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The molybdenum iron–sulphur protein from *Desulfovibrio gigas* as a form of aldehyde oxidase

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The molybdenum iron–sulphur protein originally isolated from *Desulfovibrio gigas* by Moura, Xavier, Bruschi, Le Gall, Hall & Cammack [(1976) Biochem. Biophys. Res. Commun. **72**, 782–789] has been further investigated by e.p.r. spectroscopy of molybdenum(V). The signal obtained on extended reduction of the protein with sodium dithionite has been shown, by studies at 9 and 35 HGz in ^1H_2O and ^2H_2O and computer simulations, to have parameters corresponding to those of the Slow signal from the inactive desulpho form of various molybdenum-containing hydroxylases. Another signal obtained on brief reduction of the protein with small amounts of dithionite was shown by e.p.r. difference techniques to be a Rapid type 2 signal, like that from the active form of such enzymes. In confirmation that the protein is a molybdenum-containing hydroxylase, activity measurements revealed that it had aldehyde:2,6-dichlorophenol-indophenol oxidoreductase activity. No such activity towards xanthine or purine was observed. Salicylaldehyde was a particularly good substrate, and treatment of the protein with it also gave rise to the Rapid signal. Molybdenum cofactor liberated from the protein was active in the *nit-1 Neurospora crassa* nitrate reductase assay. It is concluded that the protein is a form of an aldehyde oxidase or dehydrogenase. From the intensity of the e.p.r. signals and from enzyme activity measurements, 10–30% of the protein in the sample examined appeared to be in the functional form. The evolutionary significance of the protein, which may represent a primitive form of the enzyme rather than a degradation product, is discussed briefly.

INTRODUCTION

Molybdenum-containing hydroxylases are an important class of molybdoenzymes that includes xanthine oxidase, xanthine dehydrogenase and aldehyde oxidase. These enzymes (Bray, 1975), isolated from a wide variety of sources, all contain molybdenum, FAD and iron–sulphur centres, and appear generally very similar to one another. Reducing substrates react at the molybdenum and oxygen at the flavin. Whether the enzymes are oxidases or dehydrogenases depends on the precise environment of the flavin in the protein. Removal of the flavin appears not to affect the molybdenum. Extensive studies relating to the structures and reaction mechanisms of the molybdenum centres of these enzymes have been carried out by e.p.r. and by e.x.a.f.s. (Bray, 1980, 1984; Cramer, 1983; Bray & George, 1985; Hille & Massey, 1986). In the catalytically active form of the enzymes the molybdenum bears a sulphido ligand (Gutteridge *et al.*, 1978; Bray, 1984). A peculiarity is that this ligand is rather readily converted, by loss of sulphide, into an oxo ligand, with resultant loss of catalytic activity. The active forms of molybdenum-containing hydroxylases are readily distinguished from the desulpho forms so produced by e.p.r. of molybdenum(V) (Gutteridge *et al.*, 1978; see also Dalton *et al.*, 1976; Barber *et al.*, 1976). Though desulpho forms can, and do, arise by degradation of the active enzymes, there is also evidence (Wahl *et al.*, 1982; Ikegami *et al.*, 1984) that they can in some circumstances occur *in vivo*.

About 10 years ago, Moura *et al.* (1976) described a molybdenum iron–sulphur protein isolated from *Desulfovibrio gigas*. No catalytic activity has been described for the protein, which has, however, been studied by e.p.r. (Moura *et al.*, 1978) and by e.x.a.f.s. (Cramer *et al.*, 1984). Though e.p.r. signals due to molybdenum(V) were not described in detail, some analogy with the desulpho form of xanthine oxidase was noted (Moura *et al.*, 1978) in reactions of the metal in the protein with oxidizing and reducing agents. Similarly, Cramer *et al.* (1984) reported that the e.x.a.f.s. spectrum of its molybdenum indicated an environment of the metal similar to that in desulpho xanthine oxidase. However, no conclusions about the nature of the *Desulfovibrio gigas* protein were deduced. We now report evidence that this protein is a form of an aldehyde oxidase or dehydrogenase.

MATERIALS AND METHODS

Protein and enzyme samples

The *Desulfovibrio gigas* protein was prepared essentially as described by Moura *et al.* (1978). Concentrations of the protein were determined by using a ϵ_{320} value of 101000 $M \cdot cm^{-1}$. The molybdenum content of the sample used for all the work described was colorimetrically determined (Hart *et al.*, 1970) as 1.05 mol of Mo/mol of protein. Xanthine oxidase was prepared as described by Hart *et al.* (1970) and Bray (1982) and its molybdenum content was determined colorimetrically.

