Mössbauer studies of the effects of ligand binding and redox changes in the active center of the [NiFe]-hydrogenase from Allochromatium vinosum

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Mössbauer studies of the purified, membrane-bound Ni-Fe hydrogenase from Allochromatium vinosum in nine different states have been performed, focusing on the properties of the low-spin Fe(II) ion in the hetero-binuclear active site. The light sensitivity of several states has been exploited by carrying out in situ illuminations of hydrogenase samples, in the Mössbauer cryostat, at various temperatures between 4.2 K and 200 K. The identification of changes in the Mössbauer parameters of one Fe site in the presence of the ten Fe ions belonging to the Fe-S clusters is a difficult task. In this study we generated pairs of samples such that the contribution of the Fe-S clusters cancels when difference spectra are analyzed. Thus, the difference spectra reflect changes at the active site Fe.

The Ni-Fe site in the active enzyme has been characterized in three different redox states. In the most oxidized state (Nia-S) and the most reduced state (Nia-SR) the nickel is divalent. The intermediate state (Nia-C*) contains a Ni3+ bound to a hydrogen species, that is photo-dissociated at low temperatures. We have analyzed difference spectra for the inactive (Nir-S) and active states (Nia-S·CO, Nia-C* and Nia-SR), the CO-bound (Ni-S.CO) and Ni-S states (obtained by illumination of Ni-S.CO), and the Ni-C* state and its illuminated pair state, Ni-L*. It is known that in the inactive enzyme the active site contains an oxygen ligand in a position bridging between the Ni3+ and the Fe2+ ions. This bridging ligand is removed through activation of the enzyme. The Mössbauer spectra of the active-site iron in the inactive enzyme are significantly different from those of the iron in the Nia-S·CO, Nia-C* and Nia-SR states (activated states). This poster will discuss the new information provided by the Mössbauer spectra about the involvement of the Fe in the chemistry of the Ni-Fe center.