Biochimica et Biophysica Acta, 724 (1983) 309–316
Elsevier

BBA 41352

MAGNETIC INTERACTION OF NICKEL(III) AND THE IRON-SULPHUR CLUSTER IN HYDROGENASE FROM *CHROMATIUM VINOSUM*

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(Received April 13th, 1983)

Key words: Hydrogenase; Nickel(III); Iron-sulfur cluster; ESR; (C vinosum)

Upon partial reduction of hydrogenase from *Chromatium vinosum* with ascorbate plus phenazine methosulphate, EPR signals due to Ni(III) and a [3Fe-*x*S] cluster appear simultaneously and with equal intensities. Since the intact enzyme shows no $S = 1/2$ signals, it is concluded that Ni(III) and a [4Fe-4S]$^{3+}$ cluster interact magnetically in such a way as to prevent the detection of the two paramagnets as individual $S = 1/2$ systems. This interaction is thought to be the origin of a signal in which Fe is involved and which is not due to an $S = 1/2$ system (Albracht, S.P.J., Albrecht-Ellmer, K.J., Schmedding, D.J.M. and Slater, E.C. (1982) Biochim. Biophys. Acta 681, 330–334). A variable fraction of the enzyme preparation shows signals due to Ni(III) and a [3Fe-*x*S] cluster with equal intensities without any further treatment. These are thought to be derived from irreversibly inactivated enzyme molecules. The enzyme contains no selenium.

Introduction

Hydrogenase (hydrogen : (acceptor) oxidoreductase, EC 1.12.-.-) from *Chromatium vinosum* was first purified by Gitlitz and Krasna [1] using a detergent to solubilize the enzyme. These investigators found that the enzyme contains 4 Fe and 4 acid-labile S atoms per molecule and exhibits an EPR signal like that of a [4Fe-4S]$^{3+(3+,2+)}$ cluster. Further evidence for the presence of such a cluster came from optical spectra of the enzyme in 80% non-aqueous solvents [2], which were analogous to those of the [4Fe-4S]$^{3+(3+,2+)}$ cluster in Hipip from *C. vinosum*, but quite distinct from the [2Fe-2S] cluster in spinach ferredoxin under the same conditions. No information was provided about the absolute intensity of the EPR signal.

In this laboratory a different purification procedure was developed [3,4], based on the observation that at least half of the enzyme activity is soluble in the absence of detergent. In addition to the Fe-S signal, already observed by Krasna and coworkers [1], further referred to as signal 1, our preparations showed a second signal, signal 2, that is not due to an $S = 1/2$ system [4]. Since signal 2 but not signal 1 disappears on removal of oxygen from the enzyme preparation or on addition of β-mercaptoethanol, we first thought that oxygen was involved in this signal [3]. This was, however, ruled out when we found that re-oxidation of the reduced enzyme with excess of ferricytochrome *c* under strictly anaerobic conditions caused the reappearance of both signals 1 and 2 to their original intensities [4]. Two molecules of ferricytochrome *c* were reduced per molecule of hydrogenase. That iron is involved in both signals was shown by the fact that introduction of ^{57}Fe ($I = 1/2$) into the enzyme caused a clear splitting in signal 1 and a broadening of signal 2 [5]. It was observed that the intensity of signal 1 varies from preparation to preparation and accounts for a spin

Abbreviations: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; Hipip, high-potential Fe-S protein.

concentration of only 10–50% of the enzyme concentration. After a deoxygenation/oxygenation cycle, the spin concentration increases to one spin per molecule of enzyme [4]. We also provided experimental evidence that signal 1 is caused by a [3Fe-*x*S] cluster and that in the enzyme preparation as isolated, molecules containing such a cluster cannot be catalytically active [5]. We proposed that active molecules contain a $[4Fe\text{-}4S]^{3+(3+,2+)}$ cluster which is in magnetic interaction with the second redox group in the enzyme. Signal 2 is probably a reflection of this interaction. Since nickel raised the hydrogenase activity of *C. vinosum* 3- to 6-fold, and since all our preparations showed very small EPR signals with g values and a temperature dependence like that of Ni(III) in hydrogenase of *Methanobacterium thermoautotrophicum* [6], we proposed nickel as a candidate for the second redox group. Replacement of natural nickel by ^{61}Ni ($I = 3/2$) had, however, no effect on the EPR signals 1 and 2, whereas the Ni-like signals were too weak for a proper comparison [5].