Abbreviation used: e.x.a.f.s., extended X-ray absorption fine structure.

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Enzyme activity measurements

Purine and aldehyde dehydrogenase activities of the *Desulfovibrio gigas* protein were measured spectrophotometrically at 600 nm with 2,6-dichlorophenolindophenol (10 μM) as acceptor, at 25 °C in 50 mM-Na^+/Bicine buffer, pH 8.2. A 4 cm light path was used, and appropriate corrections were made where necessary for bleaching of the acceptor by the enzyme in the absence of substrates. The decrease of ϵ for indophenol reduction was taken as 21000 $M^{-1} \cdot cm^{-1}$.

Determination of the molybdenum cofactor content of the *Desulfovibrio gigas* protein

The procedures described by Hawkes & Bray (1984*a*) were applied. All operations, except for incubation of the complementation mixture, were carried out anaerobically in a glove cabinet operating at an oxygen content of 1 p.p.m. The protein, after dilution with 25 mM-potassium phosphate buffer, pH 7.4, containing 1 mM-EDTA, was denatured by addition to an excess of dimethyl sulphoxide containing 1–2 mM-$Na_2S_2O_4$ as described by Hawkes & Bray (1984*a*). Alternative denaturation procedures employed SDS (Hawkes & Bray, 1984*a*) or *N*-methylformamide (Hawkes & Bray, 1984*b*). Assay of the cofactor in the extracts was carried out as described by the former workers, by aerobic complementation for 24 h at 3.5 °C, with the apo nitrate reductase of partially purified extracts of the *nit-1* mutant of *Neurospora crassa*, to yield active nitrate reductase. Complementation was carried out in the presence of Na_2MoO_4 (10 mM).

E.p.r. measurements

E.p.r. spectra were recorded on a Varian E9 e.p.r. spectrometer operating at 9.3 or 35.3 GHz, interfaced to a computer (Bray *et al.*, 1978). Running conditions were generally as follows: temperature, 120 K; microwave power, 10 mW (or 10 dB attenuation for Q-band); modulation amplitude, 1.6 mT (5 mT for Q-band). Difference spectra were obtained as described by Bray *et al.* (1978), and computer simulations were performed as outlined by Bray & George (1985).

RESULTS

Molybdenum(V) e.p.r. studies of the *Desulfovibrio gigas* protein

In agreement with previous work (Moura *et al.*, 1978), untreated *Desulfovibrio gigas* protein gave an e.p.r. signal presumed to be due to molybdenum(V). The exact form of this signal varied somewhat, depending on the previous history of the sample. We did not investigate such signals, collectively referred to as the 'resting signals', in detail, but their form was found not to change if the solvent was changed from ^1H_2O to ^2H_2O. A resting signal is illustrated below in Fig. 3(*a*). Integrations corresponded to 1–7% of the molybdenum in the samples.

Again, in agreement with previous work (Moura *et al.*, 1978), we found that, on treatment of the protein with an excess of sodium dithionite for relatively long times (e.g. 15–30 min), the resting signals declined and were replaced by another signal, illustrated in Figs. 1 and 2. By analogy (to be discussed in detail below) with xanthine oxidase, we shall refer to this signal as the 'Slow' signal (Bray, 1980). We developed the Slow signal from the *Desulfovibrio gigas* protein both in ^1H_2O and ^2H_2O and recorded the spectra both at X-band (Fig. 1) and Q-band (Fig. 2). As illustrated, we were able to simulate satisfactorily all four spectra by using a single set of parameters (Table 1). This is strong evidence that a single species of molybdenum(V) is involved. (Residual resting signal is difficult to distinguish, since its main features coincide with those of the Slow signal; traces of resting signal are just discernible in Fig. 1*c*.) Integration of the Slow signal corresponded to 15–20% of the molybdenum in our experiments. This is comparable with the yield of the Slow signal from desulpho forms of various molybdenum-containing hydroxylases, though it is lower than the value of 85% conversion previously reported (Moura *et al.*, 1978).

