Communication

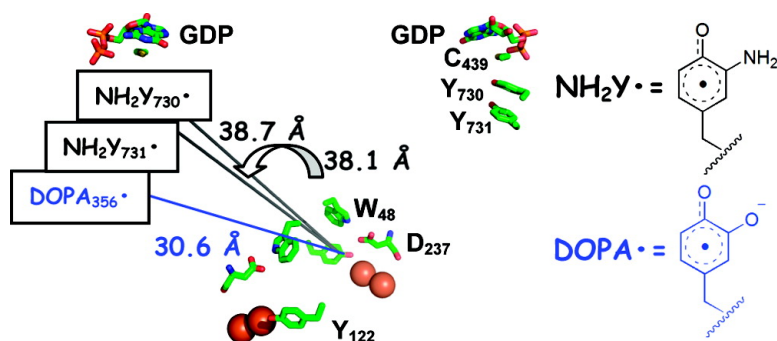# PELDOR Spectroscopy with DOPA-β2 and NHY-2s: Distance Measurements between Residues Involved in the Radical Propagation Pathway of *E. coli* Ribonucleotide Reductase

Mohammad R. Seyedsayamdost, Clement T. Y. Chan,
Veronica Mugnaini, JoAnne Stubbe, and Marina Bennati

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

PELDOR Spectroscopy with DOPA-β2 and NH$_2$Y-α2s: Distance Measurements between Residues Involved in the Radical Propagation Pathway of *E. coli* Ribonucleotide Reductase

Mohammad R. Seyedsayamdost,[†] Clement T. Y. Chan,[†] Veronica Mugnaini,[§,¶]
JoAnne Stubbe,*,[†,‡] and Marina Bennati*,[§,#]

Departments of Chemistry and Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, Massachusetts 02139-4307, and Institute for Physical and Theoretical Chemistry and BMRZ, University of Frankfurt, D-60439, Frankfurt, Germany

Received August 28, 2007; E-mail: stubbe@mit.edu; marina.bennati@mpibpc.mpg.de

Escherichia coli ribonucleotide reductase (RNR) catalyzes the conversion of nucleotides to 2′-deoxynucleotides and consists of a 1:1 complex of homodimeric subunits α2 and β2.[1] α2 is the site of nucleotide reduction and contains binding sites for allosteric effectors, which control the rate and specificity of turnover,[2] and β2 harbors a diferric tyrosyl radical (Y$_{122}$•) cofactor, which is essential for catalysis.[3] Each turnover requires radical propagation from the Y$_{122}$• in β2 to the active site in α2,[4] where a cysteinyl radical (C$_{439}$•) initiates nucleotide reduction.[5] A model for radical propagation has been postulated based on in silico docking of the individual structures of α2 and β2.[4,6] This model suggests that the Y$_{122}$• is >35 Å removed from C$_{439}$ in α2. For radical propagation to occur at a reasonable rate over this long distance,[7] a pathway, proposed to consist of the conserved residues shown in Figure 1, is required. Each active α2β2 complex contains two pathways for radical initiation, one within each $\alpha\beta$ pair (Figure 1). However, experimental validation of the positions of aromatic pathway residues that are involved in radical transfer, specifically those at Y$_{356}$, Y$_{730}$, and Y$_{731}$, remains elusive.[8,9] Direct spectroscopic analysis of these residues is hampered by the transient nature of their oxidized forms.[10] In this study, we assess the positions of these residues using PELDOR (pulsed electron−electron double resonance) spectroscopy, an emerging method of choice for distance measurements between two unpaired spins separated by 15−80 Å.[11] The Y$_{122}$• in β is one paramagnetic species; a second one, required for PELDOR studies, must be generated. We have recently developed a method to site-specifically incorporate 3-hydroxytyrosine (DOPA) into β2 and 3-aminotyrosine (NH$_2$Y) into α2 and have shown that they function as radical traps within the α2β2 complex in the presence of substrate and effector.[8,9] The DOPA radical (DOPA•) and NH$_2$Y radical (NH$_2$Y•) thus generated provide the second required radical for PELDOR experiments. We now report the first distance measurements between residues DOPA$_{356}$•, NH$_2$Y$_{731}$•, or NH$_2$Y$_{730}$• in one $\alpha\beta$ pair and the Y$_{122}$• in the second $\alpha\beta$ pair.[12] The distances provide the first structural constraints for the essential tyrosine residues in the pathway and further support the docking model.

