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Identification of a membrane-bound hydrogenase of *Desulfovibrio vulgaris* (Hildenborough)

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Two hydrogenase activities from *Desulfovibrio vulgaris* (Hildenborough) could be distinguished immunologically and biochemically. The first activity, described as hydrogenase I, corresponded to the soluble enzyme located in the periplasmic space of *D. vulgaris*. Hydrogenase I had a high specific activity and was sensitive to inhibition by CO. The second activity, hydrogenase II, was located in the membrane fraction, had a lower specific activity and was not affected by CO. The enzymes exhibited different electrophoretic mobilities in polyacrylamide gels, and reacted differently when exposed to proteases. Antibodies raised against purified periplasmic hydrogenase of *D. vulgaris* reacted with hydrogenase I, but not with hydrogenase II.

Introduction

Hydrogenase catalyses the reversible reaction $H_2 \leftrightarrows 2H^+ + 2e^-$ and has been postulated to play a central role in the hydrogen metabolism of sulphate-reducing bacteria [1]. Hydrogen may be used as an energy source acting as the initial electron donor to the electron-transport chain that leads to sulphate reduction. Various hydrogenases have been described in *Desulfovibrio* spp. that could fulfil this role; membrane-bound enzymes in *Desulfovibrio desulfuricans* (strain Norway 4) [2] and *Desulfovibrio vulgaris* (Miyazaki) [3]; periplasmic enzymes of *D. vulgaris* (Hildenborough) [4]; *Desulfovibrio gigas* [5] and *D. desulfiricans* NRC (National Research Council, Ottawa, Canada) 49001 [6]. These enzymes differ in their cellular location and in their physical parameters. However, all can catalyse the reversible reaction in vitro and use cytochrome c_3 as a natural electron acceptor in the H_2 consumption reaction.

Several hypotheses, however, have been presented that would require the presence of both hydrogen-producing and hydrogen-consuming enzymes in *Desulfovibrio*. One such hypothesis is that of obligate hydrogen cycling linked to chemiosmotic energy transduction [7]. In this scheme H_2 is produced in the cytoplasm during lactate oxidation, then transferred to the periplasm where its consumption is linked directly to the generation of a proton-motive force. Thus energy is conserved and excess reductant is removed from the cell. Another model [8] envisages an internal hydrogenase acting to transform trace amounts of H_2 to control the redox state of internal electron carriers involved in chemiosmotic energy conservation.

Alternatively, a H_2-producing activity could be linked to some other function of the cell. Such an activity could be associated, for example, with carbon monoxide dehydrogenase and be stimulated by CO [9]. Most hydrogenases studied, however, have been found to be sensitive to inhibition

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by CO [10] with the exceptions of the hydrogenases of *Alcaligenes eutrophus* [11] and *Methanobacterium formicicum* [12]. In addition, in this laboratory we have observed an hydrogenase activity from *D. vulgaris* (Hildenborough) that is apparently not inhibited by CO. This H_2 consumption activity was revealed upon polyacrylamide gel electrophoresis of whole cell extract [13].

Circumstantial evidence has already been presented suggesting that *Desulfovibrio* contains more than one hydrogenase activity. Martin et al. [14] had reported more than one band of activity upon polyacrylamide gel electrophoresis of whole cell extracts of *D. desulfuricans*, though it was not clear that the membrane-bound activity was different to the periplasmic activity described therein. Another line of evidence is the increase in hydrogenase activity observed upon the addition of Triton X-100 to whole cells of *D. vulgaris* [7]. This could be explained by the detergent causing the cell membrane to become permeable to benzyl viologen, the reaction substrate, thus allowing more hydrogenase from inside the cells to have access to the substrate. However Triton X-100 can have a stimulating effect upon hydrogenases (Pankhania, I., unpublished data; see also Ref. 15), so again this evidence is not unequivocal.

Recently, more convincing evidence has been obtained indicating the presence of more than one hydrogenase in *Desulfovibrio*. There are reports of two hydrogenases in *D. vulgaris* (Miyazaki) differing in molecular weight [16], and three different enzymes have been found in *D. desulfuricans* (strain Norway 4) present in the periplasm, the membranes and the cytoplasm [17].