In this report experimental evidence is presented that the second redox component is indeed nickel and that, by partial reduction of the active enzyme in air, the proposed magnetic coupling between Ni and the $[4Fe\text{-}4S]^{3+}$ cluster can be broken so that both paramagnets become EPR detectable. It was further established that, in contrast to hydrogenase from *Methanobacter vanillii* [7], no selenium is present in the enzyme from *C. vinosum*.

Materials and Methods

C. vinosum, strain DSM 185, was grown in a 700-l batch culture [4] in a medium essentially as described by Hendley [8]. The NaCl and Na_2S were omitted from the growth medium and 10 μM Ni was added. The presence of Ni ensured a maximal hydrogenase content of the cells [5]. We noticed that in the presence of nickel at this concentration there was very little accumulation of sulphur granules in the cells, whereby cell growth was accelerated due to the diminished light limitation. The enzyme was purified exactly as described previously [4] and was always dissolved in 50 mM Tris-HCl buffer (pH 8.0). Nickel was determined by atomic absorption spectrometry. Selenium was determined in two ways: (1) by atomic absorption spectrometry and (2) by activation analysis. Enzyme activities and EPR measurements were performed as described before [4,5]. Gel electrophoresis in the presence or absence of SDS was performed in 8–10% polyacrylamide gels, using the buffer system of Laemmli [9].

Results

Evidence for magnetic interaction between Ni and the Fe-S cluster

In addition to the strong signals 1 and 2 around $g = 2$ (Fig. 1, trace A), our enzyme preparations show very weak EPR lines around $g = 2.3$ and 2.2 which can still be observed at 70 K (Fig. 1, trace B). The EPR characteristics of these signals are the same as those for Ni(III) in hydrogenase from *M. thermoautotrophicum,* strain Marburg [6], *Desulphovibrio gigas* [10–12] and *M. thermoauto-*

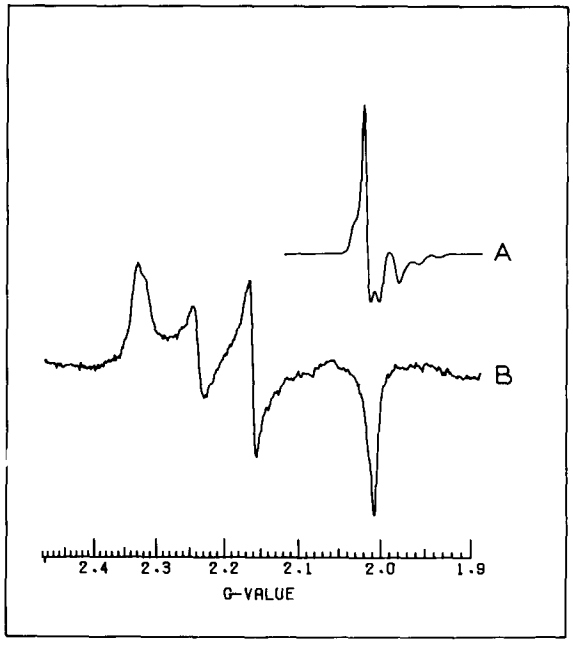


Fig. 1. EPR spectra of hydrogenase as isolated (specific hydrogen-production activity 17.5 U/mg). EPR conditions: microwave frequency, 9245.7 MHz; temperature, 14 K (A) and 70 K (B); microwave power, 2.2 mW; modulation amplitude, 0.63 mT. The gain for trace B is 160-times that of trace A. The modulation frequency for these and all other spectra in this report is 100 kHz. All spectra within one figure in this report have been scaled to the same g value scale [4].

trophicum, strain ΔH [13], except that trace B in Fig. 1 represents two overlapping signals (see later). We reported previously [4,5] that most of the $[4Fe\text{-}4S]^{3+}$ clusters in our enzyme preparations are, as such, EPR undetectable, but that after a deoxygenation/oxygenation cycle all clusters became detectable as [3Fe-*x*S] clusters. We proposed that these clusters were originally $[4Fe\text{-}4S]^{3+}$ clusters that were EPR silent due to a magnetic interaction with Ni(III) in the enzyme. If this is so, it might be expected that after a deoxygenation/oxygenation treatment the Ni(III) would also become EPR detectable. The signals in Fig. 1, trace B, however, did not change during such a treatment although signals 1 and 2 (Fig. 1, trace A) behaved in the same way as observed earlier [4].

