SUPPORTING INFORMATION

Two Distinct Mechanisms of Inactivation of the Class Ic Ribonucleotide Reductase from *Chlamydia trachomatis* by Hydroxyurea: Implications for the Protein Gating of Inter-subunit Electron Transfer

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MATERIALS AND METHODS

Construction of Vectors to Over-express His_6-$\Delta(1\text{-}248)\alpha_2$ Variants Y991F, Y990F and C672S. The substitutions were introduced by the polymerase chain reaction (PCR) with the pET-28a-*Ct*R1-Δ(1-248)-wt as the template (*1*). The primers and restriction enzymes are listed in Table 1. In each case, the gene was amplified in two fragments by PCR. Fragment 1 was amplified by using global primer **A** and specific primer **1**. Fragment 2 was amplified by using specific primer **2** and global primer **B**. After PCR, both fragments were purified by gel electrophoresis, extracted from the gel by using the Qiagen (Valencia, CA) Qiaquick system as instructed by the manufacturer, and restricted with the appropriate enzymes according to the primers. The products were re-purified and ligated in a single step with pET28a that had previously been restricted with *Nde*I and *Xho*I and gel-purified. The ligation reaction was used to transform BL21(DE3) cells to generate the over-producing strain. The sequence of the entire coding region of vector was verified by ACGT, Inc. (Wheeling, IL.).

TABLE

Table 1: Primers and restriction enzymes used for the construction of vectors to over-express His_6-Δ(1-248)α_2 variants Y991F, Y990F, and C672S.

	Primers (underline: mutation; **bold**: restriction site)	enzyme
global	A: GGT TCT ACC TAT **CAT ATG** ACG CAT TCG CAG TTG TTG G	*Nde*I
	B: GTG GTG GTG GTG **CTC GAG** TAA TTA CTG ACA TGC TTC AC	*Xho*I
Y991F	1: CGT TGC TGA TGA AC**T TCG AA**G AAA ATA CGT AGT TTT C	*BstB*I
	2: CTA CGT ATT TTC **TTC GAA** GTT CAT CAG CAA CGA CCG	
Y990F	1: CGT TGC TGA TGA AC**T TCG AA**G ATA AAA CGT AGT TTT C	*BstB*I
	2: CTA CGT TTT ATC **TTC GAA** GTT CAT CAG CAA CGA CCG	
C672S	1: GCA GTT TAA CAA AAT CTC CGT A**CT TAA G**TT GGA ACA ACG C	*Afl*II
	2: GCG TTG TTC CAA **CTT AAG** TAC GGA GAT TTT GTT AAA CTG C	

FIGURES

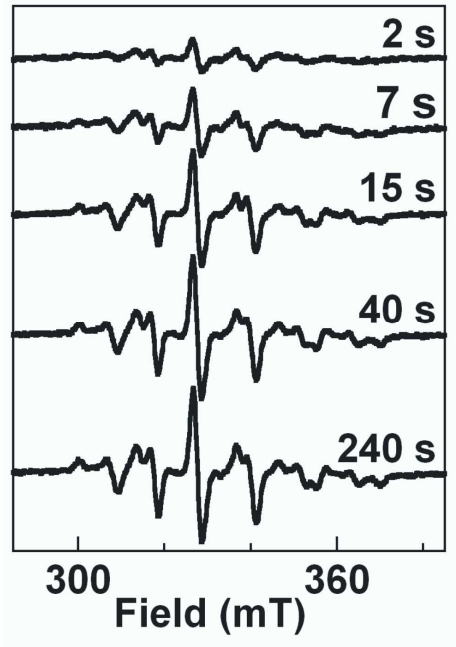

Figure S1: X-band EPR spectra defining the kinetics of formation of the homogeneous Mn^{III}/Fe^{III}-β_2 complex in the presence of His_6-$\Delta(1\text{-}248)\alpha_2$, CDP, and ATP. Samples were freeze-quenched at the indicated reaction times under the turnover conditions indicated in the legend to Figure 3A. The spectrum of a sample frozen at 20 ms was subtracted from the experimental spectrum of each sample to generate the spectra shown. Spectrometer conditions: temperature, (14.0 ± 0.2) K; frequency, 9.45 GHz; power, 200 μW; modulation frequency, 100 kHz; modulation amplitude, 1 mT; scan time, 167 s; time constant, 167 ms.