In this paper we describe the further characterisation of the CO-insensitive hydrogenase activity in *D. vulgaris* (Hildenborough) and confirm that there are at least two hydrogenases present.

Materials and Methods

Growth of organism

Desulfovibrio vulgaris strain (Hildenborough), National Collection of Industrial Bacteria, Aberdeen, Scotland, NCIB 8303, was grown at 35°C in Saunders Medium N [18] under N_2 or N_2/CO_2 (80 : 20) and cells were harvested at the end of the exponential phase of growth (approx. 1 g wet weight of cells/l). (If H_2 was used as gas-phase it was found that hydrogenase activities were extremely sensitive to inactivation by O_2 (Pankhania I., unpublished data).)

Hydrogenase assays, electrophoretic and immunoelectrophoretic procedures

Hydrogenase was assayed at 35°C by two methods. The rate of H_2 evolution was measured with a hydrogen electrode (Hansatech Ltd., King's Lynn, Norfolk, U.K.). 5 mM methyl viologen in 50 mM potassium phosphate (pH 6.0) was reduced with dithionite prior to the addition of enzyme. The electrode was calibrated by adding known quantities of hydrogen-saturated water at 35°C. The solubility of H_2 at 35°C is 74.56 mM/100 μl distilled water. Hydrogenase activity was also measured spectrophotometrically by following the reduction of methyl viologen at 578 nm (extinction coefficient, 9.7 $mM \cdot cm^{-1}$). Stoppered cuvettes, containing 2 mM methyl viologen, 50 mM Tris-HCl (pH 8.8), 10 mM glucose and 5 units per ml glucose oxidase (to remove final traces of O_2), were repeatedly (5 times) evacuated and gassed with 100% H_2. One unit of enzyme activity was defined as 1 μmol H_2 reduced per min. Activity on immunoplates or polyacrylamide gels was located by immersion in 100 mM potassium phosphate (pH 7.0), 0.5 mM benzyl viologen, 1 mM triphenyl tetrazolium chloride and incubation under H_2 for up to 24 h at 24°C which resulted in the appearance of intense red zones. Protein was determined by the method of Lowry [19]. Electrophoretic analysis and crossed immunoelectrophoresis were performed as described previously [15].

Purification of periplasmic hydrogenase

Periplasmic hydrogenase was extracted [13,20] then purified as follows: ammonium sulphate precipitation was carried out and the 70–100% (w/v) fraction (containing 90% of the hydrogenase activity) retained and dialysed against 10 mM Tris-HCl (pH 8.0)/20 mM NaCl/0.02% (w/v) NaN_3. The dialysed solution was applied to a column of DEAE Sephacel (1.6 × 20 cm) equilibrated in the dialysis buffer then eluted with 120 mM NaCl in the same buffer. The combined fractions were concentrated using an Amicon ultrafiltration ap-

paratus with a YM 30 membrane, then applied to a Sephadex G-75 column (1.5 × 80 cm) equilibrated with 10 mM potassium phosphate (pH 7.5) 0.02% (w/v) NaN_3. Fractions containing hydrogenase activity were pooled and applied to a hydroxylapatite column (1.6 × 15 cm) equilibrated with 10 mM potassium phosphate (pH 7.5). Hydrogenase was eluted with 100 mM potassium phosphate (pH 7.5), then finally concentrated on the Amicon ultrafiltration apparatus. A value of 46 000 was found for the subunit molecular weight on sodium dodecyl sulphate-polyacrylamide gel electrophoresis [21]. This compares with 49 000 found by Van der Westen et al. [20] for this monomeric enzyme.

Preparation of whole cell extract and raising of antibodies

The cells were suspended (1 g wet weight/4 ml buffer) in 50 mM Tris-HCl (pH 7.5)/5 mM benzamidine HCl and broken by passage through a French pressure cell. Cellular debris was removed by centrifugation at 10 000 × *g* for 15 min and the supernatant was used as the cell extract. The cell extract (15 mg of protein/ml) immunogen was prepared by adding Triton X-100 to 1% (w/v) concentration and then mixing with an equal volume of Freund's complete Adjuvant. This was injected into rabbits intramuscularly twice with a 2-week interval between injections and the rabbits were bled regularly from a week after the second injection. Antibodies were stored at −80°C.

