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Probing the role of the bridging C509 between the $[Fe_4S_4]$ cubane and the $[Ni_pNi_d]$ centre in the A-cluster of acetyl-coenzyme A synthase†

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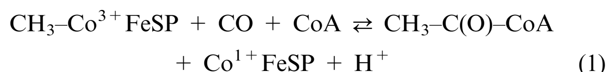
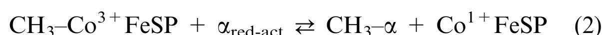
The A-cluster of acetyl-coenzyme A synthase consists of an $[Fe_4S_4]$ cubane bridged to a $[Ni_pNi_d]$ centre *via* C509 cysteinate. The bridging cysteinate, which could be substituted by histidine imidazole, mediates "communication" between the $[Fe_4S_4]$ cubane and the $[Ni_pNi_d]$ centre during the synthesis of acetyl-coenzyme A.

Metalloproteins are becoming a new focus, and the exciting work concerning their intriguing issues have recently been highlighted in *Nature*.[1] The metalloprotein acetyl-coenzyme A synthase (ACS)/carbon monoxide dehydrogenase (CODH) is a bifunctional metalloenzyme found in anaerobic archaea and bacteria that grow hemoautotrophically on CO or CO_2, and is significant for biological carbon fixation and understanding the origin of life.[2] The ACS/CODH from *Moorella thermoacetica* is an $\alpha_2\beta_2$ tetramer containing seven metal clusters connected by a molecular tunnel network.[3] The active site A-cluster, which is located in the α subunit of ACS, consists of an $[Fe_4S_4]$ cluster coordinated through a bridging cysteinate to a dinuclear nickel sub-component (Fig. 1A) and catalyzes the synthesis of acetyl-coenzyme A from CO, coenzyme A (CoA) and a methyl group donated from a corrinoid-iron sulfur protein (CoFeSP), in accordance with reaction (1):[3b,c]



$$CH_3\text{–}Co^{3+}FeSP + CO + CoA \rightleftarrows CH_3\text{–}C(O)\text{–}CoA + Co^{1+}FeSP + H^+ \quad (1)$$

During catalysis of ACS, the substrates, CO and methyl group are thought to randomly bind to the proximal nickel site (Ni_p), and nucleophilic attack by CoA on the resulting $CH_3C(O)\text{–}Ni_p$ group yields acetyl-CoA.[2b,5] Once reduced by Ti^{3+}-citrate or dithionite, the Ni-activated recombinant α subunit can accept a methyl group by reaction (2):[4]



$$CH_3\text{–}Co^{3+}FeSP + \alpha_{red\text{-}act} \rightleftarrows CH_3\text{–}\alpha + Co^{1+}FeSP \quad (2)$$

However, before activation with $NiCl_2$, the recombinant apo-α subunit without Ni_p cannot accept a methyl group, indicating that the methyl group has been transferred to the Ni_p of the A-cluster. Therefore, this methyl transfer reaction has been used as a reporter for acetyl coenzyme A synthase activity.[4]

In the last ten years, great progress concerning the structure, function and kinetic mechanism of metalloenzyme ACS/CODH has been made,[2–6] and several state forms of the A-cluster have been identified or proposed to understand the reductive activation of the A-cluster ($A_{red\text{-}act}$).[7] However, the roles of the sub-components of the active site of the A-cluster remain elusive. The Ni_p^{2+} ion is proposed to be reduced to either the Ni_p^{1+} or Ni_p^{0} oxidation state prior to accepting the methyl group cation from the CH_3–CoFeSP.[7d,e] The $[Fe_4S_4]^{2+}$ cubane may be a depository for one of the electrons used in the reductive activation, generating the $[Fe_4S_4]^{1+}$ state, but it may alternatively serve a non-redox function to stabilize Ni_p and modulate the reduction potential(s) involving it. Determining the catalytic $A_{red\text{-}act}$ electronic configuration and assigning the specific function of each sub-component of the A-cluster are exceedingly difficult due to problems surrounding the activation of the enzyme and its inherent heterogeneity.[7a,8] For instance, pinning down the role of the bridging C509 is one of the key issues.

