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ACETYL-COA SYNTHASE IN THEORY*

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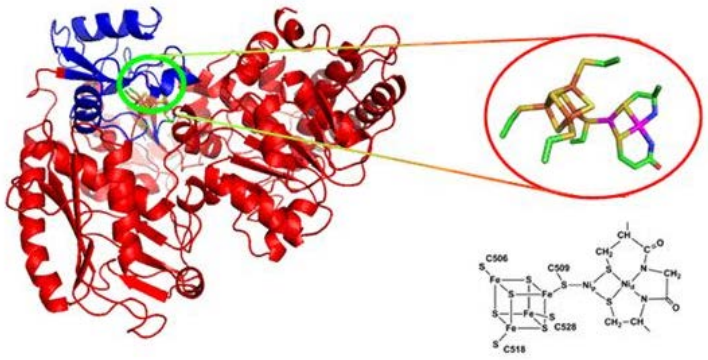
At the interface between chemistry and bioinformatics, we developed a quantum chemical model of Acetyl-CoA synthase (ACS). Our approach goes beyond the "cluster modeling", very popular in the theoretical enzymology field, and allows to get a clear picture on the structure of a recent ACS mutant.



A relevant portion of proteins includes metal ions that are required for their biological activity: such "metalloproteins" are the subject of continuous research efforts not only by experimental chemists and biochemists working on purified samples, but also by theoretical chemists working on computational models of proteins and enzymes. While experimentalists usually face the challenge of characterizing elusive species, the theoretical chemist can reproduce the properties of the latter in a computational representation that is obviously not subject to any instability, and is ready for comparison to the available experimental data. A particularly interesting subgroup of proteins including metal ions of functional relevance are the metalloenzymes, i.e. biological catalysts whose active sites include transition metals, that are mainly exploited for their ability to facilitate redox reactions. Notably, the biological utilization of small inorganic molecules like N_2, H_2, CO, CO_2 usually depends on metalloenzymes, which are able to perform reactions of wide relevance for industrial and environmental applications, at very mild physiological conditions. A fact of

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particular relevance in the context of the present discussion is that many microorganisms express the ability of processing carbon monoxide. It is needless to stress the importance of this microbial activity for the environment: for example, *Oligotropha carboxidovorans* removes ca. $2x10^8$ metric tons of CO from the atmosphere annually[1]. The variety of metal ions complexed with their respective apoproteins to compose CO-oxidizing enzymes is impressive: in such context, nature uses Ni, Fe, Cu, Mo, in various combinations. In particular, in the present contribution we investigated a NiFe-based metalloenzyme - the Acetyl-CoA synthase/carbon monoxide dehydrogenase - with specific reference to the structural properties of the apoprotein and of the embedded A-cluster. The latter is the protein's active site that catalyzes the synthesis of acetyl-CoA (i.e. CoA-S(CO)CH_3) as follows:

CH_3-Corr + CO + CoA $\rightarrow$ CH_3-C)O)-CoA (1)

where "Corr" is a corrinoid-iron sulfur protein which donates the methyl group involved in the reaction.

The A-cluster consists of an [Fe_4S_4] cubane bridged to a [Ni_pNi_d] centre via C509 cysteinate (see the sketch in the lower-right corner of Fig. 1). Recently, the role of the bridging cysteinate has been experimentally probed by means of mutation towards histidine[2]. Notably, the H509 mutant turned out to be catalytically active. Such conservation of function was assumed to correspond to complete preservation of the structural role of the C509 residue in the mutant, meaning that a rare histidinate was proposed to be able to bridge the metal sites (see the inset in Fig. 1)[1].

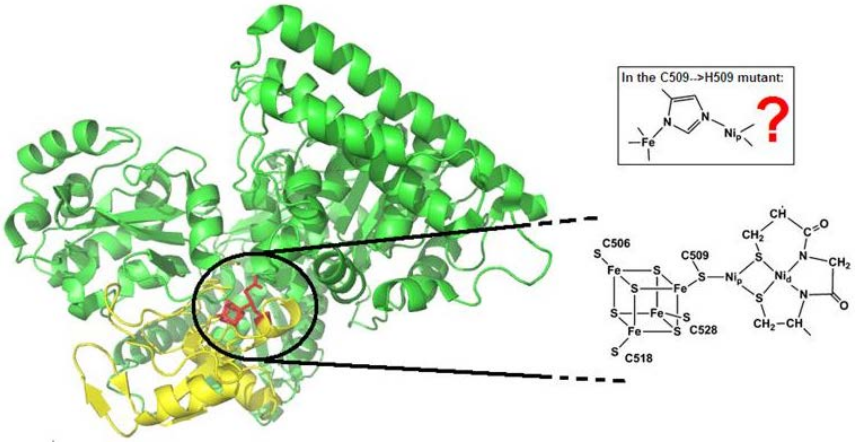

Fig. 1 - On the left: the Acetyl-CoA synthase, with the A-cluster colored in red. On the right: the sketch and inset schematically represent the latter cluster in its wild-type and mutated forms, respectively