The purified periplasmic hydrogenase gave one zone with hydrogenase activity after electrophoresis in non-denaturing polyacrylamide gels. This zone was excised, the hydrogenase eluted and mixed with an equal volume of Freund's complete Adjuvant before injection into the rabbit as described above.

Solubilisation of membranes

Whole cell extract was fractionated by centrifugation and the membrane fraction (100 000 × *g*) was incubated in 4% (w/v) Triton X-100 for 20 min at 4°C. Insoluble material was removed by centrifugation (100 000 × *g*, 60 min).

Immunoprecipitaton

Immunoprecipitation was carried out as described previously [22].

Results

Hydrogenase activities on polyacrylamide gel electrophoresis

Periplasmic hydrogenase shows a distinct activity stain ($R_f = 0.45$) on tube gels. (The purified hydrogenase revealed only three bands that stained for protein – the band of R_f value 0.45 apparently comprising more than 90% of the total protein stained on the gel). Whole-cell extract reveals a similar activity stain ($R_f = 0.45$) with some more activity visible at the top of the gel [13]. To clarify these findings whole cell extract was fractionated (see next section) and the soluble components separated from the membrane-bound components of the cell. These membranes were solubilized with the detergent Triton X-100.

Fig. 1a shows the activity stain found in the soluble fraction (fraction V) from cell fractionation. The R_f value is 0.45, the same as that of periplasmic hydrogenase. Activity staining of solubilized membranes run on polyacrylamide gel electrophoresis revealed two red-staining bands 2 and 1, R_f values 0.12 and 0.45 (Fig. 1b), respectively. In none of the cases described above did an activity appear under incubation with N_2. Thus H_2 is an essential substrate for these activities.

Gels incubated with H_2, but in the presence of CO did not show the activity stain of R_f value 0.45, in the case of solubilized membranes (Fig. 1c), or soluble cell fraction or purified periplasmic hydrogenase. This band did subsequently develop, however, upon removal of CO. Thus CO is reversibly inhibiting the hydrogenase activity of mobility 0.45. To aid clarity, this CO-sensitive activity will now be referred to as hydrogenase I.

The activity of lower mobility (R_f value, 0.12) present in solubilized membranes was still visible upon incubation with H_2 in the presence of CO. Subsequently, we refer to this CO-insensitive activity as hydrogenase II.

These results suggest that there is a discrete hydrogenase enzyme present in the membranes that is not inhibited by CO. It should be noted that the solubilized membranes also contain some CO-sensitive hydrogenase I. This is believed to be only loosely associated with the membrane (see next subsection).

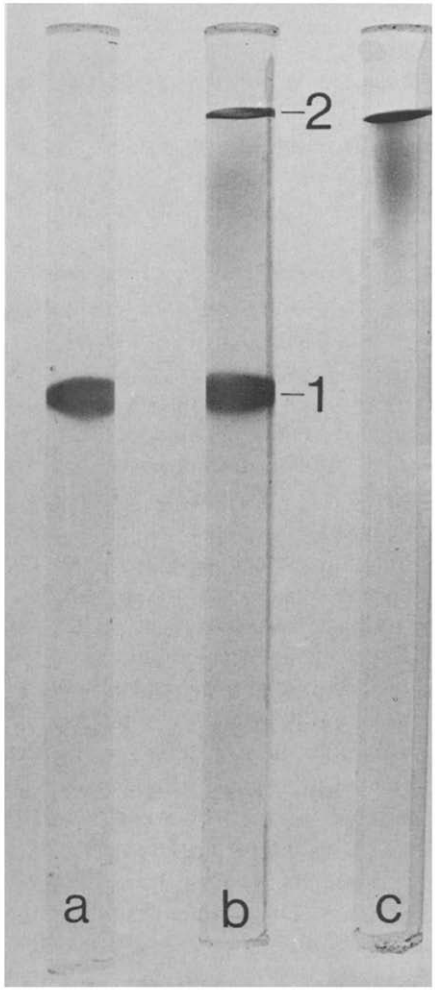

Fig. 1. Analysis of cell fractions for hydrogenase activity by non-dissociating polyacrylamide (tube) gel electrophoresis. Samples were electrophoresed on a 7.5% (w/v) polyacrylamide gel and subsequently stained for hydrogenase activity. Samples analysed were: (a) 5 μg fraction V from Table I (high speed supernatant); (b) 20 μg Triton X-100 dispersed membrane protein; (c) 20 μg Triton X-100 dispersed membrane protein + CO (approx. 0.5 mM).

