Modelling the Catalytic Site of The Membrane Bound Hydrogenase

A QM/MM Study

Enzymes are biological catalysts. These macromolecules are able to drastically accelerate almost all biochemical reactions in living organisms. The catalytic activity takes place at the active site. In many enzymes the catalytic site consists of a metal cofactor. For example in hydrogenases, the active site harbors nickel and iron atoms; the cytochrome C oxidase harbors two important copper ions; the xanthine oxidase contains molybdenum and iron atoms in the active site and so on [1].

In numerous cases the three dimensional structures are available, some of them even with very high atomic resolution [2,3]. However this high atomic resolution may not be applicable to metal centers since these sensitive active sites are often prone to radiation damage [4]. Spectroscopic measurements can be used not only to determine the extent of radiation damage, but they can also provide crucial structural and electronic information about the metal centers and their environment, such as stoichiometry, coordination and redox state of the metal ions and nature and protonation state of exogenous ligands [5 – 7].

Among the different spectroscopical techniques, resonance Raman (RR) spectroscopy is a powerful technique to selectively probe the vibrational modes of the chromophore site in proteins. Metal complexes, usually absorb in the visible range as a consequence of electronic transitions with charge-transfer character. Thus their Raman intensities may be strongly enhanced if they are irradiated with light in the red and far-red region of the visible spectrum [8].

Uncertain Attribution
This report is dedicated to describe the use of a theoretical-spectroscopical approach to elucidate and refine the three-dimensional structure of the active site of the membrane bound [NiFe]-hydrogenase (MBH) from Ralstonia eutropha [3]. The [NiFe]-hydrogenase is an enzyme that catalyzes the reversible cleavage of
molecular hydrogen in protons and electrons. The catalytic site harbors one nickel and one iron which are embedded in the protein matrix by two bridging cysteiny1 thiolates, and a third bridging position which serves as cleavage site for hydrogen [3].

Moreover, the iron is coordinated to one carbonyl- and two cyanide- ligands as depicted in figure 1.

Different redox species of the bimetallic center are defined by the oxidation state of the nickel and the chemical nature of the ligand at the third bridging position between the two metals. The crystal structure has been solved with high atomic resolution [3, 6]. However, CO and CN- groups are isoelectronic thus a definite assignment of their structural arrangement in the active site pocket by means of X-ray diffraction is not possible. Since, elucidation of the exact orientation of the atomic ligands and geometry of the active site is a prerequisite for understanding the catalytic mechanism; we refined and corrected the existing crystal structure with the help of vibrational spectroscopy and quantum chemical calculations [9,10].

The IR spectrum of the MBH exhibits three characteristic absorption bands between 2100 and 1900 cm-1: one prominent low frequency band assigned to the carbonyl stretching mode and two less intense absorptions at higher wavenumbers assigned to the cyanide stretching modes (fig. 2). These three modes are highly sensitive to a) the geometry of the active site b) the oxidation state of the metal atoms and c) the electronic and structural properties of the local environment. In particular, regarding the polarity of the local environment, one can identify three different pockets around the cyanide and carbonyl groups (fig. 1). While one pocket, containing the Leu533, is hydrophobic, the other two, characterized by the presence of the Arg530 and Thr553 are hydrophilic. These two amino acids are able to form hydrogen bonds to the respective inorganic ligands in the vicinity. Thus three different geometrical arrangements of the inorganic ligands in the active site pocket are possible.
Furthermore, the RR spectra of the H2-reduced MBH, measured with 458 nm excitation, display distinct bands between 400 and 650 cm⁻¹ (fig. 3), a spectral range characteristic of Fe-CO/CN stretching and bending modes. These bands are absent in the RR-spectra of the oxidized and as-isolated enzyme.

**Clarifying the Orientations**

In order to clarify absolute orientation of the diatomic ligands at the active site of the MBH in the oxidize state and the structure and nature of the active site in the reduced state, hybrid quantum mechanical/molecular mechanics (QM/MM) computations of the IR and Raman spectra were performed and compared with the corresponding experimental data. QM/MM is a theoretical approach, commonly used to efficiently model the active site in enzymes [11], where the electronically relevant part, namely the active site, is treated at a quantum mechanical (QM) level, while the remaining protein matrix and solvent water is described with a molecular mechanics force field. In our QM/MM calculations we used the BP86 density functional to describe the four coordinating cysteines, Cys75, Cys78, Cys597 and Cys600, His82, Arg530, Leu533, Thr553, and the active site with a hydrogen anion in the third bridging position. In addition, the 6-31g(d) basis set for all atoms excluding nickel and iron for which Ahlrichs triple-zeta polarization all-electron basis set (TZVP) was employed [12,13]. For the molecular mechanics (MM) part, the empirical CHARMM22 force field was used [14]. Three structural models of the oxidized MBH were built representing the three possible geometrical arrangements of the inorganic ligands in the active site. For the H2- reduced MBH, three models were considered representing a) the paramagnetic Ni-C state with Ni(III) and a hydride ligand at the bridging site, b) the diamagnetic Ni-SR with Ni(II) and c) the Ni-L state formed by irradiating Ni-C and characterized by absence of the bridging hydride. Finally, in order to sample structural flexibility resulting from thermal fluctuations several snapshots were extracted out of the molecular dynamics simulation performed for each structural model. The QM/MM optimized snapshot structures were later used for frequency, IR-intensity and Raman intensity calculations.

**How Does The Calculation Compare?**

Comparison of the experimental IR spectrum of the MBH with the IR spectra computed for the three models show that an excellent agreement between experiments and theory is only achieved for the structural model where the carbonyl group is located trans to the substrate binding site in a hydrophobic pocket characterized by the absence of hydrogen bonds (COtrans). Here, the computed CO and CN- stretching frequencies are only 3 cm⁻¹ and c.a. 16 cm⁻¹, respectively, shifted with respect to the experimental values (fig. 2). Deviations larger that 60 cm⁻¹ are predicted for the remaining two configurations, namely the
so called COArg and COThr models. Thus, these calculated QM/MM spectra allow a clear discrimination between different possible configurations of the diatomic ligands coordinated to the iron and the identification of their correct orientation in the active site pocket.

The RR spectrum of the MBH shows clear peaks at 609, 559, 496, 447 and 410 cm⁻¹ [10]. The position as well as the Raman intensity of these bands can be perfectly reproduced with the Ni-L model, as shown in figure 3. Furthermore, with the help of these calculations it was possible to assign the experimental bands to Fe-CO stretching and bending modes (see table 1, ref. 10). These results indicate that upon irradiation at 458 nm, the Ni-C state is photo-converted into the Ni-L state, in agreement with previous spectroscopic studies [16,17]. Summarizing, the combination of vibrational spectroscopy together with hybrid QM/MM calculations is a powerful tool for elucidating and refining the structure of metal clusters in complex heterogeneous environments, such as the active site of numerous enzymes.

References

More references are available from the author.

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