Electronic Supplementary Information

Probing the role of the bridging C509 between [Fe₄S₄] cubane and [Ni₅Ni₃] centre in the A-Cluster of Acetyl-CoA Synthase

Yi Liua, Xiaofei Zhu a, Feng Wangle, Tianlei Ying a, Pingwei Li c, Zhongxian Huanga and Xiangshi Tan a *

a Department of Chemistry & Institutes of Biomedical Sciences, Fudan University, Shanghai 200433, China. E-mail: xstan@fudan.edu.cn; Fax: +86-21-65641740; Tel: +86-21-55664475
b Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
c Department of Biochemistry & Biophysics, Texas A & M University, College Station, TX 77843 (USA)

Experimental Procedure

Materials: Restriction enzymes were purchased from New England Biolabs. The pQE-60 expression vector was purchased from Novagen. Moorella thermoacetica strain 39073 was purchased from ATCC. E. coli strain JM109 was from Agilent Technologies. Pfu Turbo polymerase and E. coli strain XL1-Blue were from Stratagene. Platinum Pfx DNA polymerase was purchased from Invitrogen. T4 DNA ligase, Wizard® Genomic DNA Purification Kit and Wizard® Plus SV Mini-Prep DNA Purification System were from Promega. Buffers, purification reagents and other chemicals were purchased from Sigma-Aldrich, unless otherwise stated. The oligonucleotide primer pairs below, labeled P1-P9, were synthesized and sequenced by Shanghai Invitrogen Biotech Co. Ltd.

P1 [5’-GGCGGACATGTCTGATTTTGATAAAATC-3’ (F), 5’-GGCGGAGATCTCATAATGGGATCCATGG-3’ (R)]
P2 [5’-CTCCTGGCTCTCGCAGTCGCTTTGCCC-3’ (F), 5’-GGGCAAAGGACTGGGGAGGACGCAGGAG-3’ (R)]
P3 [5’-CTCCTGGCTCTCCACCAGTCGCTTTGCCC-3’ (F), 5’-GCAAAGGACTGGGGAGGACGCAGGAGA-3’ (R)]
P4 [5’-CTCCTGGCTCTCAGTCTGCCAGTCGCTTTGCCC-3’ (F), 5’-GGGCAAAGGACTGGGGAGGACGCAGGAG-3’ (R)]
5’-GCAAAGGACTGGGAGAGGACGCAGGAG-3’(R) 
P5 [5’-CTCCTGCGTCCTCGTCCAGTCCTTTGC-3’ (F),
5’-GCAAAGGACTGGGAGAGGACGCAGGAG-3’(R)]
P6 [5’-CCTCGCCCAGGCCTTTGCCCCCAACGCTGTTTGTTATTTG-3’ (F),
5’-CAATACACACAGCGTTGGGGGCAAAGGCCCTGGGCGAGG-3’(R)]

**Mutagenesis and cloning of acsB:** Genomic DNA of *Moorella thermoacetica* was isolated using the DNA isolation kit as described. Gene *acsB* encoding ACS-α subunit was cloned using the genomic DNA with primer pair P1 and the plasmid pLHK05 was isolated as reported. Site-directed mutagenesis of *acsB* was performed using the QuikChange™ Site-Directed Mutagenesis Kit (Strategene) with plasmid pLHK05 as template. Genes C509A, C509H, C509S, C509V were constructed similarly, using primers P2, P3, P4, and P5 respectively. Mutant gene Δbridge (C509A/H516A/S511A) was constructed using the P6 primer pair with C509A as template. In each case, the resulting PCR products were transformed into *E. coli* XL-1 Blue cells (Strategene). Transformed cells were selected on ampicillin plates. The cloned regions of the plasmids were sequenced (Shanghai Invitrogen Biotech Co. Ltd) to verify the fidelity of the PCR reactions; Proteins corresponding to the genes above will be referred to C509A, C509H, C509S, C509V, Δbridge, respectively.

**Protein Purification:** *E. coli* strain JM109 cells were transformed with each plasmid; cells were grown in 10 L of Begg's media at 37°C under anaerobic conditions, induced with 0.5 mM IPTG, harvested anaerobically and then frozen in liquid N₂. All protein purifications and manipulations were performed in an inert atmosphere (N₂) glove box (MBraun). Cells were thawed, suspended in anaerobic Buffer A (50 mM Tris pH 8.0 plus 1 mM sodium dithionite), and sonicated as described. The supernatant fraction of the cell extract was loaded onto a Ni-NTA agarose column (2.6 x 15 cm) that had been equilibrated in Buffer A. After washing with 5 column volumes of buffer A containing 10 mM imidazole, the protein was eluted using Buffer A plus 100 mM imidazole. Combined fractions were concentrated and passed through a column of Sephadex-G25 equilibrated in 50 mM Tris-HCl pH 8.0. The resulting proteins were found to be at least 90% pure based on the densitometry of denaturing polyacrylamide gels stained with Coomasssie Brilliant Blue. Aliquots were frozen in liquid N₂. For
Ni-activation, protein aliquots in 50 mM Tris-HCl buffer pH 8.0 were incubated with 4 molar equivalents of NiCl₂ per mol α protein, and then subjected to a column of Sephadex G-25 (equilibrated in Buffer A) to remove excessive NiCl₂.\textsuperscript{S2,S3}

\textit{M. thermoacetica} cells were grown in a 10 L bioreactor and harvested as described.\textsuperscript{S4,S5} The Corrinoid protein (CoFeSP) and methyltransferase (MeTr) from \textit{M. thermoacetica} cells were purified as reported.\textsuperscript{S4,S5} Ti(III) citrate, Co\textsuperscript{3+}FeSP and methylated Co\textsuperscript{3+}FeSP were prepared as described.\textsuperscript{S4} Protein concentrations were determined by the Bradford method.\textsuperscript{S6}

\textbf{Characterization of Proteins}: Proteins were activated with NiCl₂ and then subjected to a PD10 column to remove excessive nickel ions. The metal content of proteins was determined by overnight digestion in 1M HNO₃ (Trace Metal Grade). Digested samples were diluted to 0.2 M HNO₃ for analysis with a thermo X7 ICPMS (Thermo Electron Corp., USA). UV/Vis absorption spectra were recorded using a HP8453 (Hewlett-Packard, Palo Alto, CA, USA) UV/vis spectrophotometer. Pre-steady-state stopped-flow experiments were performed at 25°C with an SF-61 DX2 Double-Mixing Stopped-Flow instrument (Hi-Tech Limited, UK) installed in a glove box (Braun, MB100 with an O₂ analyzer to assure that the O₂ concentration was < 1 ppm). Methyl group transfer was monitored at 390 nm.

\textbf{Kinetics of [Fe₄S₄]²⁺ Reduction}: The stopped-flow kinetics of reduction in variants, including Δ bridge, C509A, C509V and C509S, were preformed as described.\textsuperscript{S4} The reduction reaction was monitored at 420 nm and the results were shown as in SF1.
**SF1.** Reduction of ACS variants with Ti-citrate (1.0 mM) in Tris-HCl pH 8.0. Concentrations of variants were 40 μM for each before mixing.
References