We have previously shown [4] that partial reduction of the enzyme with ascorbate plus phenazine methosulphate likewise results in an increase in the intensity of signal 1. We now find that in this case there is a simultaneous increase in the intensity of the signals in the $g = 2.2$ to 2.3 region. Signal 2 disappears, as reported earlier [4].

Fig. 2 shows the spectra of the enzyme preparation used in Fig. 1, 12 min after the addition of ascorbate plus phenazine methosulphate. Trace B could be well simulated by addition of two $S = 1/2$ signals in a ratio of 1 : 0.55 (Fig. 2, trace C). We ascribe the individual signals to two different species of Ni(III). The time course of the changes in the signals, in an experiment with a different

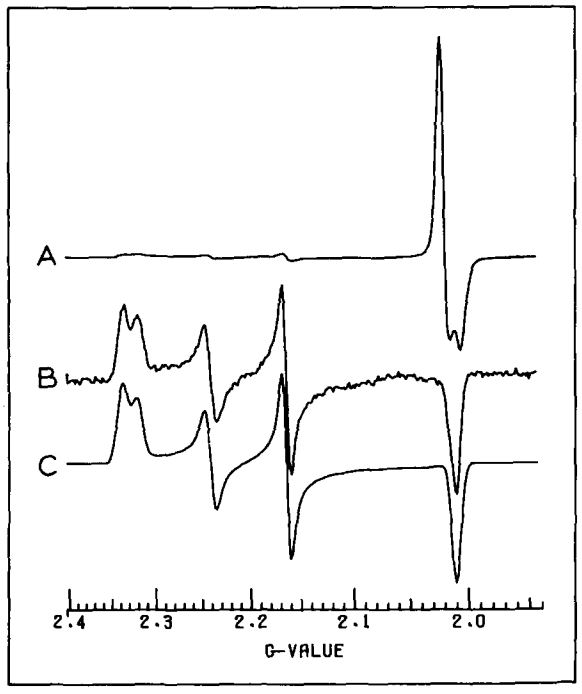


Fig. 2. EPR spectra of hydrogenase (same preparation as in Fig. 1) that has reacted with 20 mM ascorbate plus 5 μM phenazine methosulphate for 12 min at 20°C. EPR conditions: microwave frequency, 9248.1 MHz; temperature, 14 K (A) or 70 K (B); microwave power, 0.26 mW (A) or 2.2 mW (B); modulation amplitude, 0.63 mT. The gain for trace B is 25-times that of trace A. Trace C is a computer simulation for trace B. It is the sum of two $S = 1/2$ signals, using the following parameters. For signal a: $g_{x,y,z} = 2.337, 2.1637, 2.0114$; width $(x,y,z) = 1.45, 1.02, 1.3$ mT and relative intensity = 1. For signal b: $g_{x,y,z} = 2.321, 2.2411, 2.0114$; width $(x,y,z) = 1.55, 1.29, 1.3$ mT and relative intensity = 0.55.

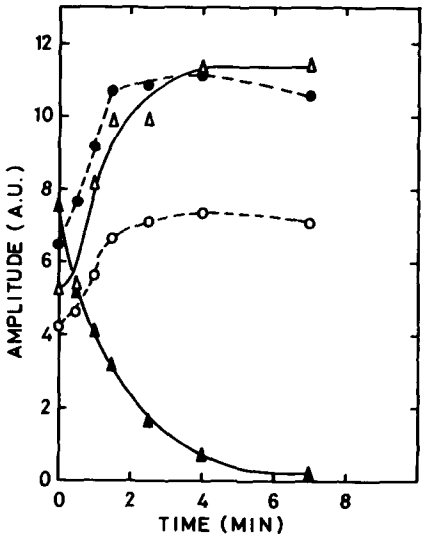


Fig. 3. Time course of the changes of the EPR signals of hydrogenase (specific hydrogen-production activity 6.7 U/mg) after addition of 20 mM ascorbate plus 5 μM phenazine methosulphate. At $t = 0$, the enzyme was mixed in an EPR tube with ascorbate and phenazine methosulphate at 20°C and after 30 s the tube was immersed in liquid nitrogen, whereafter EPR spectra at 14 and 70 K were recorded. The tube was then immersed in a water bath of 20°C and after a certain time, not including the time needed for thawing, the enzyme was again frozen in liquid nitrogen for EPR inspection. This procedure was repeated several times. No changes were observed if phenazine methosulphate was omitted. (△———△) Amplitude of signal 1, (▲———▲) amplitude of signal 2 measured at $g = 1.98$, (○———○) amplitude of the line at $g = 2.16$, (●———●) amplitude of the line at $g = 2.24$.