The objective of this study is to probe the specific role of the bridging C509 of the A-cluster in ACS by genetic and biochemical/biophysical approaches. To evaluate the role of the bridging C509, four single-substitution mutants of wild type ACS were constructed, in which the bridging C509 was replaced by Ala, His, Ser and Val (C509A, C509H, C509S and C509V), respectively. A triple-substitution mutant (C509A/H516A/S511A, called Δbridge), in which C509 and two nearby potential bridging residues (H516 and S511) were replaced with alanines, was also prepared. The relevant properties of

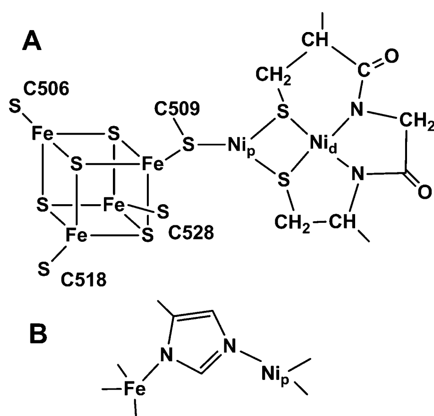


Fig. 1 (A) The structure of the A-cluster and (B) a suggested coordination model of the imidazole bridge in C509H.

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Table 1 Metal analysis and stopped-flow kinetic parameters of ACS proteins[a]

	[Ni]/[α]	[Fe]/[α]	k_{red}/S^{-1}	k_{meth}/S^{-1}	$[CH_3]/[α]$
WT	2.3	4.1	0.025	16	0.35
C509H	2.1	3.8	0.017	10	0.25
C509A	2.2	3.7	0.010	0.06	0.10
C509S	2.4	4.0	0.030	0	0
C509V	1.8	3.7	0.047	0	0
Δbridge	2.1	3.6	0.010	0	0

[a] All data represent average values (mol mol^{-1} α). The Ni content refers to that after Ni activation. k_{red} (S^{-1}) indicates the $[Fe_4S_4]$ reduction rate constant of various ACS proteins (40 μM) mixed with Ti^{3+}-citrate (1.0 mM) in Tris-HCl (pH 8.0). k_{meth} (S^{-1}) and $[CH_3]/[α]$[4a,c] represent methyl transfer rate constants and methyl groups transferred per ACS protein, respectively.

these variants were evaluated and interpreted to provide new insights into the respective functions of the sub-components in the A-cluster. This study also revealed how the three sub-components of the A-cluster work together to conduct the function of ACS.

All ACS variants prepared in this investigation were activated by treatment with $NiCl_2$, and then subsequently passed through a gel filtration column to remove excess $NiCl_2$. Metal analysis indicated that each mutant contained ~2 Ni ions per molecule (Table 1). This is consistent with the presence of the $[Ni_pNi_d]$ sub-site in these mutants. All brown proteins of ACS (C509H, C509S, C509V, C509A and Δbridges) contained ~4 Fe per molecule, respectively, suggesting that the Δbridge and C509 mutants (C509H, C509S, C509V and C509A) contained an $[Fe_4S_4]$ cubane. These Fe-containing variants exhibited a broad absorption at ~420 nm, a characteristic feature of $[Fe_4S_4]^{2+}$ clusters. Treatment of these variants with dithionite or Ti^3-citrate bleached this absorption at ~420 nm to generate the $[Fe_4S_4]^{1+}$ state with different rate constants (Fig. 2, SF1†, Table 1).

