ENDOR spectroscopy and DFT calculations: evidence for the hydrogen bond network within α2 in the PCET of E. coli ribonucleotide reductase

Supporting Information

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Figure S1. DFT model 1 with two waters with $g_x = 2.0049$, $g_y = 2.0041$ and $g_z = 2.0018$.

Figure S2. Alternative DFT model 2 with two waters with $g_x = 2.0048$, $g_y = 2.0038$ and $g_z = 2.0018$. 
**Figure S3.** Alternative DFT model 3 with two waters with $g_x = 2.0055$, $g_y = 2.0041$ and $g_z = 2.0019$.

**Fig. S4.** Alternative DFT model 5 with one water but shorter HO-H O-NH$_2$Y$_{730}$ distance (1.9 Å) with $g_x = 2.0048$, $g_y = 2.0040$ and $g_z = 2.0018$. 
Figure S5. DFT model 6 without water (see Table 1 for g-values).

Figure S6. (a) Cluster model for model 1. The residues involved in the PCET (NH$_2$Y$_{730}$*, Y$_{731}$ and C$_{439}$) and the water molecules are shown in stick representation, the remaining residues are shown in the wire representation.
Influence of second sphere residues on hydrogen bond interactions to NH$_2$Y$_{730}^\bullet$

The g-tensor calculations on model systems of different size and composition (see Table 2) show a substantial effect of the second sphere residues on the g$_x$-value (compare entries 2 and 4). Analyzing the structural properties it can be seen that, when removing these outer residues, the hydrogen bond between the central phenoxy group and WAT1 shrinks significantly by about 0.4 Å (from 2.16 Å in entry 2 to 1.78 Å in entry 4). This suggests that the influence of the surrounding residues on the calculated g-tensor can be rationalized by their steric influence on the hydrogen bonds to the oxygen of NH$_2$Y$_{730}^\bullet$. In order to analyse this further we additionally calculated the g-values as a function of the WAT1-NH$_2$Y$_{730}^\bullet$ hydrogen bond.
distance using the small model shown in Table 2, entry 4. The results of this calculation are shown in Figure S7. It can be seen that the gₓ-value is a steep function of the hydrogen bond distance. At a short hydrogen bond length of 1.8 Å (as in the small model, entry 4 in Table 2) we have a similar gₓ-value as in the small model, at a hydrogen bond distance of 2.2 Å (as in the large model, Table 2, entry 2) the gₓ-value of the large model is reached. At an even longer hydrogen bond distance of 3.0 Å the “water-free limit” of the gₓ-value is reached – as in the small model without WAT1 (Table 2, entry 5) and in the large model without WAT1 (Table 2, entry 3). Summing up these results it can be concluded that the gₓ-value is a sensitive function of the WAT1-NH₂Y₇₃₀• hydrogen bond distance and at the same time the strength of this hydrogen bond is strongly influenced by the interaction of WAT1 with the surrounding second sphere residues, i.e. the effect of the second sphere residues on the gₓ-value is of steric nature, modulating the strength of the WAT1-NH₂Y₇₃₀• hydrogen bond, and herewith influencing the gₓ-value.

**Figure S7.** gₓ value as a function of the hydrogen bond distance between Y₇₃₀ and WAT1. A relaxed surface scan was carried out with model NH₂Y₇₃₀•-Y₇₃₁-C₄₃-WAT1 (Table 2, entry 4) constraining the distance between O(Y₇₃₀) and H(WAT1).
Figure S8. Overlaying structures 2X0X (red, X-ray resolution 2.3 Å), 2XO4 (blue, X-ray resolution 2.5 Å) and 4R1R (green, X-ray resolution 3.2 Å) (10, 32, 33). Only the residues used in model 7 (Figure S6) were used for the overlaying procedure and are displayed. In the overlay procedure the $C_\alpha$ atoms of 2XO4 and 4R1R are fitted onto the respective $C_\alpha$ atoms of 2X0X in such a way, that the RMS deviation of their distances is smallest using the “Pair Fitting” method in PyMOL (Version 1.4.1). The RMS deviation of the pairwise $C_\alpha$ atom distances is a measure for the similarity of the backbone structures. Note that GDP and WAT1 are not used in the overlay procedure and in the calculation of the RMS deviation. The RMS deviation of 2XO4 to 2X0X is 0.106 Å and the RMS deviation of 4R1R to 2X0X is 0.201 Å. All three structures are thus quite similar regarding their backbones. The visualized overlay shows, that also the side chain orientations are similar. Only the side chain of C225 in 4R1R shows small rearrangements compared to 2X0X and 2XO4, which is due to the binding of the substrate.
Figure S9.  (a) Spin density plot (isocontour surfaces for $+0.005\,\text{Å}^3$ and $-0.005\,\text{Å}^3$ are shown as blue and red surface representations) for the $Y_{731}\leftrightarrow Y_{730}$ radical transfer transition state structure. Model 7 (Figure S6b) was used for the calculations. For better visualization only the PCET residues are shown.

(b) Spin density plot (isocontour surfaces for $+0.005\,\text{Å}^3$ and $-0.005\,\text{Å}^3$ are shown as blue and red surface representations) for the $Y_{730}\leftrightarrow C_{439}$ radical transfer transition state structure. Model 7 (Figure S6b) was used for the calculations. For better visualization only the PCET residues are shown.