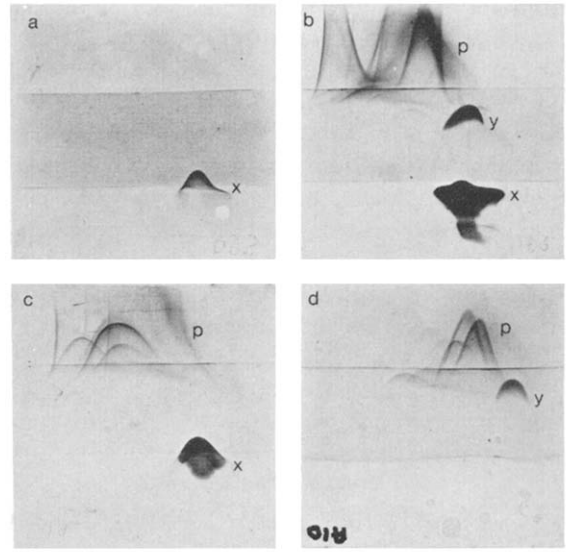

Fig. 2. Crossed immunoelectrophoresis. Aliquots of (a) purified hydrogenase I (2 μg), (b) Triton X-100 dispersed membranes (20 μg), (c) Fraction V (30 μg) and (d) sample 'b', (5 μg) the first protein peak after passage through a Sephadex G100 column (1.6 × 90 cm) were electrophoresed in the first horizontal dimension. Electrophoresis in the second vertical dimension was into agarose containing antiserum to purified hydrogenase I then into antiserum to whole cells. Plates were stained for hydrogenase activity ('x' and 'y'), then subsequently stained for protein ('p'). 'x' arcs show the presence of an activity equivalent to hydrogenase I (plates a, b, c). 'y' arcs indicate the presence of another activity found in membranes (plates b, d). 'p' arcs are due to proteins interacting with anti 'whole cell' serum, but not associated with hydrogenase activity.

Cellular location of hydrogenase activities

Table I details the distribution of hydrogenase activity found during subcellular fractionation of *D. vulgaris* cell extract. In the whole-cell extract most of the hydrogenase activity (more than 95%) is inhibited in the presence of CO. This CO-sensitive activity (hydrogenase I) is mainly located in the soluble fraction of the cell and thus is found in the supernatant fractions. However, in the membrane fraction (fraction IV) more than 20% of recovered activity was not sensitive to CO (hydrogenase II). The specific activity of hydrogenase II is highest in fraction IV, but is very much lower than hydrogenase-I specific activity in cells grown on Saunders medium. CO-sensitive activity in fraction IV may be due to trapped soluble hydrogenase I or a membrane-associated form of hydrogenase I. Repeated washings and further centrifugation at 100 000 × *g* resulted in a lowering of hydrogenase I activity in the pellet (data not shown). This supports the theory that hydrogenase I is loosely associated with the membranes and can be removed by repeated washings.

As hydrogenase II is present in solution with hydrogenase I these data do not exclude the possi-

TABLE I

SUBCELLULAR FRACTIONATION OF *DESULFOVIBRIO VULGARIS* (HILDENBOROUGH)

Cells (1.5 g wet weight) were harvested and suspended in 50 mM Tris-HCl (pH 7.5)/50 mM NaCl/1 mM dithiothreitol and broken by passage through a French pressure cell. The cell extract thus obtained was centrifuged at $10000 \times g$ (20 min). The $10000 \times g$ supernatant was centrifuged at $100000 \times g$ (60 min). Hydrogen consumption activity was then measured in each fraction in the presence and absence of CO (approx. 0.25 mM).