The stopped-flow method was applied to evaluate the activity of ACS variants in accepting a methyl group from the CH_3–Co^{3+}FeSP.[4] All Ni-activated and Ti^{3+}-citrate-reduced ACS variants were reacted with CH_3–Co^{3+}FeSP, and the formation of the Co^{1+}FeSP product was monitored at 390 nm.[4] To confirm whether the methyl group had been transferred to the active site Ni_p ion or not, control experiments were performed, in which the apo-ACS before Ni activation was treated with Ti^{3+}-citrate and then reacted with CH_3–Co^{3+}FeSP. None of the control experiments exhibited a detectable methyl transfer activity. Ni-activated and

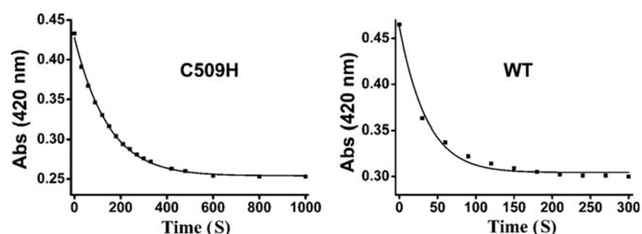

Fig. 2 Reduction of WT ACS and mutant C509H with 5 mM dithionite. The concentration of the proteins were each 80 μM.

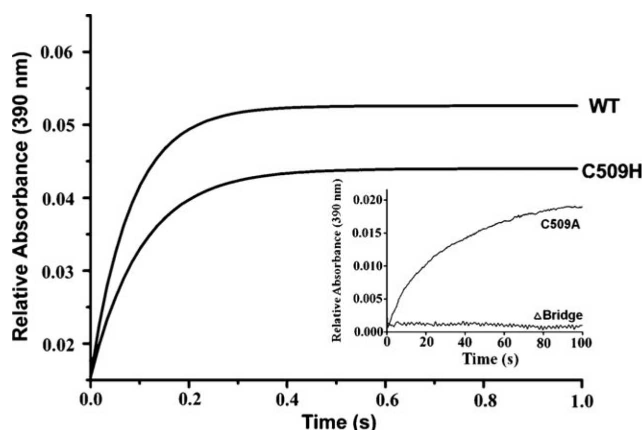

Fig. 3 The stopped-flow reaction of CH_3–Co^{3+}FeSP (10 μM) mixed with various variant proteins (20 μM/[α]) in 50 mM Tris-HCl buffer (pH 8.0) monitored at 390 nm. All α proteins and CH_3–Co^{3+}FeSP were pre-incubated with Ti^{3+}-citrate (1.0 mM) for 30 min before mixing. The concentration of the reductant and proteins are those before mixing in stopped-flow. The recombinant α protein exhibits only a 30–50% catalytic activity due to its inherent heterogeneity.[7a,8]

Ti^{3+}-reduced recombinant ACS accepted a methyl group (Fig. 3), similar to those reported in earlier studies.[4]

We then began to evaluate the role of the bridging C509 with the mutant C509A, in which the bridging C509 was replaced by alanine to interrupt the interaction between the $[Fe_4S_4]$ cubane and the $[Ni_pNi_d]$ centre. The C509A exhibited a 100-fold slower methyl transfer activity than that of wild-type (WT) ACS. This revealed that the C509 bridge is necessary and that interruption of the bridge dramatically influences the activity of the A-cluster. There is a possibility that nearby residues H516 or S511 (Fig. 4) could conceivably function as a bridge in the absence of C509, but somewhat altering the conformation of the sub-unit. The other possibility is that the cubane and Ni_p in C509A are sufficiently close together without a bridge.

In the open conformation of the α subunit in $ACS/CODH_{Mt}$, the distances between the imidazole group of H516 and Ni_p, and the Fe of the cubane coordinated to C509 are 6.0 and 4.5 Å, respectively. The distances between the O atom of S511 and these metals are 7.8 and 7.9 Å,

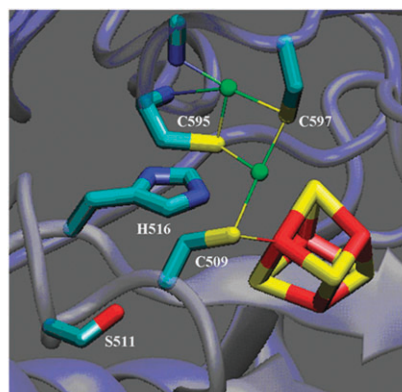

Fig. 4 The structure of wild-type α focused on the A-cluster region of ACS (drawn in VMD viewer). H516 or S511 may act as a surrogate bridge for C509.