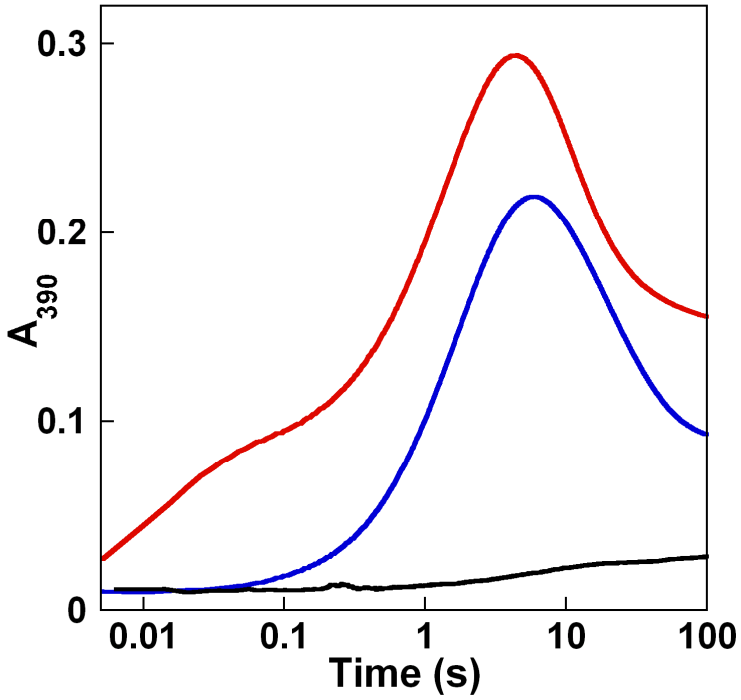

Figure S2: Absorbance-versus-time traces (at 390 nm) from the reaction between H_2O_2 and various forms of Mn/Fe-β_2. The red and blue lines are from reaction of the Mn^{II}/Fe^{II} and Mn^{III}/Fe^{III} forms, respectively, which we reported previously (*2*). The black line was acquired after mixing a solution of H_2O_2 (100 mM) with an equal volume of a solution prepared by prior treatment of Mn^{IV}/Fe^{III}-β_2 (0.40 mM) with 100 mM HU for 2 hours at ambient temperature. All reactions were carried out at 5 °C.

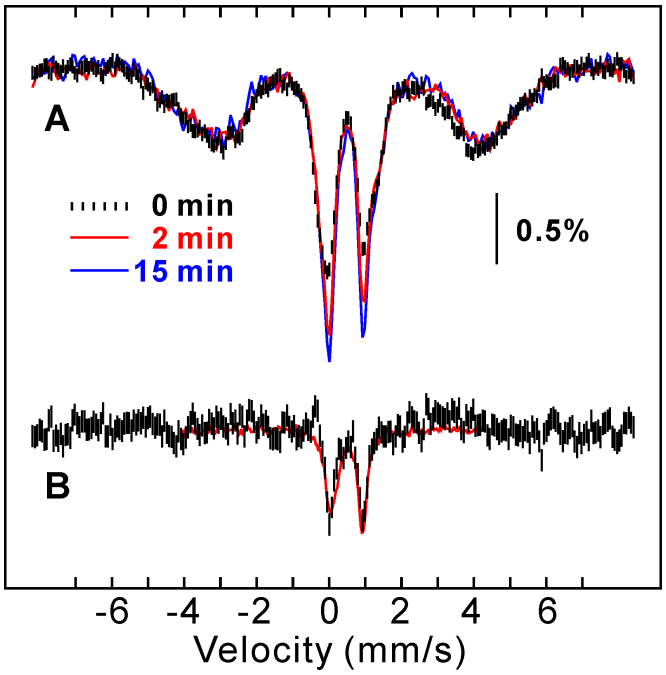

Figure S3: 4.2-K/53-mT Mössbauer spectra of samples prepared by treating Mn^{III}/Fe^{III}-β_2 with dissolved NO. (**A**) Mn^{III}/Fe^{III}-β_2 was prepared by treatment of a solution of Mn^{IV}/Fe^{III}-β_2 (2.3 mM monomer) with sodium dithionite (1.5 equiv per β) for 1 h at ambient temperature. The solution was subsequently exposed to air to remove residual dithionite and then deoxygenated again. The spectrum of this sample is shown as black vertical bars in **A**. An aliquot of an NO-saturated buffer solution was added (1 equiv. NO per Mn/Fe cluster), and the solution was incubated for 2 min or 15 min before freezing. The spectra of these samples are shown in **A** as red and blue lines, respectively. (**B**) The difference between the spectra of the sample reacted with NO for 15 min and the starting Mn^{III}/Fe^{III}-β_2 is shown as black vertical bars. The overlaid red solid line is the reference spectrum of the novel Fe^{III}-containing diamagnetic species, as described in the text. It is plotted at an intensity corresponding to 11% of the total absorption of the experimental

spectrum of the 15 min NO sample.

