SUPPOR TING INFORMATION

SPECTROSCOPIC STUDIES ON THE [4Fe-4S] CLUSTER IN ADENOSINE 5′-PHOSPHOSULFATE REDUCTASE FROM MYCOBACTERIUM TUBERCULOSIS

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Running head: FeS Cluster Function in Assimilatory APS Reductase

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Fig. 1. Structure based sequence alignment of 17 APS reductases from prokaryotes. The ClustalW Multiple Sequence Alignment program was used. The bar graph indicates the degree of conservation per position. Strictly conserved residues are outlined in red, red letters indicate conserved residues and conserved regions are boxed in blue. Alignment pictures were rendered with the server ESPript 2.2 (http://espript.ibcp.fr).

Supplementary Fig. 2. UV-vis absorption spectra of MtAPR. UV-vis absorption of 10μM MtAPR in 50 mM Tris–HCl, 150 mM NaCl (pH 8.5 at 4 °C) and 10% glycerol, before (—) and after (••••) the addition of 2-fold stoichiometric excess of APS. Inset shows the corresponding difference spectrum resulting from complex formation between MtAPR and APS. The difference spectrum is the spectrum of the mixture minus spectrum of enzyme alone.

Supplementary Fig. 3. 4.2-K/53-mT Mössbauer spectra of 1 mM MtAPR in the absence (vertical bars) or presence of 2 mM APS (solid line).

Supplementary Fig. 4. Rate and equilibrium constants for MtAPR. A. Single-turnover reduction of APS by Lys144Ala MtAPR performed under subsaturating conditions as described in Materials and Methods. Single-turnover reaction to measure $k_{cat}/K_m$ was performed with 5 nM wild-type MtAPR or 20 μM Lys144Ala MtAPR and 0.25 nM APS. B. Single-turnover reduction of APS by wild-type (filled circles) and Lys144Ala (filled triangles) MtAPR performed under saturating conditions as described in Materials and Methods. Single-turnover reactions to measure $k_{max}$ were performed with 150 μM enzyme and 50 μM APS. C. APS binding to Lys144Ala MtAPR measured by ultrafiltration as described in Materials and Methods.

Supplementary Fig. 5. EPR spectra of radiolytically cryoreduced 250 μM MtAPR in the absence (red) or pre-incubated with 1 mM APS (blue) as described in Materials and Methods. Conditions: temperature, 77 K; microwave frequency, 9.45 GHz; microwave power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 10 G; scan time, 167 s; time constant, 167 ms. Asterisks indicate the position of the intense signal at $g = 2$ emanating from free radicals generated during cryoreduction and the position of the peaks of hydrogen atoms formed during cryoreduction.

Supplementary Fig. 6. 4.2-K/53-mT Mössbauer spectra of 250 μM MtAPR in the absence (top) or pre-incubated with 1 mM APS (bottom) after γ-irradiation (vertical bars) as described in Materials and Methods. In these experiments, 50% of the starting material is unchanged (red solid line). Spectral
changes that result from different orientations of the externally applied 53-mT magnetic field are highlighted by the black and blue arrows.

Supplementary Fig. 7. EPR spectra of oxidized wild-type MtAPR. A. Spectra of 250 µM MtAPR treated with a stoichiometric amount of ferricyanide in buffer containing 50 mM Tris–HCl, 150 mM NaCl (pH 8.5 at 4 °C) and 10% glycerol. B. Expanded version of panel A around $g = 2$. Prior to freezing, samples with protein were incubated with ferricyanide for 5 min at rt. Unreacted potassium ferricyanide is present in the sample. However, controls reactions carried out in the absence of protein indicate that it does not contribute to features in the spectrum. Conditions: temperature, 10 K; microwave frequency, 9.38 GHz; microwave power, 10 mW.
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4

A

Fraction P

0.5

0.4

0.3

0.2

0.1

0

Time (min)

0

10

20

30

40

K144A

B

Fraction P

1

0.8

0.6

0.4

0.2

0

Time (min)

0

10

20

30

40

50

WT

K144A

C

E/A_{total}

0.5

0.4

0.3

0.2

0.1

0

K144A (μM)

0

20

40

60

80

100
Supplementary Figure 5
Supplementary Figure 6