Fraction	Total activity (Units)		Specific activity (Units/mg protein)	
	−CO	+CO	−CO	+CO
I whole-cell extract	385	5.8	2.5	0.004
II $10000 \times g$ pellet	20	0.3	0.7	0.001
III $10000 \times g$ supernatant	360	5.5	2.9	0.004
IV $100000 \times g$ pellet	20	4.3	0.8	0.017
V $100000 \times g$ supernatant	322	1.0	3.3	0.001

bility that hydrogenase II is to some degree sensitive to inhibition by CO. However, further work using immunoprecipitation to separate the two activities indicates that hydrogenase II is not inhibited by CO (see next section and Fig. 4).

Routinely fraction IV was recentrifuged at $100000 \times g$, to remove traces of soluble hydrogenase then the pellet was dispersed with Triton X-100 (4% w/v) to obtain solubilized membranes.

Immunological analysis of the hydrogenase species

Antibodies specific to hydrogenase I (periplasmic hydrogenase) were prepared. These antibodies gave a single major protein-staining and hydrogenase-activity-staining arc when analysed by crossed immunoelectrophoresis with purified hydrogenase I as antigen (Fig. 2a). Anti 'hydrogenase I' serum also formed an activity staining arc with Triton X-100 dispersed membranes (Fig. 2b'x') and with fraction V, the soluble cell fraction (Fig. 2c'x').

This shows that the membranes as prepared contain the hydrogenase of periplasmic origin. This would be expected to constitute band I seen in the activity stained gel (Fig. 1b). Thus all three samples analysed, i.e., purified hydrogenase I, soluble cell fraction and solubilized membranes, reacted with antibodies to hydrogenase I. This strongly suggests that all three samples contain hydrogenase I and this is supported by the results of polyacrylamide gel electrophoresis.

The membrane fraction was also examined by crossed immunoelectrophoresis employing anti-serum prepared to the total extract, on the same cross plate as that with antiserum to hydrogenase I (Fig. 2b). A single precipitin arc exhibiting hydrogenase activity was observed (Fig. 2b'y'). This activity could be separated from hydrogenase I by passing the membrane fraction through a Sephadex G-100 column (Fig. 2d'y'). Hydrogenase I had a greater retention time than this second activity, which eluted in the void volume of the column.

When purified hydrogenase I was analysed by crossed immunoelectrophoresis against the anti-'whole cell' serum neither a hydrogenase-staining nor a protein-staining precipitin arc was seen (data not shown). Anti-'whole cell' serum then does not cross-react with periplasmic hydrogenase. The soluble cell fraction did not show an activity arc with anti-'whole cell' serum (data not shown). Protein-staining arcs were evident on crossed immunoelectrophoresis (Fig. 2c'p') after precipitation of hydrogenase I with antiserum (Fig. 2c'x').

It appears, therefore, that the anti-'whole cell' serum does not cross-react with the periplasmic enzyme (hydrogenase I), but does interact with a component exhibiting hydrogenase activity in the membrane fraction. Further analysis on non-denaturing polyacrylamide gels of solubilized membranes in which the components separated in the gel are electrophoresed into agarose layers containing antiserum (Fig. 3a and b) revealed that anti-'whole cell' serum reacted with the hydrogenase component of band 2 only (Fig. 3a'y'). A diffuse activity stain, not an arc, is visible due to

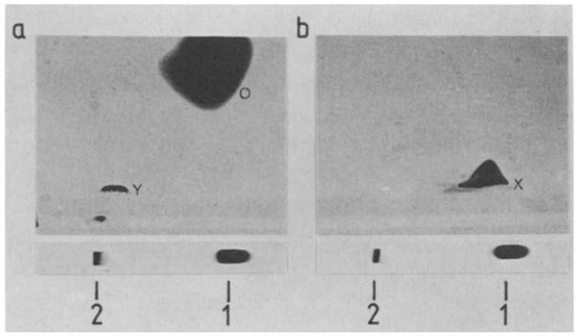

Fig. 3. Immunoplates showing that bands 1 and 2 are distinct. Triton X-100 dispersed membranes (80 μg protein) were mixed with 2.5 μg of purified periplasmic hydrogenase and fractionated by electrophoresis in a non-dissociating cylindrical; 7.5% (w/v) polyacrylamide gel in the first, horizontal dimension. Electrophoresis in the second dimension was into agarose containing antiserum, to (a) whole cells and (b) periplasmic hydrogenase. The immunoplates were stained for hydrogenase activity. A control gel not subjected to the second dimension and stained for hydrogenase activity is shown below each immunoplate. Band 1 corresponds to the mobility of periplasmic hydrogenase, band 2 to that of membrane bound hydrogenase. 'x' is an activity arc due to periplasmic hydrogenase. 'y' is an activity arc due to membrane bound hydrogenase. 'o' is a diffuse stain due to the presence of periplasmic hydrogenase.