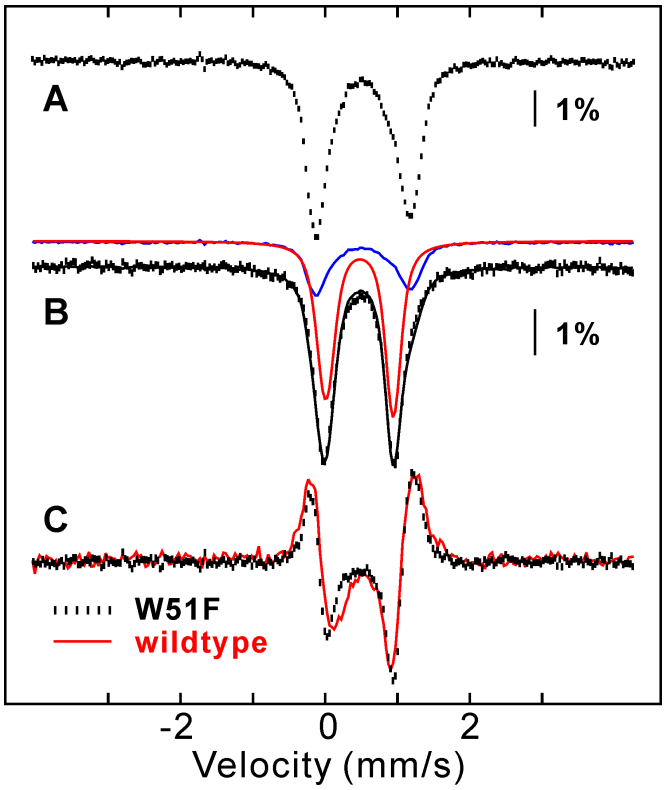

Figure S4: 4.2-K/zero-field Mössbauer spectra monitoring the reaction of Mn^{IV}/Fe^{III}-β_2-W51F with HU. **A**: The spectrum of a sample of Mn^{IV}/Fe^{III}-β_2-W51F (3 mM β monomer). **B**: The spectrum after treatment of the same sample with 50 mM HU for 40 min at ambient temperature. The blue line is a simulation of the spectrum of the Mn^{IV}/Fe^{III} cluster (28% of the total intensity) using the parameters quoted in the manuscript. The red line is a simulation of the spectrum of the new, Fe^{III}-containing, diamagnetic species (85% of the total intensity) with δ = 0.48 mm/s and ΔE_Q = 0.93 mm/s. The black solid line overlaid with the experimental data is the sum of the two theoretical sub-spectra. **C**: The change is illustrated by the difference spectrum **B** – **A** (black hashed vertical bars). The red line is the corresponding difference spectrum obtained for wild type β_2. It was generated from spectra shown in Figure 3B in the main manuscript.

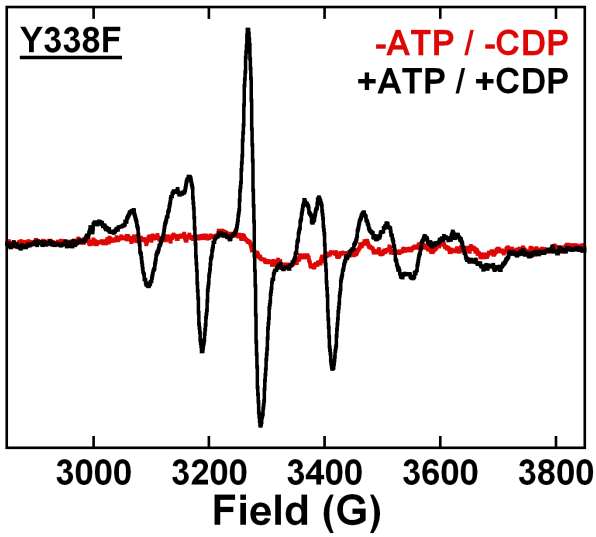

Figure S5: X-band EPR spectra showing that reduction of Mn^{IV}/Fe^{III}- β_2–Y338F to the Mn^{IV}/Fe^{III} complex by hydroxyurea (HU) requires substrate and/or allosteric effector. Both samples contained 150 μM β_2-Y338F (0.75 equiv Mn^{IV}/Fe^{III} per β), 600 μM His_6-Δ(1-248)α_2, and 10 mM DTT. The presence (black) and absence (red) of nucleotides, including 1 mM ATP and 4 mM CDP, are indicated on the figure. These components were quickly pre-mixed, and 20 mM HU was then added. Samples were incubated for 5 min at ambient temperature before freezing. Spectrometer conditions: temperature, (14.0 ± 0.2) K; frequency, 9.45 GHz; power, 200 μW; modulation frequency, 100 kHz; modulation amplitude, 1 mT; scan time, 167 s; time constant, 167 ms.

REFERENCES

1. Jiang, W., Yun, D., Saleh, L., Barr, E. W., Xing, G., Hoffart, L. M., Maslak, M.-A., Krebs, C., and Bollinger, J. M., Jr. (2007) A manganese(IV)/iron(III) cofactor in *Chlamydia trachomatis* ribonucleotide reductase, *Science* 316, 1188-1191.

2. Jiang, W., Xie, J., Nørgaard, H., Bollinger, J. M., Jr., and Krebs, C. (2008) Rapid and quantitative activation of *Chlamydia trachomatis* ribonucleotide reductase by hydrogen peroxide, *Biochemistry 47*, 4477-4483.