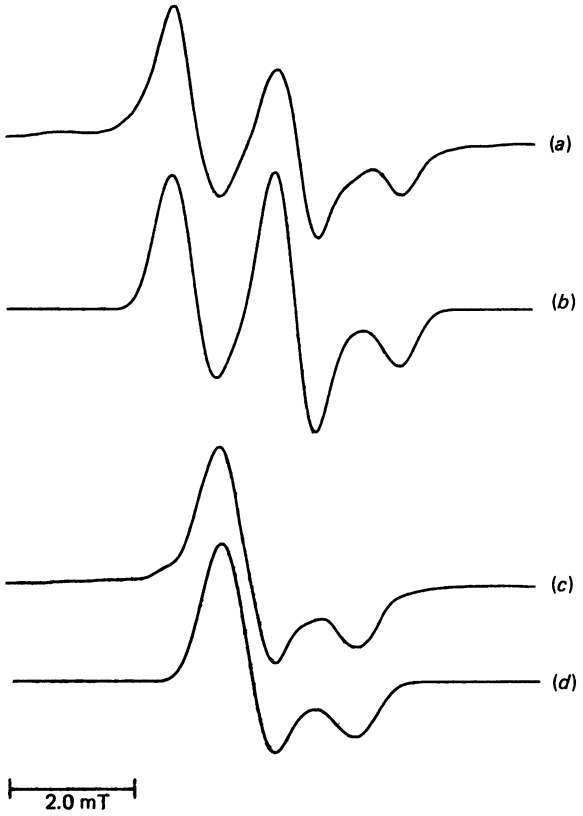

Fig. 1. E.p.r. spectra at X-band of the Slow Mo(V) signal from the *Desulfovibrio gigas* protein in ^1H_2O and in ^2H_2O

Samples of the protein in (*a*) ^1H_2O, or in (*c*) ^2H_2O, were reduced with 5 mM-sodium dithionite for 15 min then frozen. E.p.r. spectra were recorded and simulated as described in the Materials and methods section. (*b*) Corresponds to a simulation of (*a*), and (*d*) to a simulation (*c*). Parameters used for simulations are given in Table 1.

Active molybdenum-containing hydroxylases yielding the Rapid signal are normally contaminated (Bray, 1980) with the inactive desulpho form, yielding the Slow signal. During reduction of such preparations with dithionite, both signals are seen. However, the kinetics and thermodynamics of reduction of molybdenum(VI) in the two enzyme forms, through molybdenum(V) to molybdenum(IV), differ markedly (Bray *et al.*, 1975). Thus brief reduction with small amounts of dithionite yields the Rapid signal and longer reduction with larger amounts yields the Slow signal.

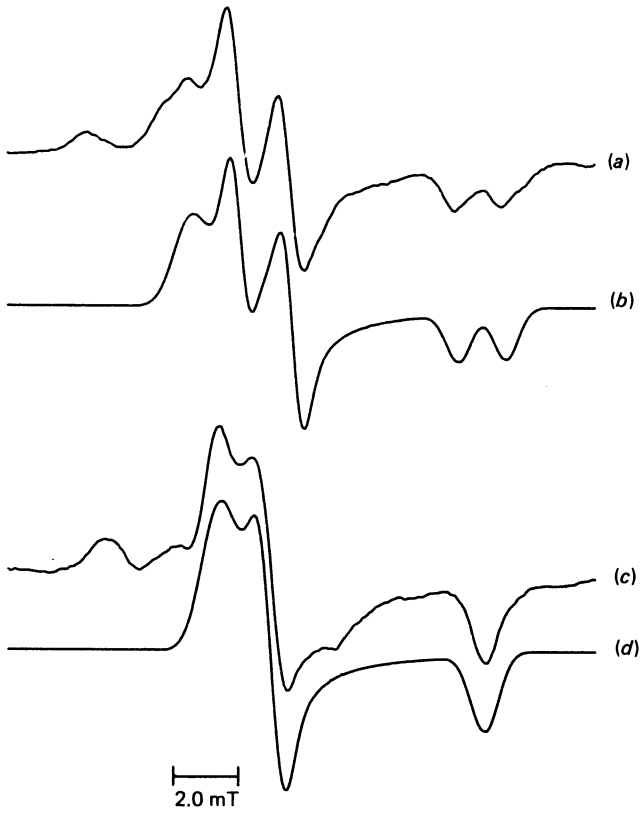

Fig. 2. E.p.r. spectra at Q-band of the Slow Mo(V) signal from the *Desulfovibrio gigas* protein in ^1H_2O and in ^2H_2O

The experiment and lettering of the spectra are analogous to those in Fig. 1, except that Q-band was used. A single set of parameters (apart from linewidths) was used for all the simulations.