To generate a stable pathway radical at the desired residue, we have taken advantage of expressed protein ligation and suppressor tRNA/aminoacyl-tRNA synthetase methodologies to site-specifically replace Y$_{356}$ in β2 and Y$_{730}$/Y$_{731}$ in α2, respectively. In the former case, we inserted DOPA, which has a reduction potential 260 mV lower than that of Tyr (pH 7),[13] and demonstrated formation of a DOPA• in a kinetically competent fashion only in
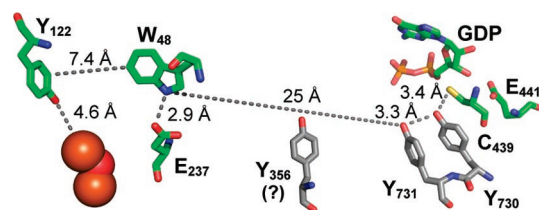
† Department of Chemistry, Massachusetts Institute of Technology.
‡ Department of Biology, Massachusetts Institute of Technology.
§ University of Frankfurt.
# Present address: Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.
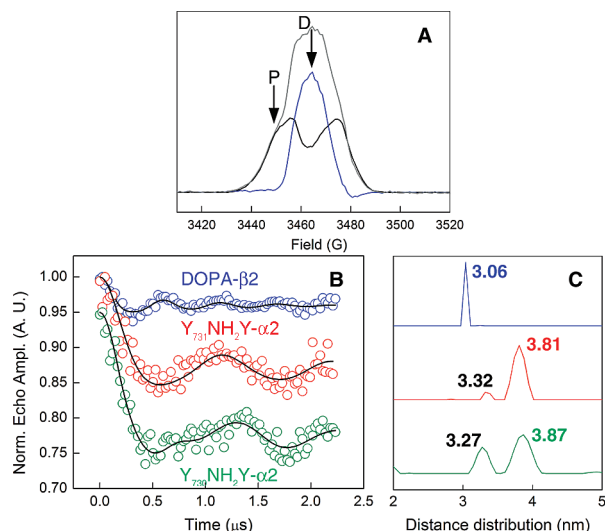¶ Present address: CSIC, Campus Universitari, Bellaterra, Cerdanyola, Spain.

Figure 1. The proposed radical initiation pathway within an $\alpha\beta$ pair.[4,6] Residues in gray have been shown to be redox-active using DOPA-β2[8] and NH$_2$Y-α2s,[9] respectively. Note that the position of Y$_{356}$ is unknown.

the presence of substrate (GDP or CDP) and/or effector (TTP or ATP). In the latter case, we incorporated NH$_2$Y, which has a reduction potential 190 mV lower than that of Tyr (pH 7),[13] and observed kinetically competent formation of an NH$_2$Y• in the presence of CDP/ATP.[14] We have now used these unnatural α2 and β2 variants to measure the distance between the newly formed radical in one $\alpha\beta$ pair and the remaining Y$_{122}$• in the adjacent $\alpha\beta$ pair. At least 25% of the wt β2 population contains 2 Y$_{122}$•/dimer which allows PELDOR interactions diagonally across the α2β2 complex to be observed.[15,16]

DOPA-β2[8] (0.3 Y$_{122}$•/dimer) and pre-reduced α2 were concentrated to 250 μM. Formation of DOPA• was induced by addition of GDP/TTP. After 30 s, the sample was supplemented with glycerol (final 6% v/v) and the reaction was quenched by hand-freezing in liquid N$_2$. Figure 2A (gray trace) shows the X-band spin−echo detected absorption spectrum of this reaction along with the pump and detect frequencies used in the four-pulse DEER sequence.[17] The spectrum is a composite of DOPA• and Y$_{122}$• signals. Subtraction of the Y$_{122}$• component (black, 54%) yields the DOPA• (blue, 46%). Figure 2B (blue) shows the echo modulation trace recorded at the detection frequency after subtraction of a monoexponential signal decay function.[15,18] The modulation frequency of this spectrum is indicative of the distance between Y$_{122}$•−DOPA•, the modulation depth, λ, is a function of the concentration of radical pairs.[19] Analysis of this trace using the distance-domain Tikhonov regularization procedure[20] results in the distance distribution profile in Figure 2C (blue), which indicates a distance of 30.6 ± 0.5 Å[21] between Y$_{122}$• and DOPA•. Residue 356 lies on the disordered C-terminal tail of β2; thus, the distance determined here is the first structural information for this residue, which is invisible in all crystal structures solved to date.