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respectively. Moreover, both H516 and S511 residues are located on flexible loops (Fig. 4).

A kinetic study with the triple mutant Δbridge, in which C509, H516 and S511 were replaced with Ala, revealed that the Δbridge did not exhibit methyl group transfer activity. The results suggest that the interaction between the cubane and the di-nickel centre *via* the C509 bridge is required for methyl transfer activity and that H516 or S511 may act as a surrogate bridge in the absence of C509.

In addition, a series of mutants of ACS (C509S, C509H and C509V), in which the bridging Cys509 was replaced by Ser, His and Val, respectively, were investigated. C509S, in which the cysteinate bridge C509 might be replaced by a serine oxide, exhibited no detectable methyl transfer activity. Oxygen is a harder donor than sulfide, and the electronic coupling between the cubane and the di-nickel site may differ relative to sulfide. C509H can accept a methyl group from CH_3–Co^{3+}FeSP at a > 70% extent (calculated from $[CH_3]/[\alpha]$ values).[4a,c] The near-WT-level of methyl group transfer activity for C509H indicates that the di-nickel site is assembled well in this mutant, and strongly suggests that an imidazole group could bridge the di-nickel site to the cubane of the A-cluster. Therefore, the histidine that replaces the bridging cysteine 509 might function as a bridge, with one nitrogen of the imidazole ring coordinating to a cubane Fe and the other nitrogen coordinating to Ni_p, as shown in Fig. 1b. The steric effect of His and the electronic coupling efficiency mediated by the imidazole bridge might be the factors that influence its methyl transfer activity. Thus, we observed that the amplitude with the C509H mutant was about 22% lower than that found with WT ACS. Other possibilities could also exist: the histidine might also only coordinate Ni_p or the imidazole nitrogen coordinates neither the Fe nor Ni_p, or there is an exogenous (*e.g.* ^{-}OH) bridging ligand. However, in these two cases, one would expect that the mutant lacking a bridge (*e.g.* C509V) would exhibit the same methyl transfer activity as C509H, but they did not. This favours the speculation that H509 bridges the cubane and the di-nickel site. There are some examples of histidine coordination to $[Fe_4S_4]$ cubanes, including in [NiFe] hydrogenase and [FeFe] hydrogenase.[9]

As mentioned above, C509A showed a significantly diminished methyl transfer activity, but the mutant C509V exhibited no detectable methyl group transfer activity due to it lacking a bridging coordinating atom. Unlike Ala, Val is more bulky and has greater steric hindrance. Thus, it might also prevent the bridging candidates from serving as a bridge. The disruption of this linkage may alter the redox potential of the cubane and/or Ni_p. The absence of methyl transfer activity in C509V indicates that the cubane and the di-nickel site "communicate" with each other through a bridge to activate functionality. The result also supports the hypothesis that there is a candidate bridge in variant C509A. The absence of methyl transfer activity in C509S indicates that an O bridge is not sufficient for this communication.

In conclusion, the bridging cysteinate of C509, which is crucial for the methylation activity of ACS, could mediate an interaction between the $[Fe_4S_4]$ cubane and the $[Ni_pNi_d]$ sub-site, influencing the redox potential of both sub-components. The imidazole of histidine (but not the hydroxyl of serine) can replace the thiolate of C509 to bridge functionally the $[Fe_4S_4]$ and $[Ni_pNi_d]$ sub-components of the A-cluster. The three sub-components of the A-cluster form a co-operative unit to conduct the function of ACS. Detailed structural and mechanistic studies of C509A and C509H are under way in our laboratory.

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References

1 (*a*) J. C. Fontecilla-Camps, P. Amara, C. Cavazza, Y. Nicolet and A. Volbeda, *Nature*, 2009, **460**, 814–822; (*b*) Y. Lu, N. Yeung, N. Sieracki and N. M. Marshall, *Nature*, 2009, **460**, 855–862; (*c*) R. Lill, *Nature*, 2009, **460**, 831–838; (*d*) G. Schwarz, R. R. Mendel and M. W. Ribbe, *Nature*, 2009, **460**, 839–847; (*e*) B. Alsion and S. Moriah, *Nature*, 2009, **460**, 848–854; (*f*) K. J. Waldron, J. C. Rutherford, D. Ford and N. J. Robinson, *Nature*, 2009, **460**, 823–829.