TABLE I

THE AMOUNTS OF NICKEL AND [3Fe-xS] CLUSTER DETECTABLE IN THE ENZYME AS ISOLATED AND AFTER PARTIAL REDUCTION WITH ASCORBATE PLUS PHENAZINE METHOSULPHATE

Experimental conditions as in Fig. 3.

Preparation	Specific H_2-production activity (U/mg protein)	Concentration of the paramagnets								
		Before reduction			After reduction			Difference		
		[3Fe-xS] (μM)	Ni (μM)	Ni/[3Fe-xS]	[3Fe-xS] (μM)	Ni (μM)	Ni/[3Fe-xS]	[3Fe-xS] (μM)	Ni (μM)	Ni/[3Fe-xS]
a	17.5	26	20.2	0.78	43.8	38.5	0.88	17.8	18.3	1.03
b	6.7	38.3	44	1.15	75.2	78	1.04	36.9	34	0.93
c	6.1	5.2	4.1	0.79	7.9	6.4	0.81	2.7	2.3	0.85
Mean				0.91			0.91			0.93

enzyme preparation, is given in Fig. 3. During the first 4 min, there is a simultaneous increase in the signal amplitude of both the $g = 2.16$ and the $g = 2.24$ lines and of signal 1. At the same time, signal 2 decreases in amplitude. The slight difference in the time course of the decline in signal 2 and the increase in the other signals is probably due to the fact that upon longer incubation both Ni signals and the [3Fe-xS] signal decrease in intensity, possibly as a result of reduction. For the Ni signals this is already apparent after 7 min. The spin concentrations represented by the Ni and the [3Fe-xS] cluster signals are given in Table I. In the untreated enzyme approximately equal amounts of Ni(III) and [3Fe-xS] cluster are EPR detectable, the average ratio of Ni/[3Fe-xS] being 0.9. After treatment with reductant, both intensities increase maximally 1.5–2-times and again the average ratio of extra induced intensities due to Ni and Fe-S cluster is 0.9. We conclude that the same amounts of Ni(III) and [3Fe-xS] cluster, that were not detectable as such before reduction, now become EPR detectable as independent $S = 1/2$ systems. Since the spin state of the disappearing signal 2 is not known, the spin concentration cannot be calculated. Although Fig. 3 suggests a relationship between the disappearance of signal 2 and the appearance of the signals due to Ni and the 3Fe cluster, no quantitative conclusion is possible. Signal 2 also disappears after removal of oxygen or the addition of β-mercaptoethanol, without any change in the Fe-S and the Ni signals. As ascorbate plus phenazine methosulphate will certainly consume most of the oxygen in the enzyme solution under the conditions used in the experiments of Table I, the disappearance of signal 2 might also be related to the disappearance of oxygen. A small increase in the $g = 4.3$ signal, representing aspecifically bound Fe^{3+}, was only sometimes observed. Ascorbate alone had virtually no effect within 40 min. Addition of 0.2 mM TMPD had an effect similar to that of phenazine methosulphate, but the rate of the changes was more than 5-times slower.

Detection of different forms of the enzyme

During the purification of the enzyme we noticed that the activity was present in two overlapping peaks in the eluate from the Ultrogel AcA-44 column, following the procedure described by Van Heerikhuizen et al. [4]. Two fractions, taken at positions where the overlap was considered to be minimal, revealed different specific activities (Table II) and EPR spectra. The main fraction, called Type-I enzyme, has the EPR properties as already described in Figs. 1–3. At low temperatures, additional signals of unknown origin were observed with this enzyme in the $g = 2$–2.3 region (Fig. 4) that disappeared when ascorbate plus phenazine methosulphate was added. These signals broaden at higher temperatures, leaving the signals with the characteristics of Ni in hydrogenase. No optical absorption bands of cytochromes could be detected in the 400–650 nm region. Electrophoresis on polyacrylamide gels in the presence of 0.1% SDS showed several contaminating protein bands

TABLE II

PROPERTIES OF THREE FORMS OF HYDROGENASE ACTIVITY THUS FAR ENCOUNTERED DURING PURIFICATION OF THE ENZYME FROM *C. VINOSUM* AS DESCRIBED BY VAN HEERIKHUIZEN ET AL. [4]

n.d., not determined.