A similar analysis has been carried out with Y$_{730}$NH$_2$Y-α2 and Y$_{731}$NH$_2$Y-α2.[9] In these cases, a 1:1 complex of β2 (1.2 Y$_{122}$•/ dimer) and NH$_2$Y-α2 was concentrated to 80−100 μM. Formation of the NH$_2$Y•, quenching, data acquisition, and analysis were carried out as described for DOPA-β2 above.[15,18] The modulation traces for Y$_{731}$NH$_2$Y-α2 (red) and Y$_{730}$NH$_2$Y-α2 (green) are shown in Figure 2B. The λ observed in Figure 2B is proportional to the radical

Figure 2. (A) Spin—echo detected EPR spectrum after reaction of DOPA-$\beta2\alpha2$ with GDP/TTP. The spectrum of unreacted $Y_{122}\bullet$ (black) has been subtracted from the observed spectrum (gray), yielding the DOPA$\bullet$ spectrum (blue). Pump (P) and detect (D) frequencies are indicated by arrows. (B) Normalized four-pulse DEER at 6 K for DOPA-$\beta2\alpha2$ (blue) and NH_2Y-$\alpha2\beta2$ (red and green) with GDP/TTP. The overlaid black lines describe fits using the Tikhonov regularization procedure. (C) Resulting distance distributions obtained from the analysis in (B) are shown for each NH_2Y-$\alpha2$ and DOPA-$\beta2$ variant. The distances of 3.32 and 3.27 nm (black) are related to $Y_{122}\bullet$—$Y_{122}\bullet$ pairs.[15]
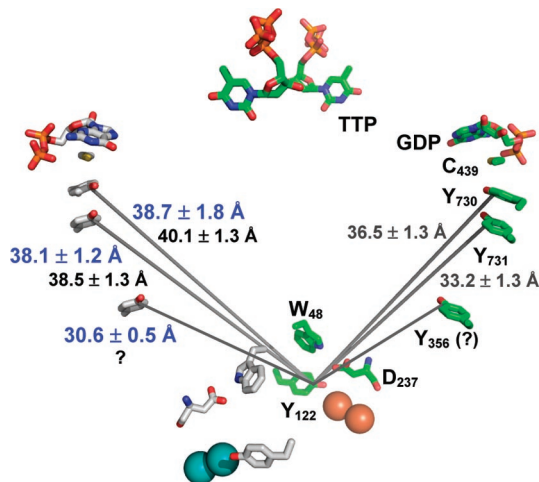

Figure 3. Diagonal and linear distances predicted by the docking model (black) and measured diagonal distances obtained by PELDOR in this study (blue). The residues constituting a pathway in one $\alpha\beta$ pair are colored in light gray (Fe shown in teal); residues in the second pathway are shown in green (Fe shown in orange). For clarity, the residues and ligands in only one pathway (right) have been labeled. Distances from the model (black) represent averages from both pathways in the complex and have been measured from the aromatic C4 atom of each Tyr residue. The position of Y_{356} is unknown.

pair content of the $\beta2$ variant before and after the reaction.[22] Analysis of the reaction with $Y_{731}NH_2Y$-$\alpha2$ gives a dominant distance distribution peak at 38.1 ± 1.2 Å[21] between $NH_2Y_{731}\bullet$ and $Y_{122}\bullet$ (Figure 2C). A small peak corresponding to the $Y_{122}\bullet$—$Y_{122}\bullet$ pair is also observed.[15] In case of $Y_{730}NH_2Y$-$\alpha2$, a distance of 38.7 ± 1.8 Å[21] is observed along with a peak related to unreacted $Y_{122}\bullet$—$Y_{122}\bullet$.[23]

The data are consistent with recent findings of half-site reactivity in the $\alpha2\beta2$ complex,[8,18,24] which contains two radical transfer pathways, structurally related by C_2-symmetry (Figure 3). In the first turnover, radical transfer occurs within only one of these

pathways. In the case of DOPA-$\beta2$ and NH_2Y-$\alpha2$, radical transfer results in formation of a new DOPA$\bullet$ or $NH_2Y\bullet$ at the expense of $Y_{122}\bullet$ in one $\alpha\beta$ pair. The $Y_{122}\bullet$ within the other $\alpha\beta$ pair, where radical initiation does not occur, is preserved, thus allowing distance measurements diagonally across the $\alpha2\beta2$ complex between these two radicals.

To analyze the distances, we consider the docking model, generated from the individual structures of $\alpha2$ and $\beta2$ based on shape and charge complementarities and conserved residues (Figure 3).[4,6] As noted above, Y_{356} is not visible in any structures of $\beta2$ and its position is unknown. Thus, the data provide the first structural constraint for residue Y_{356} in the active $\alpha2\beta2$ complex. On the basis of the docking model, the diagonal distances across the $\alpha2\beta2$ dimer interface for the Y_{122}—Y_{731} and Y_{122}—Y_{730} pairs are 38.5 ± 1.3 and 40.1 ± 1.3 Å, respectively. Within error, these distances are similar to the 38.1 and 38.7 Å that we report here. Accordingly, our studies establish the position of the critical Tyr pathway residues in the $\alpha2\beta2$ complex, as presaged by the docking model, and also support a long-range radical transfer mechanism for $C_{439}\bullet$ formation. This study sets the stage for further X-band and high-field PELDOR experiments[25] which could be used to measure the orientation between two radicals and ultimately report on the distance from Y_{356} to Y_{731}.