We decided to investigate the effects of brief reduction with small amounts of dithionite on the *Desulfovibrio gigas* protein. Under these conditions, in contrast with the situation when large amounts of the reducing agent were used as described above, the resting signal did not decrease in intensity. However, close examination revealed that weak additional features were present also in the spectra. By using difference techniques to subtract out the resting signals, the spectrum of Fig. 3(*e*) was obtained. This shows a distinct resemblance to Rapid type 2 signals (Bray, 1980) from molybdenum-containing hydroxylases.

This finding encouraged us to look for xanthine oxidase-like and aldehyde oxidase-like catalytic activity from the *Desulfovibrio gigas* protein. As discussed in the next subsection, this work revealed salicylaldehyde, among other compounds, to be a substrate. We then tried the effect (Figs. 3*a*–3*d*) of using salicylaldehyde as reductant on the e.p.r. spectrum. Resting signals initially present are illustrated in Fig. 3(*a*). Addition of salicylaldehyde changed the spectrum to that in Fig. 3(*b*). Subtraction of the spectrum of Fig. 3(*a*) from that of Fig. 3(*b*) yielded Fig. 3(*c*). This spectrum is scarcely distinguishable from that of Fig. 3(*e*), referred to above and obtained with dithionite.

We carried out further experiments, in ^2H_2O in place of ^1H_2O as solvent, on the effects of small additions of dithionite on the spectrum. A difference spectrum obtained under these conditions is illustrated in Fig. 3(*f*). A simulation of the spectrum of Fig. 3(*c*) is illustrated in Fig. 3(*d*), and one of that in Fig. 3(*f*) in Fig. 3(*g*). These simulations used a single set of parameters, as given in Table 1. In view of the relative weakness of the signals, we did not attempt to study them at Q-band. Because of this, and of possible differences between the signals generated with dithionite and salicylaldehyde (perhaps due to contamination of the former by traces of the Slow signal), the parameters must be regarded as somewhat preliminary. However, the assignment of these signals from the *Desulfovibrio gigas* protein to the Rapid class is quite unambiguous. Intensity of the Rapid signal generated with salicylaldehyde corresponded to about 5% of the molybdenum.

Catalytic activity

The obvious analogies between the e.p.r. spectra of the *Desulfovibrio gigas* protein and the molybdenum-containing hydroxylases encouraged us to look for catalytic activity. In the absence of flavin, on which oxidase activity of the enzymes depends, we looked for dehydrogenase activity with 2,6-dichlorophenol-indophenol as electron acceptor. Data from a limited range of substrates are illustrated in Table 2 and are compared with corresponding data from xanthine oxidase. We used fixed substrate concentrations that were near to the optimal values for xanthine oxidase. Because of inhibition of xanthine oxidase by excesses both of purine substrates (Hofstee, 1955; Hille & Stewart, 1984) and of 2,6-dichlorophenol-indophenol (Gurtoo & Johns, 1971), we did not attempt to determine K_m values.

Aldehyde dehydrogenase activity was clearly observed for the *Desulfovibrio gigas* protein with all the three aldehydes which were tested (Table 2). Activities of this protein, as a fraction of that of 100% functional xanthine oxidase, ranged, under the conditions of measurement, from 6 to 16%, the highest value being recorded with salicylaldehyde. On the other hand, no catalytic activity was observed with purine or xanthine. For these, activity relative to that of xanthine oxidase was lower by at least three orders of magnitude than that for aldehyde substrates.

Molybdenum cofactor activity

The molybdenum cofactor may be liberated from xanthine oxidase and under appropriate conditions transferred quantitatively to the apo nitrate reductase in extracts from the *nit-1* mutant of *Neurospora crassa* to yield active nitrate reductase (Hawkes & Bray, 1984*a*). This procedure, followed by assay of the nitrate reductase activity, forms the basis for the standard assay of molybdenum-cofactor activity.