Enzyme	Specific activity (U/mg)		Relative amount (%)	EPR signals				Ni contents (μmol/g protein)
	H_2 production	H_2 uptake		1	2	Ni-a	Ni-b	
Type I	17.5	147	55	+	+	+	+	29.2
Type II	57	455	45	+ [b]	–	+	–	28.6
Type III	27	0	12 [a]	–	–	–	–	n.d.

[a] This is the amount found in the eluate of the DEAE-cellulose.

[b] The signal differs somewhat from the signal 1 of the Type I enzyme (see Fig. 7).

in addition to the 60 kDa band of the hydrogenase (Fig. 5). The other active fraction, here called the Type-II enzyme, has a higher specific activity than that reported earlier for a pure enzyme [2,4] (Table II), although it appears not to be very pure in gel electrophoresis (Fig. 5). Its EPR spectrum differs from that of the Type-I enzyme in several respects (Fig. 6): (i) signal 2 is absent; (ii) although a signal 1 is present, it appears at slightly different g values (Fig. 7); (iii) there is only one Ni signal present and no other signals in the $g = 2.1$–2.3 region are observed at low temperature (Fig. 6); (iv) when ascorbate plus phenazine methosulphate is added, the intensity of signal 1 decreases somewhat in the

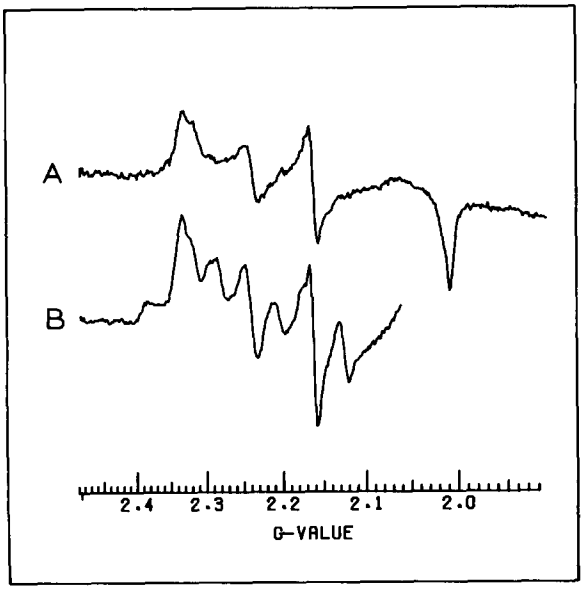


Fig. 4. Comparison of the $g = 2.1$–2.4 region in spectra of hydrogenase at two different temperatures. The enzyme was the same as used in Fig. 1. EPR conditions: microwave frequency, 9243.3 MHz; temperature, 58 K (A) or 18 K (B); microwave power, 2.2 mW; modulation amplitude, 0.63 mT. The gain for trace A is 1.25-times that for trace B.

