report our findings with specifically labeled substrate analogues [2′-^{2}H]N_3UDP[1] and [2′-^{15}N]N_3UDP. Our results clearly indicate formation of the same radical species as observed by Sjöberg et al. Results with the [2′-^{15}N]N_3UDP and [2′-^{2}H]N_3UDP indicate that the new radical is indeed located on a nitrogen originally at the 2′-position of the substrate and that the observed coupling of this species to hydrogen is not caused by the hydrogen on the 2′-carbon. Structures proposed by Sjöberg et al. for this radical are inconsistent with these results.

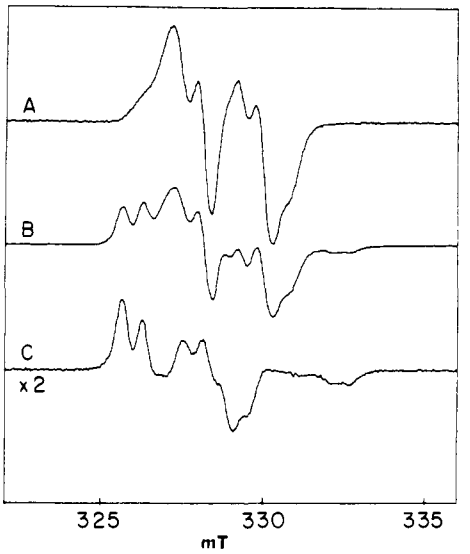

Figure 1. EPR spectra of RDPR with N_3UDP: (A) RDPR in the absence of N_3UDP, (B) 7 min after the addition of N_3UDP, (C) after subtraction of the remaining tyrosine radical signal A from B. Spectrometer conditions: microwave frequency, 9.224 GHz; microwave power, 10 μW; modulation amplitude, 0.2 mT; temperature, 13 K; scanning rate, 16 mT/min; time constant, 0.0645.

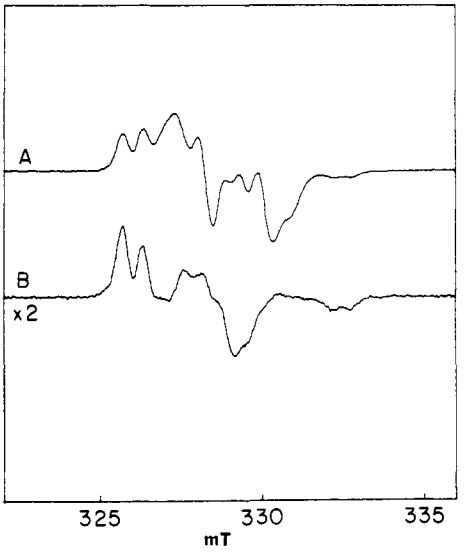

Figure 2. EPR spectra of RDPR with [2′-^{2}H]N_3UDP: (A) 7 min after the addition of [2′-^{2}H]N_3UDP to RDPR, (B) after subtraction of the remaining tyrosine radical signal from A. Spectrometer conditions are as in Figure 1.

Incubation of 17.6 nmol of B_2 under standard assay conditions[5] followed by freezing in liquid N_2 resulted in the EPR spectrum of the tyrosine radical observed in Figure 1A. The sample was then thawed and equilibrated at 25 °C, and N_3UDP (final concentration of 1.5 mM) was added. The reaction was allowed to proceed for 7 min at 25 °C and the sample again frozen in liquid N_2,[6] resulting in the spectrum indicated in Figure 1B. Figure 1C is the spectrum of the new radical species after subtraction of the remaining tyrosine radical spectrum (Figure 1A) from the spectrum of Figure 1B. This species is essentially identical with that observed by Sjöberg et al. with N_3CDP.[4] The hyperfine

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(1) Abbreviations: RDPR, ribonucleoside diphosphate reductase; N_3NDP, 2′-azido-2′-deoxynucleoside 5′-diphosphate; NDP, nucleoside diphosphate; mT, millitesla.

(2) For recent reviews: Thelander, L.; Reichard, P. *Annu. Rev. Biochem.* **1979**, *48*, 143. Reichard, P.; Ehrenberg, A. *Science (Washington, D.C.)* **1983**, 221, 514.

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(5) All EPR spectra were run in D_2O. Proteins were exchanged into D_2O by centrifugation through a 1-mL column of Sephadex G-25 with equilibrated HEPES (pD 7.2), 15 mM $MgSO_4$, 1 mM EDTA in D_2O. Typical reaction mixtures contained in a final volume of 0.3 mL: 50 mM HEPES (pD 7.2), 15 mM $MgSO_4$, 1 mM EDTA, 90 μm TTP, 0.5 mM NADPH, 0.3 mg of thioredoxin, 0.05 mg of thioredoxin reductase, 1.4 mg (17.6 nmol) of B_2, and 1.1 mg (7 nmol) of B_1.

(6) A time course of radical formation with N_3UDP derivatives showed maximum radical formation at about 7 min.