Typical data obtained on the *Desulfovibrio gigas* protein in comparison with xanthine oxidase are illustrated in Fig. 4. The molybdenum cofactor was clearly liberated from this protein by treatment with dimethyl sulphoxide and complemented the apo nitrate reductase. However, the molybdenum-cofactor activity observed was lower than that for xanthine oxidase, calculated on the basis of the molybdenum content of the starting proteins. Values obtained, for the molybdenum-cofactor activity of the *Desulfovibrio gigas* protein in the experiment of Fig. 4 and in three comparable experi-

Table 1. E.p.r. parameters in Mo(V) species from the *Desulfovibrio gigas* protein in comparison with those of some molybdenum-containing hydroxylases

Parameters given for the *Desulfovibrio gigas* protein were used for the simulations in Figs. 1–3; data from the literature on various molybdenum-containing hydroxylases are given for comparison. *g*-Values are believed to be correct to ±0.0003; hyperfine couplings and half-linewidths are in mT.

		g-Values				$A(^1H)$				
Protein	Signal	1	2	3	Average	1	2	3	Average	Reference
D. gigas	Slow*	1.9705	1.9680	1.9580	1.9655	1.68	1.60	1.44	1.57	The present work
Aldehyde oxidase	Slow	1.9720	1.9668	1.9556	1.9648	1.60	1.60	1.65	1.62	N. Turner & R. C. Bray (unpublished work)
Xanthine oxidase	Slow (nitrate)	1.9689	1.9666	1.9571	1.9642	1.50	1.48	1.52	1.50	Gutteridge *et al.* (1978)
Xanthine oxidase	Slow	1.9719	1.9671	1.9551	1.9647	1.66	1.66	1.56	1.63	Gutteridge *et al.* (1978)
						0.16	0.16	0.16	0.16	
D. gigas	Rapid type 2†‡	1.9882	1.9702	1.9643	1.9742	1.15	1.66	1.26	1.36	The present work
						1.14	0.63	0.93	0.90	
Aldehyde oxidase	Rapid type 2	1.9895	1.9700	1.9622	1.9739	1.38	1.50	1.50	1.46	Bray *et al.* (1984)
						1.13	0.65	0.71	0.83	
Xanthine oxidase	Rapid type 2 (pyridine-3-aldehyde)	1.9861	1.9695	1.9623	1.9726	1.35	1.57	1.45	1.46	Bray *et al.* (1984)
						1.35	0.74	0.99	1.03	

* Linewidths at X- and Q-band respectively were 0.26, 0.23, 0.26; 0.48, 0.27 and 0.37.
† Linewidths were 0.26, 0.25 and 0.24.
‡ Note that the combination of hyperfine splittings shown is arbitrary. Though there are clearly two protons giving splittings of 1.26 and 0.93 mT in the A_3 direction, it is uncertain which of these gives the larger and which the smaller splitting in the A_2 (and A_1) directions.

ments, were 4, 6, 6 and 5 nmol of NO_2^- formed/min per pg-atom of Mo respectively. In the first two experiments (cf. Fig. 4) the denaturant was dimethyl sulphoxide, in the third it was *N*-methylformamide, and in the fourth it was SDS. In the four corresponding experiments with xanthine oxidase, the observed molybdenum-cofactor activity averaged 21 ± 1 nmol of NO_2^- formed/min per pg-atom of Mo. Thus cofactor activity of the *Desulfovibrio gigas* protein averaged only 25% of that of xanthine oxidase.

DISCUSSION

Our results make it clear that the *Desulfovibrio gigas* protein is a molybdenum-containing hydroxylase. Similarities to and differences from other enzymes of this class therefore have to be discussed in the light of our data and of that in the literature.

The molybdenum centre

Considering first the molybdenum centre, e.p.r. results indicate that this is present in both the desulpho form and the functional form. Relative intensities of the Rapid and Slow signal would suggest perhaps 30% of the active form and 70% of the desulpho forms of the protein. But these values could well be substantially in error, as we made little attempt to maximize the signal intensities. Since, on storage, functional molybdenum-containing hydroxylases gradually become degraded to the desulpho form, different samples of the protein would of course be expected to differ in their contents of these species. This, and the limitations (cf. Bray, 1984) of the e.x.a.f.s. method when dealing with mixtures, no doubt explain the failure of Cramer *et al.* (1984) to find evidence, in preparations of the *Desulfovibrio gigas* protein, of a short molybdenum–sulphur bond arising from the sulphido ligand of the metal in the active enzyme form. From work on xanthine oxidase (Bordas *et al.*, 1980) and xanthine dehydrogenase (Cramer *et al.*, 1981) such a bond is expected in functional molybdenum-containing hydroxylases.

E.p.r. parameters of the Rapid and Slow signals from the *Desulfovibrio gigas* protein are compared with those of corresponding signals from xanthine oxidase and aldehyde oxidase in Table 1. The Slow signals from the three proteins are very similar to one another. In the case of the *Desulfovibrio gigas* protein, the g_1 and g_2 values are quite close together. In this its signal is more like that of the nitrate complex of xanthine oxidase (Gutteridge *et al.*, 1978) than the one obtained from this enzyme in Bicine buffer without added anions.