hydrogenase I activity (Fig. 3a'o'). As anticipated the hydrogenase-I-specific antiserum cross-reacted only with the material which constituted band I on the gel (Fig. 3b'x'). This establishes that there are two immunologically and electrophoretically distinct hydrogenases present in the organism, one of which, the constituent of band 2, is exclusively membrane bound.

A further indication of the absence of a cross-reaction between solubilized membranes and anti-'periplasmic hydrogenase' serum is shown by immunoprecipitation. Hydrogenase I is selectively removed from solubilized membranes by incubation with antiserum to periplasmic hydrogenase (Fig. 4). The membranes fraction used (from cells in early log phase) was assayed before immunoprecipitation and it was found that 47% of the total activity was insensitive to inhibition by CO. After immunoprecipitation it was found that 42% of the total activity remained in solution, and that this activity was completely insensitive to CO. Thus the activity precipitated out with antiserum

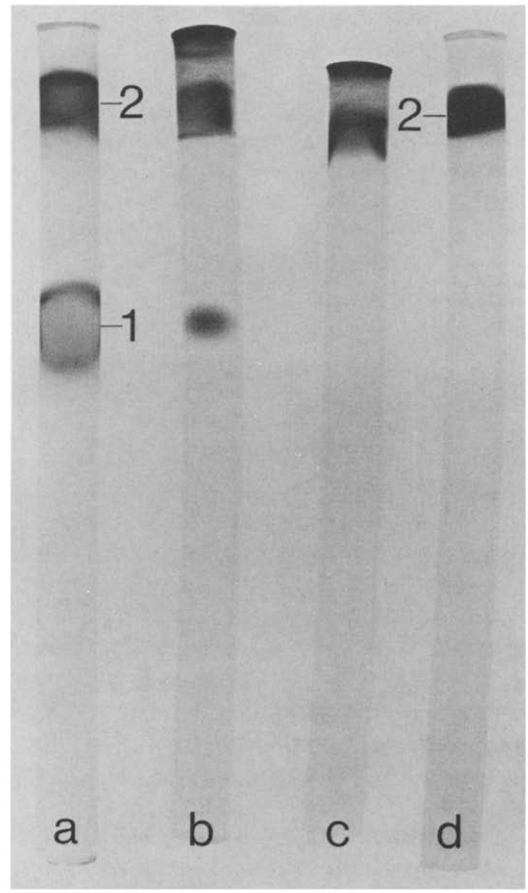

Fig. 4. Immunoprecipitation of hydrogenase I. Solubilised membranes (10 mg·ml^{-1}) were incubated for 20 min at 4°C with anti-'periplasmic hydrogenase' serum (20 mg·ml^{-1}) as follows: Oul (b) 10 μl (c) 25 μl (d) 100 μl. *Staphylococcus aureus*, Protein A (2×concentration of antiserum) was then added and incubated for 15 min at 4°C. Samples were centrifuged for 10 min at 10000× *g*. The supernatant was assayed and subjected to electrophoresis on 7.5% (v/v) polyacrylamide tube gels and then stained for hydrogenase activity. All of the gels show an activity due to hydrogenase II (band 2). Gel a shows hydrogenase I (band 1). Addition of anti-'periplasmic hydrogenase' serum causes precipitation of hydrogenase I leading to activity at the top of gels b and c. Gel d shows only hydrogenase II – all detectable hydrogenase I has precipitated and been removed by centrifugation.

to hydrogenase I corresponds to that activity sensitive to inhibition by CO.