References

(1) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705.
(2) Jordan, A.; Reichard, P. *Annu. Rev. Biochem.* **1998**, *67*, 71.
(3) Ehrenberg, A.; Reichard, P. *J. Biol. Chem.* **1972**, *247*, 3485.
(4) Uhlin, U.; Eklund, H. *Nature* **1994**, *370*, 533.
(5) Stubbe, J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2723.
(6) Högbom, M.; Galander, M.; Andersson, M.; Kolberg, M.; Hofbauer, W.; Lassman, G.; Nordlund, P.; Lendzian, F. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 3209.
(7) Stubbe, J.; Nocera, D. G.; Yee, C. S.; Chang, M. C. Y. *Chem. Rev.* **2003**, *103*, 2167.
(8) Seyedsayamdost, M. R.; Stubbe, J. *J. Am. Chem. Soc.* **2006**, *128*, 2522.
(9) Seyedsayamdost, M. R.; Xie, J.; Chan, C. T.; Schultz, P. G.; Stubbe, J. *J. Am. Chem. Soc.* **2007**, in press.
(10) Ge, J.; Yu, G.; Ator, M. A.; Stubbe, J. *Biochemisty* **2003**, *42*, 10071.
(11) (a) Schiemann, O.; Prisner, T. F. *Q. Rev. Biophys.* **2007**, *40*, 1. (b) Milov, A. D.; Maryasov, A. G.; Tsvetkov, Y. D. *Appl. Magn. Reson.* **1998**, *15*, 107.
(12) With the radical traps DOPA-$\beta2$ (ref 8), NH_2Y-$\alpha2$s (ref 9), and N_3NDP (ref 18), consumption of $Y_{122}\bullet$ and formation of a new radical occur in only one $\alpha\beta$ pair. There is no radical transfer in the second $\alpha\beta$ pair; thus, the $Y_{122}\bullet$ in this pair remains.
(13) See refs 8 and 9 and references therein.
(14) Note that the kinetics of DOPA$\bullet$/$NH_2Y\bullet$ formation and its requirement for NDP/(d)NTP implicate pathway dependence. This issue will be further addressed in another study.
(15) Bennati, M.; Weber, A.; Antonic, J.; Perlstein, D. L.; Robblee, J. H.; Stubbe, J. *J. Am. Chem. Soc.* **2003**, *125*, 14988.
(16) With DOPA-$\beta2$, only 4—8% of the total population contained 2 $Y_{122}\bullet$/ dimer resulting in a diminished λ in the DEER trace (see Figure 2).
(17) (a) Pannier, M.; Veit, S.; Godt, A.; Jeschke, G.; Spiess, H. W. *J. Magn. Reson.* **2000**, *142*, 331. (b) Jeschke, G.; Bender, A.; Paulsen, H.; Zimmermann, H.; Godt, A. *J. Magn. Reson.* **2004**, *169*, 1.
(18) Bennati, M.; Robblee, J. H.; Mugnaini, V.; Stubbe, J.; Freed, J. H.; Borbat, P. *J. Am. Chem. Soc.* **2005**, *127*, 15014.
(19) Bode, B. E.; Margraf, D.; Plackmeyer, J.; Durner, G.; Prisner, T. F.; Schiemann, O. *J. Am. Chem. Soc.* **2007**, *129*, 6736.
(20) Chiang, J. W.; Borbat, P. P.; Freed, J. H. *J. Magn. Reson.* **2005**, *172*, 279.
(21) Note that the half-peak widths in the distance distributions have been indicated as the error in the PELDOR measurements.
(22) The λ values for DOPA-$\beta2$ and wt $\beta2$ are 0.07 ± 0.01 and 0.30 ± 0.01, respectively. Due to the instability of DOPA$\bullet$/$NH_2Y\bullet$, λ is reduced after the reaction.
(23) The basis for residual $Y_{122}\bullet$—$Y_{122}\bullet$ is related to differences in the formation rate constant and stability of $NH_2Y\bullet$ at residue 730 vs 731.
(24) Seyedsayamdost, M. R.; Stubbe, J. *J. Am. Chem. Soc.* **2007**, *129*, 2226.
(25) Denysenkov, V. P.; Prisner, T. F.; Stubbe, J.; Bennati, M. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 13386.

JA076459B