MINISYMPOSIA

1. *Ni-Biochemistry*
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DESULFOVIBRIO GIGAS HYDROGENASE; CATALYTIC CYCLE AND ACTIVATION PROCESS

Desulfovibrio gigas [NiFe] hydrogenase (E.C. 1.12.2.1) has a molecular weight of 89 kD (two subunits of 63 kD and 26 kD) and contains 1 gatm of nickel, 11 gatm of iron and 11-12 gatm of sulfide [1].

1 — NATIVE STATE

IRON-SULFUR-CENTERS

Mössbauer and EPR spectroscopic studies established that in the purified enzyme the iron-sulfur clusters are arranged in a $[Fe_3S_x]_{ox}$ cluster (EPR active) and two $[Fe_4S_4]^{2+}$ clusters (EPR silent) [2]. The $[Fe_3S_x]_{ox}$ cluster is the origin of an almost isotropic EPR signal centered around g = 2.02, observable below 30 K. The Mössbauer parameters of the $[Fe_4S_4]$ clusters (quadrupole splitting of 1.16 mm/s and isomeric shift of 0.46 mm/s, at 4.2 K) are typical of 4Fe centers in the +2 oxidation level [2].

NICKEL CENTER

In the native preparations, a rhombic EPR signal with g-values at 2.31, 2.23 and 2.02 (*Ni-signal A*) is observed up to 120 K (Fig. 1). This rhombic signal, assigned to nickel(III), accounts for 50-100% of the chemically detectable nickel depending on preparation. This assignment was confirmed by the observation of hyperfine coupling in ^{61}Ni-isotopic replaced hydrogenase [3]. A minor species can also be detected at g-values 2.33, 2.16 and ~2.0 (*Ni-Signal B*, Fig. 1). The relative intensities of *Ni-Signals A* and *B* varies with preparation and can be altered by anaerobic redox cycling of the enzyme. This indicates that there exists different Ni(III) environments in the oxidized enzyme.

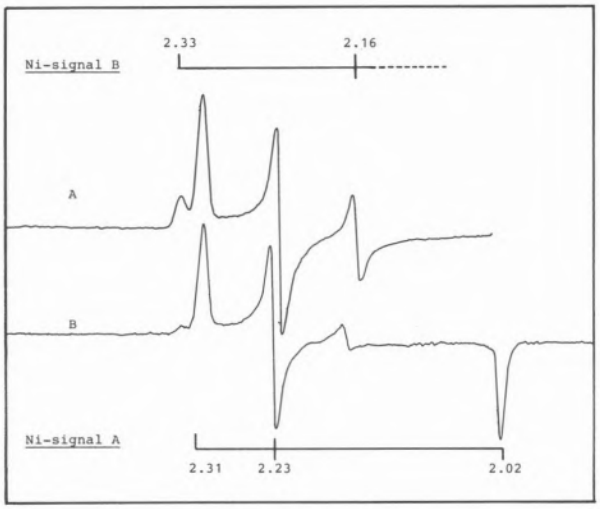


Fig. 1
EPR spectra of D. gigas *[NiFe] hydrogenase, recorded in a Bruker ER-200 tt spectrometer, equipped with an Oxford Instruments continuous flow cryostat.*
A) and B) — Two different preparations of the enzyme. Temperature 100 K, modulation amplitude 1 mT, microwave power 2 mW, frequency 9.34 GHz

2 — INTERMEDIATE OXIDATION STATES

The first event occuring during the anaerobic reduction of *D. gigas* with hydrogen is the disappearance of *Ni-Signals A* and *B* and the isotropic g = 2.02 signal due to the $[Fe_3S_x]$ clusters [4]. An EPR silent state is then attained. Further reduction of the enzyme under H_2 atmosphere is accompanied by the development of a new rhombic EPR signal with g-values at 2.19, 2.16 and 2.02 (*Ni-Signal C*, Fig. 2-A). This signal was also attributed to a nickel species by the ^{61}Ni isotopic substitution [3].

During the course of the reduction experiment *Ni-Signal C* attains a maximum intensity (40-60% of the chemically detectable nickel). Longer incubation time under H_2 yields an EPR silent state, when measured at 77 K. At low temperature (below 15 K) EPR signals typical of $[Fe_4S_4]^{1+}$ clusters are observed [5].

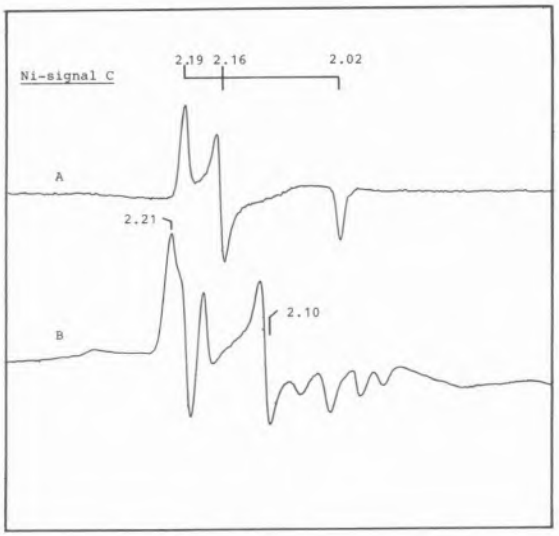


Fig. 2

EPR spectra of intermediate redox states of D. gigas *[NiFe] hydrogenase, in the presence of hydrogen. Experimental conditions as in Fig. 1.*

A) Temperature 77 K, microwave power 2 mW.