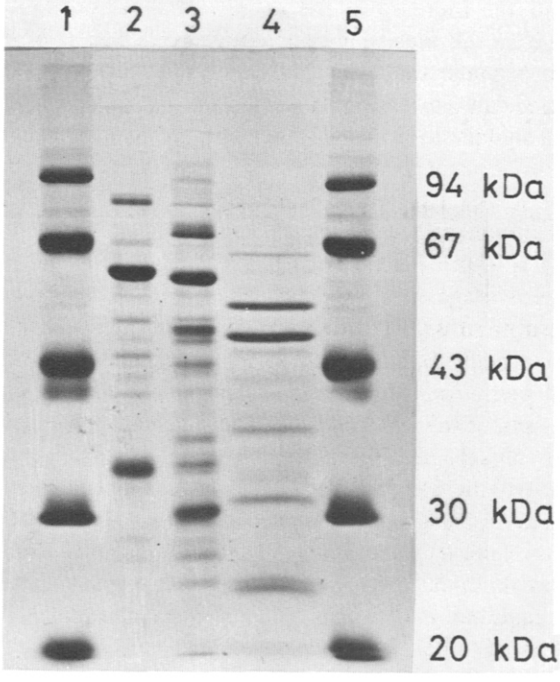


Fig. 5. SDS gel electrophoresis on a 10% acrylamide slab gel of the three types of hydrogenase activity. The two outer lanes contain proteins used for calibration (phosphorylase *b*, 94 kDa; albumin, 67 kDa; ovalbumin, 43 kDa; carbonic anhydrase, 30 kDa and trypsin inhibitor, 20 kDa). Lanes 2, 3 and 4 contain the Type-I, Type-II and Type-III enzymes, respectively.

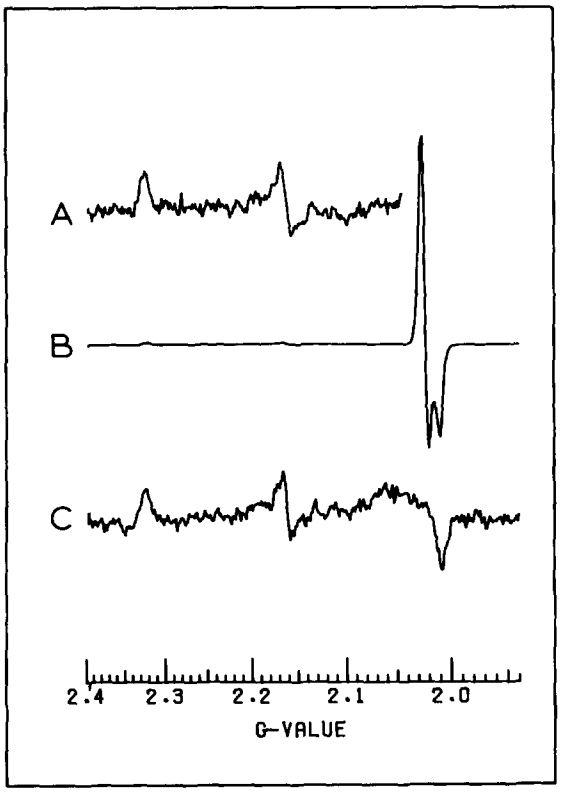


Fig. 6. EPR spectra of the Type-II enzyme (specific hydrogen-production activity 57.2 U/mg). EPR conditions: microwave frequency, 9242.6 MHz; temperature, 14 K (A and B) or 70 K (C); microwave power, 0.26 mW (A and B) or 2.2 mW (C); modulation amplitude, 0.63 mT. The relative gains for A, B and C were 25, 1 and 25, respectively.

first minute (20°C) to a steady level corresponding to a spin concentration equal to that of the Ni, without any change in the intensity of the nickel signal. The two types of enzyme could be distinguished already at the DEAE-cellulose column, where they eluted with different salt concentrations (0.29 M NaCl for the Type-I and 0.35 M for the Type-II enzyme). A third minor fraction, showing only hydrogen-production activity, was sometimes eluted from this column already with 0.21 M NaCl. It did not show any EPR signals at all and its activity appeared to be much less stable during storage than those of the other two types of enzyme preparations. In gel electrophoresis with 0.1% SDS, the main bands appeared to have a molecular mass of about 50 kDa rather than 60 kDa (Fig. 5).

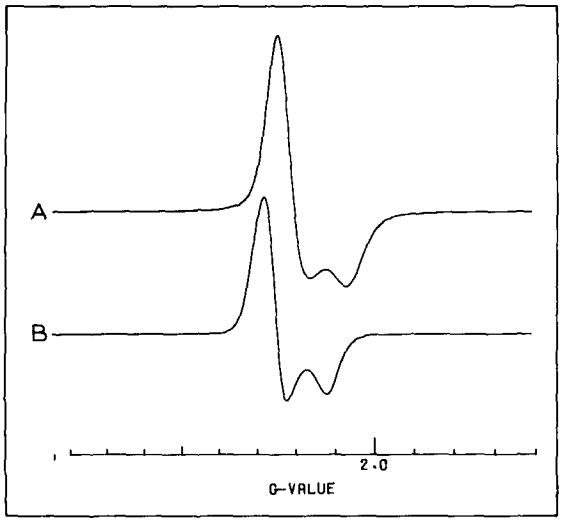


Fig. 7. Comparison of the Fe-S signals of the Type-I and Type-II enzymes. (A) Expanded scan of the Fe-S signal of the Type-I enzyme used in Fig. 2. (B) Expanded scan of the Fe-S signal of the Type-II enzyme used in Fig. 6. EPR conditions as in Fig. 1. Each division of the g value scale is 0.01.