The Rapid signal from *Desulfovibrio gigas* protein is of the type 2 variety. Molybdenum-containing hydroxylases give type 1 or type 2 Rapid signals, or mixtures of these. Though the distinction between the two types is somewhat blurred (Bray *et al.*, 1984), the type 2 Rapid signals show two strongly coupled protons exchangeable with the solvent, and type 1 signals show one strongly coupled and one weakly coupled proton. For the type 2 Rapid signal from the *Desulfovibrio gigas* protein, the two strongly coupled protons are equally coupled in the A_1 direction, but unequally coupled in the A_2 and A_3 directions. In this respect this signal resembles those from aldehyde oxidase and those obtained from xanthine oxidase with pyridine-3-aldehyde (Bray *et al.*, 1984), but differs from other Rapid type 2 signals (Malthouse *et al.*,

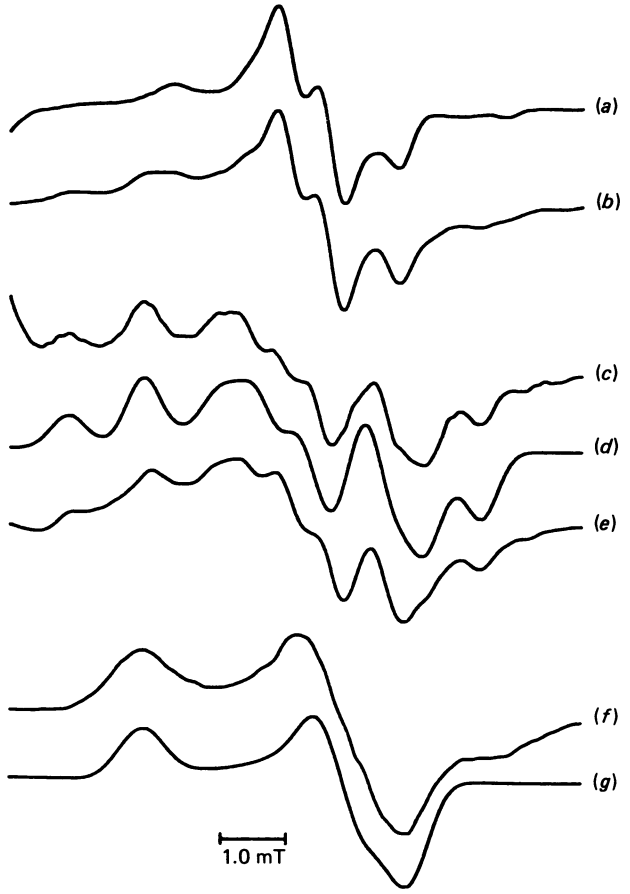

Fig. 3. The Resting signal, and the Rapid signal obtained by difference, from the *Desulfovibrio gigas* protein

The Resting signal from the protein is shown in (*a*) (slight variations in the form of this signal were noted, depending on the history of the sample). (*b*) Corresponds to the sample of (*a*) after anaerobic addition of salicylaldehyde (1.5 mM) and incubation for 90 s before freezing. (*c*) Is a difference spectrum obtained from (*a*) and (*b*) and corresponds to $[(b)-0.95(a)]\times 3$. (*d*) Is a simulation of (*c*) and was obtained by using the parameters given in Table 1. (*e*) Is the Rapid signal obtained by reduction of the protein with sodium dithionite (2 mM for 2 min). Again, the spectrum illustrated was obtained by difference, from spectra analogous to those in (*a*) and (*b*). All the above signals were generated in ^1H_2O. Spectrum (*f*) was obtained by reduction with dithionite in ^2H_2O analogously to spectrum (*e*). (*g*) Is a simulation of (*f*) and was obtained by using the same parameters (Table 1) as were used for (*d*).

1981) from xanthine oxidase. The similarity of the parameters, as listed in Table 1, for the Rapid type 2 signals from the three proteins is indeed striking.

It cannot be claimed that e.p.r. parameters of the *Desulfovibrio gigas* protein are more like those of aldehyde oxidase than of xanthine oxidase. On the other hand this protein resembles aldehyde oxidase in that no type 1 Rapid signals have so far been described for it. This is in sharp contrast with the situation for xanthine oxidase, for which the type I signal is probably the more common form in the considerable number of Rapid signals that have been studied.