2 (*a*) S. W. Ragsdale, *J. Inorg. Biochem.*, 2007, **101**, 1657; (*b*) P. A. Indahl and D. E. Graham, in *Metal Ions in Life Sciences*, ed. A. Sigel, H. Sigel and R. K. O. Sigel, John Wiley & Sons, Ltd., Chichester, vol. 2, 2007, pp. 357–416; (*c*) J. Seravalli and S. W. Ragsdale, *J. Biol. Chem.*, 2008, **283**, 8384–8394.

3 (*a*) T. I. Doukov, T. I. Iverson, J. Seravalli, S. W. Ragsdale and C. L. Drennan, *Science*, 2002, **298**, 567–572; (*b*) C. Darnault, A. Volbeda, E. J. Kim, P. Legrand, X. Vernède, P. A. Lindahl and J. C. Fontecilla-Camps, *Nat. Struct. Biol.*, 2003, **10**, 271–279; (*c*) T. Meins, B. Thiele, P. Romer, R. Huber and O. Meyer, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 446–451.

4 (*a*) X. Tan, C. Sewell, Q. Yang and P. A. Lindahl, *J. Am. Chem. Soc.*, 2003, **125**, 318–319; (*b*) M. R. Bramlett, X. Tan and P. A. Lindahl, *J. Am. Chem. Soc.*, 2003, **125**, 9316–9317; (*c*) X. Tan, C. Sewell and P. A. Lindahl, *J. Am. Chem. Soc.*, 2002, **124**, 6277–6284.

5 A. Volbeda and J. C. Fontecilla-Camps, *Dalton Trans.*, 2005, 3443–3450.

6 (*a*) X. Tan, I. V. Surovtsev and P. A. Lindahl, *J. Am. Chem. Soc.*, 2006, **128**, 12331–12338; (*b*) X. Tan, C. Sewell and P. A. Lindahl, *J. Am. Chem. Soc.*, 2002, **124**, 6277–6284; (*c*) S. Gencic and D. A. Grahame, *Biochemistry*, 2008, **47**, 5544–5555; (*d*) S. W. Ragsdale, *J. Inorg. Biochem.*, 2007, **101**, 1657–1666; (*e*) J. C. Fontecilla-Camps, A. Volbeda, C. Cavazza and Y. Nicolet, *Chem. Rev.*, 2007, **107**, 4273–4303.

7 (*a*) X. Tan, M. Martinho, A. Stubna, P. A. Lindahl and E. Münck, *J. Am. Chem. Soc.*, 2008, **130**, 6712–6713; (*b*) P. Amara, P. Volbeda, J. C. Fontecilla-Camps and I. J. Field, *J. Am. Chem. Soc.*, 2005, **127**, 2776–2784; (*c*) R. P. Schenker and T. C. Brunold, *J. Am. Chem. Soc.*, 2003, **125**, 13962–13963; (*d*) P. A. Lindahl, *JBIC, J. Biol. Inorg. Chem.*, 2004, **9**, 516–524; (*e*) S. W. Ragsdale, *J. Biol. Inorg. Chem.*, 2004, **9**, 511–515.

8 J. Xia, Z. Hu, C. V. Popescu, P. A. Lindahl and E. Münck, *J. Am. Chem. Soc.*, 1997, **119**, 8301.

9 (*a*) A. Volbeda, M. H. Charon, C. Piras, E. C. Hatchikian, M. Frey and J. C. Fontecilla-Camps, *Nature*, 1995, **373**, 580–587; (*b*) J. C. Fontecilla-Camps, A. Volbeda, C. Cavazza and Y. Nicolet, *Chem. Rev.*, 2007, **107**, 4273–4303.

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