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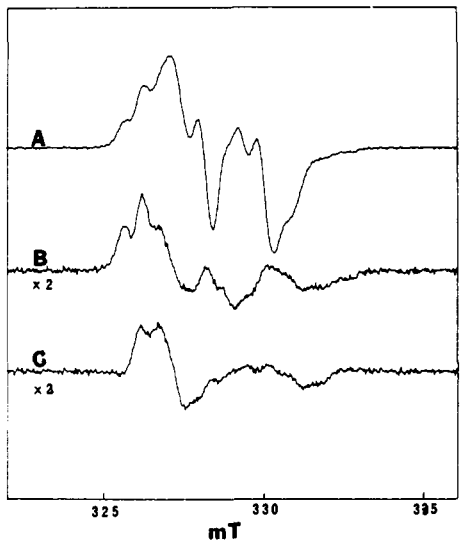
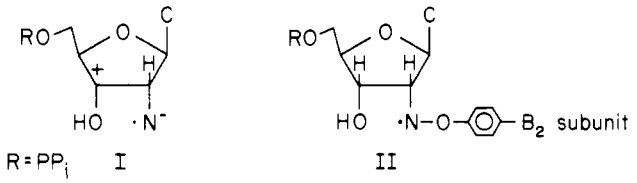


Figure 3. EPR spectra of RDPR with [2′-^{15}N]N_3UDP: (A) 7 min after the addition of [2′-^{15}N]N_3UDP, (B) after subtraction of the remnant tryosine radical from A, (C) after subtraction of the remaining $^{14}N_3$UDP signal (Figure 1C) from the spectrum in B. Spectrum conditions are as in Figure 1.

structure of this radical signal can be interpreted as arising from anisotropic interactions with an $I = 1$ nucleus (large triplet) and an $I = {}^1/_2$ nucleus (smaller doublets).

To test the hypothesis that this new radical is localized on RDPR generated structures such as I or II, specifically on the



nitrogen attached to the 2′-carbon of N_3CDP, we synthesized [2′-^{2}H]N_3UDP[7] and [2′-^{15}N]N_3UDP.[8] The results of the EPR experiments are indicated in Figures 2 and 3. The enzyme incubated for 7 min with [2′-^{2}H]N_3UDP has spectra (Figure 2A, B) identical with those of the corresponding protonated material (Figure 1B, C).

The inability to affect the hyperfine splitting by deuteration of the 2′-carbon strongly suggests that the radical is *not* attached to that carbon. On the other hand, the spectrum of the enzyme incubated with [2′-^{15}N]N_3UDP is consistent with the radical being localized on the nitrogen. Subtraction of the remnant tyrosyl radical spectrum from Figure 3A results in a spectrum (Figure 3B) composed of two radical signals having hyperfine interactions with either ^{15}N or ^{14}N.[8] Subtraction of the ^{14}N-coupled signal (e.g., the spectrum of Figure 1C) results in Figure 3C, which is consistent with ^{15}N hyperfine interactions.

These studies clearly indicate that the new radical species generated is located on a single nitrogen of the intermediate generated by RDPR action on substrate analogue N_3UDP. The hyperfine splitting is not caused by the 2′-H since replacement of the proton with deuterium has no effect on this splitting pattern. Deuterated N_3UDP with labels in the 1′- and 3′-position are being synthesized. Results from experiments with these compounds may then allow us to speculate on the structure of this new radical species and whether it is related to cleavage of the 3′-carbon–hydrogen bond.[3]

Note Added in Proof. [1′-^{2}H]N_3UDP and [3′-^{2}H]N_3UDP upon interaction with RDPR show EPR spectra identical with that in Figure 1C.

(7) Synthetic details are available upon request.
(8) In this synthesis the isotopic label is distributed equally between the nitrogens proximal and distal to the 2′-carbon.

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Neighboring Group Participation in Organic Redox Reactions. 9. Facilitation of a Disulfide Oxidative Cleavage Reaction

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We wish to report that one of the neighboring tertiary amine groups in bis(3-(dimethylamino)propyl) disulfide (**1**) accelerates the rate of oxidative cleavage of the disulfide by aqueous I_2 by a factor of $\sim 10^6$ over that found with cystine.[1] In addition to the tremendous acceleration, the kinetics of the oxidative cleavage of **1** differ greatly from the kinetics of the aqueous iodine oxidation of cystine[1] and of the bis quaternary ammonium salt $[(CH_3)_3N(CH_2)_3S]_2{\cdot}2I^-$ (**2**) and indicate that intramolecular nucleophilic assistance is concomitant with electrophilic cleavage of the disulfide bond.[2a] The formation and cleavage of the disulfide bond is important in many areas of chemistry[2a,b] and biochemistry.[2c,d] Although unusually facile reactions of disulfides with electrophiles may be due to neighboring group participation,[3] systematic studies of the effects of neighboring groups are few in number. When the kinetics of the electrophilic cleavage of several amino acid disulfides by Ag(I) and by Hg(II)[4] were reported, the differences in reactivity were discussed in terms of electrostatic effects and hydrogen bonding.

The rapid reaction of **1**[5] with aqueous I_2 at pH 4–9 yields the sulfonic and sulfinic acids.[6] The procedures used to study the spectrophotometric rates have been described previously.[8] The pseudo-first-order decay of triiodide was monitored over two half-lives, with coefficients of correlation of 0.996–0.999. The data for the I_2 oxidation at pH $\geq$7 were obtained using a stopped-flow spectrophotometer.[8c,9] In Table I the change in rate

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(5) The bis disulfide, **1**, bp 107 °C (10 torr) (Anal. ($C_{10}H_{24}N_2S_2$) C, H, N) was prepared by H_2O_2 oxidation of the thiol **3**[4a] and was derivatized as its bis quaternary salt, $[(CH_3)_3N(CH_2)_3S]_2{\cdot}2I^-$ (**2**) (Anal. ($C_{12}H_{30}N_2S_2I_2$) C, H, N).
(6) The NMR of the product indicated that approximately equimolar quantities of the two acids were formed. The sulfonic acid had been characterized earlier.[7a] The second component (^{1}H NMR δ 3.05 (m, 2), 2.8 (s, 6), 2.45 (m, 2), 2.05 (m, 2); IR (KBr pellet) 1130 (s, br), 950 (s, br) cm^{-1}) has the spectral characteristics of the sulfinic acid[7b] and could be converted to the sulfonic acid with dilute H_2O_2 in less that 1.5 h at room temperature.
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