B) Same as A, at 4.2 K, microwave power 2 mW

At redox states of the enzyme such that *Ni-Signal C* develops, low temperature studies reveal the presence of another EPR active species: below 10 K, the shape of the EPR spectra changes drastically and a new set of signals at g = 2.21, 2.10 and broad components at higher field is clearly discernible at 4 K (Fig. 2-B). This set of g-values exhibits different power dependence from that of *Ni-Signal C* (readily saturated with low microwave power, typical of a slow relaxation species). The origin of the "2.21" signals is under discussion. Since these signals are only observable at low temperature with high microwave power levels (fast relaxing species), they may originate from an iron-sulfur center. Since the g-values appear to be too high, another explanation is that they originate from the Ni-center weakly interacting with another paramagnetic center in the vicinity (*e.g.* iron-sulfur center).

3 — MID-POINT REDOX POTENTIALS

Redox transitions were observed at -70 mV (measured by the disappearance of the 2.02 signal) and -220 mV (measured by the disappearance of the *Ni-Signal A* (Fig. 3 - insert A)). Only the second redox transition is pH dependent, with a slope of ~ –60 mV per pH unit [6]. *Ni-Signal C* develops at a mid-point redox potential below -300 mV, reaches a maximum around -350 mV and disappears below -400 mV (Fig. 3).

LISSOLO *et al.* [7] determined the activity of the enzyme as a function of the redox potential. Their study indicates that the hydrogenase activation is a one-electron process with a mid-point redox potential around -340 mV (Fig. 3 - insert B). This value correlates with the appearance of *Ni-Signal C*, suggesting that this signal may represent an activated state of the enzyme.

4 — ACTIVATION PROCESS AND CATALYTIC CYCLE

The definition of the role of the nickel during the redox cycle of [NiFe] hydrogenases requires the assignment of the oxidation states involved, the characterization of the ligation mode of the nickel center, as well as the elucidation of possible interactions between the redox centers.

The simplest interpretation of our redox data involves a redox scheme that requires the transition from Ni(III) to Ni(0). However, nickel chemistry shows that the very high and very low oxidation states are not stable chemical species; very negative and very positive redox potentials are associated with the transitions Ni(I) ⇌ Ni(0) and Ni(III) ⇌ Ni(II), respectively. Also, the Ni(III)/Ni(II)

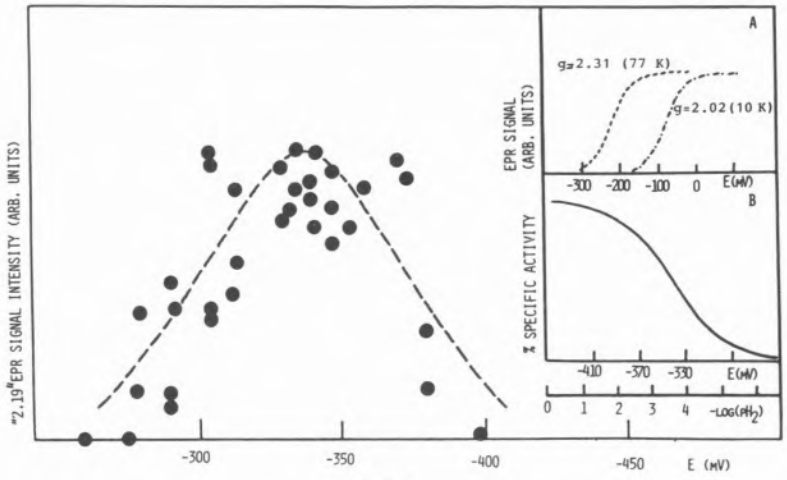


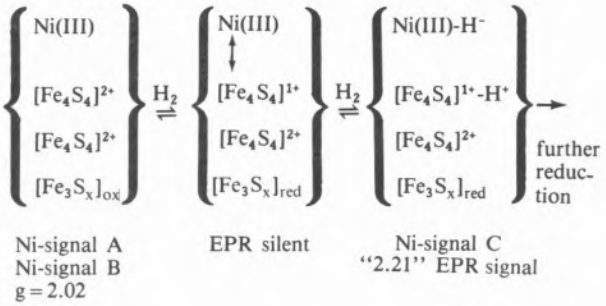
Fig. 3

EPR signal intensity (arbitrary units) of the Ni-signal C *in function of the redox potential. EPR signals were measured at 77 K. No attempt was made to fit the experimental points to a Nernst equation.*
Insert A — Redox titration followed at g = 2.02 (10 K) and g = 2.31 (77 K), data from reference [2].
Insert B — Activation profile of D. gigas *[NiFe] hydrogenase at different partial pressures of hydrogen, data from reference [7]*

chemistry offers a wide range of versatile properties namely: facile rearrangement of ligands, spin and conformational equilibria as well as alteration of the type and number of ligand in the nickel coordination sphere. The redox potential of the Ni(III)/Ni(II) couple can be brought, in principle, to physiological levels by preferential stabilization of the Ni(III) state. Thus, the utilization of fewer redox states seems more realistic in terms of the nickel chemistry.