Our measurements of catalytic activity (Table 1) are

Table 2. Enzymic activity of the *Desulfovibrio gigas* protein in comparison with xanthine oxidase

Activity was measured with various substrates, as described in the Materials and methods section, with 2,6-dichlorophenol-indophenol as electron acceptor. The xanthine oxidase sample was 65% functional, but the values given are those calculated for 100% functional enzyme. No such correction was applied to the data for the *Desulfovibrio gigas* protein.

Substrate (concn.)	Activity [$k_{cat.}$ (s^{-1})]	
	Xanthine oxidase	*D. gigas* protein
Xanthine (1 mM)	6.1	< 0.0005
Purine (1 mM)	4.4	< 0.0005
Salicylaldehyde (1 mM)	1.6	0.25
Acetaldehyde (10 mM)	2.0	0.12
Propionaldehyde (10 mM)	0.1	0.007

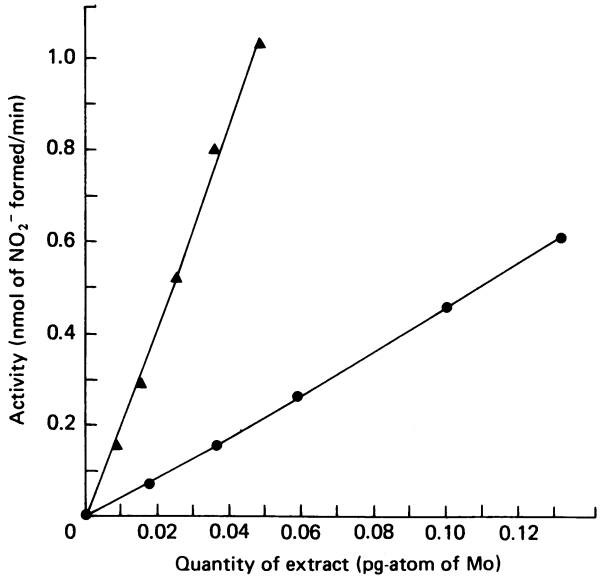

Fig. 4. Molybdenum-cofactor activity, measured by the *nit-1* complementation assay, for cofactor liberated from the *Desulfovibrio gigas* protein, in comparison with that from xanthine oxidase

Cofactor activity (in terms of nitrate reductase activity) was measured, as described in the Materials and methods section, after aerobic incubation for 24 h at 3.5 °C of various quantities of extracts, prepared by denaturation with dimethyl sulphoxide, from xanthine oxidase (▲) or from the *Desulfovibrio gigas* protein (●). Quantities of the extracts are expressed (see the Materials and methods section) in pg-atom of Mo in the protein before denaturation. A fixed quantity of partly purified *nit-1* extract was used in all cases, with the addition of Na_2MoO_4 (10 mM). The slope of the graphs corresponds to a molybdenum-cofactor activity of 21 nmol of NO_2^- formed/min per pg-atom of Mo for the cofactor from xanthine oxidase, and an activity of 4 nmol of NO_2^- formed/min per pg-atom of Mo for that from the *Desulfovibrio gigas* protein.

in no sense exhaustive, and further work is required (B. Barata & J. Moura, unpublished work). Molybdenum-containing hydroxylases show extremely wide and rather variable specificities, with even the distinction between aldehyde oxidases and xanthine oxidases being not particularly clear (Bray, 1975) and with additional molybdenum-containing hydroxylases (e.g. Mehra & Coughlan, 1984) known. Two points stand out from our data, however. The first is the complete absence of activity with xanthine and with purine. This clearly shows the protein to be of the aldehyde oxidase rather than the xanthine oxidase type (cf. Krenitsky *et al.*, 1972; Badwey *et al.*, 1981). The other point is the lowness of the activity under our conditions of measurement with aldehydes, averaging only 10% of that of xanthine oxidase. Although this might suggest a relatively low content of the functional enzyme, the data in Table 2 were obtained for the sake of comparison and may very well not represent maximal activity for the *Desulfovibrio gigas* protein.