In view of the above, we performed density functional theory (DFT) optimizations of models of the isolated A-cluster, without explicit representation of the surrounding protein matrix[3]. Such calculations - carried out by using a split-valence polarized (SVP) basis and a gradient corrected density functional termed "BP86" - showed that major structural reorganization of the protein active site would be needed to accommodate a bridging histidinate.

In order to clarify whether the presence of a bridging histidinate is compatible with the overall architecture of the enzyme, we developed an *ad hoc* model, again based on DFT. In particular, we decided to use a fully quantum chemical approach that took into account the key structural features of the enzyme, including the steric constraints imposed by the protein matrix that harbors the A-cluster. To do so, we analyzed the aminoacidic sequence, its conservation among the homologue proteins available in the current databases, and the overall folding of the protein. As a result of such effort, we found that the portion of apoprotein in proximity of the A-cluster is structurally conserved, and is included in a continuous stretch of protein sequence going from amino acid 503 to 599 (see the yellow portion of the protein in Fig. 1, to visualize the backbone region directly involved in coordination of all

Fe and Ni ions). This subsystem is composed of ≈1,900 atoms, a number that renders a straightforward implementation of high-level quantum chemical approaches way too expensive from a computational point of view. Targeted modifications of such system allowed us to overcome this problem; they include removal of a flexible loop and glycine mutation of all residues that interact with other protein residues outside the selected core. These modifications led to a ≈700 atoms model (Fig. 2), which could be treated at the quantum chemical level with modern codes and computer resources (the total number of basis functions are in the order of 4,200). Both the wild-type and the mutant proteins - with bridging histidine in either the cationic or in the neutral forms - were considered for full geometry optimizations. This clearly implies that we were able to avoid the imposition of arbitrary restraints on the position of atoms along energy minimization - a very popular procedure usually referred to as the "cluster approach" - thanks to the very large size of our model.

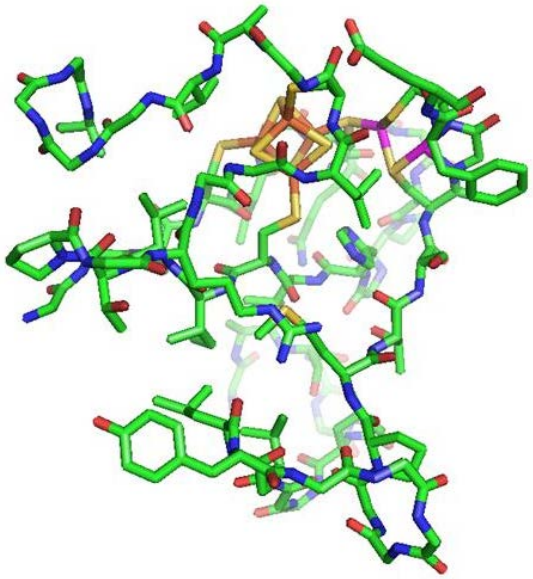

Fig. 2 - The ≈700 atoms model of the wild-type A-cluster and its surroundings, used in the present study for DFT geometry optimization (hydrogen atoms not represented)

We adopted a split-valence (SV) basis at the level of the backbone and non-coordinated side chains, while the atoms of the A-cluster and of its first coordination sphere were assigned the SVP basis. As far as the wild-type protein model is concerned, the RMSD of atomic positions with respect to the enzyme crystal structure was found to be well below 1 Å. On the other hand, geometry optimizations of the mutant allowed us to demonstrate that the presence of a bridging histidinate as represented in Fig. 1 would require major changes in the fold of the protein, namely a 6 Å displacement of a backbone region deeply buried within the core of the protein matrix. Steric stress is minimized, instead, in the case of models in which one of the N atoms of the imidazole ring is not covalently bound to any metal ion. In particular, DFT modeling points at the presence of a singly protonated (i.e. neutral) histidine sidechain, which bridges the $[Fe_4S_4]$ cubane and the $[Ni_pNi_d]$ site by direct involvement of the non-protonated N atom of the imidazole ring.
The study here presented demonstrates how large-size DFT models can be fruitfully applied for the theoretical characterization of metalloproteins, even when possible effects of mutation on protein folding need to be taken into account.

References

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