Discussion

As yet we do not understand the significance of the different forms of the enzyme isolated from *C. vinosum*. Type-III activity is not always observed. Since this form of the enzyme has no hydrogen-uptake activity at all and the main peptide bands in SDS gels correspond to a molecular mass of about 50 kDa, instead of 60 kDa for the normal enzyme [4], we consider this form to be an artefact. The other two types of enzyme have hydrogen-production/hydrogen-uptake activity ratios (Table II) similar to that found in broken cells of *C. vinosum* (about 1 : 8) and contain a 60 kDa polypeptide as the main component. Both preparations contain about 1.8 atoms of Ni per 60 kDa protein (Table II). The extra EPR signals in the $g = 2.1-2.3$ region, observed at low temperatures only, are seen exclusively with the Type-I enzyme. Since the EPR characteristics of the Ni signals in Type-I enzyme observed at 70 K are similar to those of Ni(III) in hydrogenase of *M. thermoautotrophicum*, strain Marburg [6] and other 'uptake' hydrogenases [10–13], we propose that these signals belong to the hydrogenase. This conclusion is supported by the observation that the absolute inten-

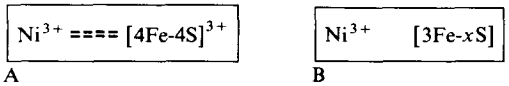
sity of these signals is equal to that of the [3Fe-xS] cluster and that on partial reduction of the enzyme preparation these signals gain in intensity simultaneously with the signal of the [3Fe-xS] cluster. Signal 2 also belongs to the hydrogenase itself, since it has been observed with the pure enzyme [4]. The Type-II enzyme, which has a specific activity which is somewhat higher than that of enzyme preparations from *C. vinosum* reported thus far [2,4], shows no signal 2 and has an Fe-S signal with slightly displaced g values. It shows only one type of Ni signal with g values ($g_{x,y,z}$ = 2.009, 2.163, 2.327) very close to the major Ni signal in the Type-I enzyme ($g_{x,y,z}$ = 2.011, 2.164, 2.337).

In considering the signals of Ni and the [3Fe-xS] cluster in the two types of preparations, it is of importance to keep in mind that the activities of our preparations are not related to the intensity of the [3Fe-xS] cluster signal but to the concentration of the EPR-undetectable $[4Fe\text{-}4S]^{3+}$ cluster, that can be converted to detectable [3Fe-xS] cluster after a deoxygenation/oxygenation treatment [5]. Since in our previous work all active fractions in the purification procedure were usually pooled, the preparations used were probably a mixture of Type-I and Type-II enzymes. In the present study, we find nearly equal amounts of EPR-detectable Ni and [3Fe-xS] cluster in both types of enzyme. From this we conclude that, in the enzyme as isolated, not only the signal of the [3Fe-xS] cluster but also those of Ni stem from irreversibly inactivated hydrogenase molecules. The experiments in Table I show that more Ni and [3Fe-xS] cluster can become EPR detectable during partial reduction. We conclude that in the active enzyme the Ni(III) and the $[4Fe\text{-}4S]^{3+}$ cluster are magnetically coupled and that signal 2, which under certain conditions is observed in the enzyme as isolated, is a reflection of this interaction. These views are summarised in Scheme I. We presume that partial reduction of the enzyme results in a destabilization of the $[4Fe\text{-}4S]^{3+}$ cluster, which then converts to an EPR-detectable [3Fe-xS] cluster [14], whereby the magnetic coupling with Ni(III) is broken. Since in hydrogenase from *D. gigas* [10,12] and *M. thermoautotrophicum*, strain ΔH [13], also only part (40–50%) of the chemically determined Ni can be observed as Ni(III) in the enzyme as isolated, the possibility exists that most of the Ni in these enzymes is also present in a magnetically coupled state. For these enzymes there is no information available as to whether or not the EPR-detectable Ni originates from irreversibly inactivated enzyme. The measured redox potential of the EPR-detectable Ni from these enzymes, −150 mV [10] to −220 mV [12], might thus not reflect the potential of the Ni in intact enzyme molecules. The Type-I enzyme in this study contains two types of Ni with a different environment, Ni-a and Ni-b, the relative ratio of the two varying in different preparations. Upon careful inspection of the published EPR spectrum of hydrogenase from *C. vinosum* as purified by Strekas et al. (Fig. 2 of Ref. 2), one can, although the authors did not comment on this, also see EPR lines at g = 2,3, 2.2 and 2.15, indicating that this enzyme closely resembles our Type-I enzyme or might be a mixture of Type I and Type II. Signal 2 cannot be observed in this spectrum, which is understandable since β-mercaptoethanol was used during the purification.