Our finding of molybdenum-cofactor activity in the protein is consistent with the other results. The low value observed could be due to a number of factors. Desulpho xanthine oxidase yields cofactor indistinguishable (Hawkes & Bray, 1984*a*,*b*; Wahl *et al.*, 1984) from that from the functional enzyme. If, on the other hand, some damaged cofactor molecules were present, this might be expected to affect not only cofactor activity, but also the e.p.r. of the enzyme. However, in view of the great similarities of the e.p.r. spectra to those of other molybdenum-containing hydroxylases, as described above, this explanation seems unlikely. Alternatively, it could be that it is more difficult to liberate the cofactor from this protein than is the case for xanthine oxidase. This explanation is rendered unlikely by the finding that similar activities were obtained with three different denaturation procedures. Thus we are left with the likelihood that the molybdenum cofactor from *Desulfovibrio gigas* may be chemically slightly different from that from other molybdenum-containing hydroxylases, resulting in its being less efficient in the complementation assay.

Iron–sulphur centres and other parts of the molecule

Aside from the molybdenum centre, the *Desulfovibrio gigas* protein, though it contains no flavin, has other similarities to molybdenum-containing hydroxylases and their degradation products. Particularly striking is the resemblance of its absorption and c.d. spectra (Moura *et al.*, 1976) to those of deflavo xanthine oxidase (Komai *et al.*, 1969). Similarly, e.p.r. signals from its iron–sulphur centres in the reduced state show *g*-values (Moura *et al.*, 1978) that are close to, or within the range of, values reported for the [2Fe–2S] centres of molybdenum-containing hydroxylases (Bray, 1975; Dalton *et al.*, 1976; Barber *et al.*, 1976, 1982; Coughlan *et al.*, 1984; Hille *et al.*, 1985). However, there are differences of Fe/Mo ratios and of M_r, with the *Desulfovibrio gigas* protein reported to have a higher Fe content (Moura *et al.*, 1978) and a lower M_r (Moura *et al.*, 1976) than those of normal molybdenum-containing hydroxylases (Bray, 1975).

Origin of the *Desulfovibrio gigas* protein

It is abundantly clear that the *Desulfovibrio gigas* protein is a form of aldehyde oxidase or aldehyde dehydrogenase. It might represent a product arising from degradation of a normal molybdenum-containing hydroxylase or, alternatively, it might be a protein with a molybdenum domain like that in molybdenum-containing hydroxylases but with the rest of the molecule substantially different. Unfortunately, available data are not adequate to distinguish unambiguously between these hypotheses.

The M_r data and the absence of flavin in the protein could be explained by proteolytic degradation. Treatment of xanthine dehydrogenase from chicken or turkey liver with subtilisin at high pH values yields flavin-free degradation products of M_r 65000 (Ni Fhaolain & Coughlan, 1977) and 120000 (Coughlan *et al.*, 1979) respectively for enzyme from the two sources. It would obviously be desirable to isolate the *Desulfovibrio gigas* protein under conditions designed to minimize proteolysis (and conversion of the functional into the desulpho form) and to determine the molybdenum and iron–sulphur contents and M_r of this material. So far there have been no indications of the presence of FAD in the partly purified protein. Though a flavoprotein co-purifies (Moura *et al.*, 1978) to some extent with it, this has not as yet been characterized or shown to be in any way related to the molybdenum iron–sulphur protein. Note that a low molybdenum content relative to iron could be due to incomplete incorporation of molybdenum into the molybdenum domain [cf. demolybdo xanthine oxidase; Hart *et al.*, 1970; A. Ventom & R. C. Bray, unpublished work]. Furthermore, the presence of much desulpho form does not unambiguously indicate degradation *in vitro*, as opposed to incomplete formation of the functional enzyme. On the whole, presently available data seem to point to the protein representing a molybdenum-containing hydroxylase, lacking flavin and with extra iron–sulphur centres.

The work has important implications from an evolutionary point of view. *Desulfovibrio gigas* performs one of the most primitive types of respiration: the dissimilatory reduction of sulphate. The question arises as to how a molybdenum-containing hydroxylase could be related to such an ancient process. Since the organism is a strict anaerobe, it is interesting, if not particularly surprising, that the enzyme (at least as currently prepared) lacks flavin, the part of the molecule with which oxygen reacts. The finding (depending primarily on activity measurements rather than on e.p.r.) that the enzyme is of the aldehyde oxidase, rather than of the xanthine oxidase, type suggests that the former evolved before the latter (cf. Krenitsky *et al.*, 1972). Finally, the occurrence of the enzyme in *Desulfovibrio gigas* is consistent with the fact that this bacterium can grow with ethanol as carbon and energy source (F. Widdel, personal communication).

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