Another important point to consider in the reactional mechanism of hydrogenase is that the so-called "oxygen stable" [NiFe] hydrogenases (*e.g. D. gigas* hydrogenase) are not fully active in the "as isolated" state. Studies of the hydrogenase activity [7] indicate that the enzyme must go through a lag phase as well as an activation one, in order to be fully active. This complex phenomenon seems to envolve the removal of oxygen (lag phase) followed by a reduction step (activation phase).

Taking into consideration the hydrogenase activity studies, the plausibility of the Ni(III) $\rightleftharpoons$ Ni(II) redox cycling scheme, and the sequence of events observed by EPR spectroscopy upon exposure to H_2 atmosphere, a model is proposed for the mechanism of the [NiFe] hydrogenases in the context of both the catalytic and the activation processes:



The "as isolated" state is fully characterized. EPR and Mössbauer studies in the enzyme "as isolated" [2] indicate that there is no magnetic interaction between these four redox centers.
The active state of the enzyme is EPR silent. During this activation process, both the isotropic g = 2.02 and the nickel signal disappear. The loss of the g = 2.02 signal is attributed to the reduction of the $[Fe_3S_x]$ cluster, $E_o = -70$ mV (EPR silent $[Fe_3S_x]_{red}$).
In order to retain the Ni(III)/Ni(II) redox scheme, the disappearance of *Ni Signal A* and/or *Ni Signal B* requires a more complicated mechanism. We propose that one of the $[Fe_4S_4]$ clusters is reduced into a $[Fe_4S_4]^{1+}$ state (S = 1/2) and the reduced cluster is spin coupled with the Ni(III) center resulting in an EPR silent state. This proposal implies that the previously determined redox potential, -220 mV, for the disappearance of *Ni-Signal A* [2] is actually the mid-point redox potential for

one of the [Fe_4S_4] clusters. Such a mechanism is supported by the optical studies which indicate that the activation process involves the reduction of iron-sulfur clusters. Preliminary Mössbauer data (our unpublished results in collaboration with B.H. Huynh) also show that approximately one [Fe_4S_4] cluster is reduced in the EPR silent state and it is possible to recognize the normal "signature" of the reduced 3Fe cluster.

The events which follow the EPR silent state are the appearance of both the *Ni-Signal C* and the "g = 2.21" signal. In accordance with the heterolytic mechanism of hydrogen activation, we propose that in the presence of the natural substrate a hydride intermediate state is obtained. The nickel center is assigned as the hydride binding site and the $[Fe_4S_4]^{+1}$ cluster is the proton binding site. The spin coupling between the Ni(III) and the $[Fe_4S_4]^{1+}$ cluster is broken in this hydride intermediate, originating *Ni-Signal C*. Thus, this signal is assumed to represent the hydride-bound Ni(III) center and the g = 2.21 is attributed to the proton--bound $[Fe_4S_4]^{1+}$ cluster. Alternatively, the g = 2.19 EPR signal could be due to a transient Ni(III) state in a different coordination, resulting from the breaking of the coupling and the g = 2.21 signal could be due to the interacting Ni(III) and $[Fe_4S_4]^{1+}$ centers bound to hydride and proton, respectively. By further incubation with H_2 the *Ni-Signal C* disappears, suggesting reduction to Ni(II) with the concomitant development of reduced [Fe_4S_4] center signals.

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MS1.2 — MO

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HYDROGENASE FROM *CHROMATIUM VINOSUM*: THE REDOX STATES OF NICKEL AND THE IRON-SULPHUR CLUSTER DURING CATALYSIS

The presence of nickel in a purified hydrogenase was first reported in 1981 by Graf and Thauer [1] for the enzyme from *Methanobacterium thermoautotrophicum*. EPR spectroscopy [2] showed that virtually all nickel was present as low-spin Ni(III) which could be reduced with H_2. No signals due to Fe-S clusters were observed. Hydrogenase from *Chromatium vinosum*, also a nickel--enzyme, clearly displays two signals in the $g = 2$ region which could be ascribed to Fe-S clusters [3]. An analysis of the 4 different EPR signals that can be detected in the enzyme as isolated led to the following hypothesis [3-5]. The preparation contains intact and defect enzyme molecules. Defect molecules, which are unreactive in the standard activity assays with viologens, contain one Ni(III) ion and one [3Fe-xS]^{ox} cluster. Two forms of Ni(III), Ni-a and Ni-b, can be detected. Their ratio varies from preparation to preparation. In-