We have recently started a systematic study of the effect of the redox potential on the behaviour and properties of the enzyme.

SCHEME I

(A) Intact enzyme: Two interacting $S = 1/2$ systems; EPR silent or signal 2. (B) Irreversibly inactivated enzyme: Two independent $S = 1/2$ systems; Ni signal and signal 1.



A B

Acknowledgements

We thank Dr. J. Kragten and Mr. B. Van Bennekom (Laboratory of Physics, University of Amsterdam) for their kind help with the Ni analysis. Dr. F.J.M.J. Maessen and Mr. P. Bank (Laboratory of Analytical Chemistry, University of Amsterdam) are acknowledged for the determination of selenium by atomic absorption spectrometry. We are further indebted to Dr. J.R.W. Woittiez (Netherlands Energy Research Foundation ECN, Petten) for selenium determination by activation analysis. Part of this research was supported by a grant from the Netherlands Organization for the Advancement of Pure Re-

search (Z.W.O.) under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.).

References

1 Gitlitz, P.H. and Krasna, A.I. (1975) Biochemistry 14, 2561–2568
2 Strekas, T., Antanaitis, B.C. and Krasna, A.I. (1980) Biochim. Biophys. Acta 616, 1–9
3 Van Heerikhuizen, H., Albracht, S.P.J., Ten Brink, B., Evers-van Twist, L. and Slater, E.C. (1978) in Hydrogenases: Their Catalytic Activity, Structure and Function (Schlegel, H.G. and Schneider, K., eds.), pp. 151–158, Goltze, Göttingen
4 Van Heerikhuizen, H., Albracht, S.P.J., Slater, E.C. and Van Rheenen, P.S. (1981) Biochim. Biophys. Acta 657, 26–39
5 Albracht, S.P.J., Albrecht-Ellmer, K.J., Schmedding, D.J.M. and Slater, E.C. (1982) Biochim. Biophys. Acta 681, 330–334
6 Albracht, S.P.J., Graf, E.-G. and Thauer, R.K. (1982) FEBS Lett. 140, 311–313
7 Yamazaki, S. (1982) J. Biol. Chem. 257, 7926–7929
8 Hendley, D.D. (1955) J. Bacteriol. 70, 625–634
9 Laemmli, U.K. (1970) Nature 227, 680–685
10 Cammack, R., Patil, D., Aquirre, R. and Hatchikian, E.C. (1982) FEBS Lett. 142, 289–292
11 Le Gall, J., Ljungdahl, P.O., Moura, I., Peck, H.D., Jr., Xavier, A.V., Moura, J.J.G., Teixeira, M., Huynh, B.H. and DerVartanian, D.V. (1982) Biochem. Biophys. Res. Commun. 106, 610–616
12 Teixeira, M., Moura, I., Xavier, A.V., DerVartanian, D.V., LeGall, J., Peck, H.D., Jr., Huynh, B.H. and Moura, J.J.G. (1983) Eur. J. Biochem. 130, 481–484.
13 Kojima, N., Fox, J.A. Hausinger, R.P., Daniels, L., Orme-Johnson, W.H. and Walsh, C. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 378–382.
14 Thomson, A.J., Robinson, A.E., Johnson, M.K., Cammack, R., Rao, K.K. and Hall, D.O. (1981) Biochim. Biophys. Acta 637, 423–432.