Exposure of hydrogenases to proteases

The results of exposure to proteases have been used as a criterion for distinguishing hydrogenase

isoenzymes in *Eschericia coli* [15]. Treatment of the soluble cell fraction with pancreatin (Fig. 5c and d) or proteinase K (data not shown) does not influence the electrophoretic mobility of the enzyme (hydrogenase I) present in the extracts. Enzyme assay revealed that there was also no change in total hydrogenase activity following exposure to the proteases. Hydrogenase I, therefore, appears unaffected by such treatment. Similar experiments with solubilized membranes, however, revealed that the activity, constituting band 2 was lost on exposure to pancreatin (Fig. 5b) and proteinase K. Two bands of low R_f value were distinguished on these slab gels – both activities sensitive to protease treatment. The two bands may be related forms of hydrogenase II. Activity measurements showed that there was an approx. 20% reduction in total hydrogenase activity following such treatment. (Much activity remains due to hydrogenase I.) These results further support the presence of two distinct hydrogenases in the membrane fraction; hydrogenase I, whose activity is not affected by these proteases, and hydrogenase II, whose activity is destroyed by treatment with these proteases.

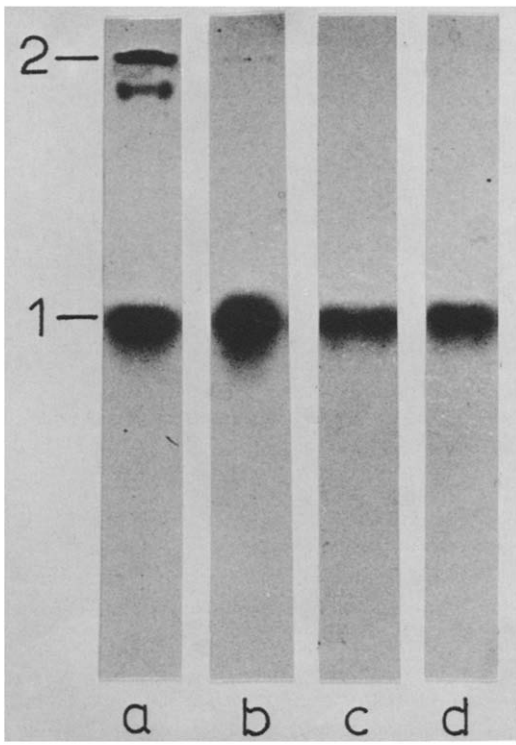

Fig. 5. Effect of pancreatin on hydrogenase activities. Samples were electrophoresed on a 7.5% (w/v) polyacrylamide slab gel and subsequently stained for hydrogenase activity. Samples analysed were: (a) 20 μg Triton X-100 dispersed membrane protein; (b) samples as in (a) after incubation with pancreatin (2% wt protease/wt. sample) at 30 °C for 30 min in 50 mM Tricine-HCl (pH 7.8); (c) lug soluble cell fraction; (d) sample as in (c) after incubation with pancreatin (2% wt protease/wt. sample) as described for (b) above. Band 1 corresponds to the mobility of periplasmic hydrogenase, band 2 to that of membrane bound hydrogenase.

Discussion

The periplasmic hydrogenase of *D. vulgaris* (Hildenborough) has already been well characterised [20,21], and it has recently been cloned in *E. coli* [23].

Our aim was to confirm that the CO-insensitive hydrogenase activity observed on polyacrylamide gel electrophoresis was due to a separate and distinct enzyme and not due to periplasmic hydrogenase activity. First of all it was necessary to be able to identify the periplasmic enzyme unequivocally. Cellular location is not a sufficiently precise guide, as hydrogenase activity with an electrophoretic mobility of 0.45 on polyacrylamide gels is found in all fractions after subcellular fractionation – even after selective extraction of periplasmic hydrogenase before cell disruption. The activity could be due to contamination by the highly active periplasmic enzyme, or to some precursor molecule or even to a completely different enzyme activity. Thus antibodies were raised to periplasmic hydrogenase to aid in identification. These antibodies reacted with hydrogenase in all cellular fractions thus the activities represent at least related, or possibly identical, forms of hydrogenase. Curiously, though fortunately for the experimenter, when antibodies were raised against the whole cell extract no cross-reaction occurred with periplasmic hydrogenase. This indicates that it is not a strongly antigenic protein in the crude extract. Anti-whole cell serum could thus be used in the identification of the second hydrogenase activity – a membrane-bound species. This hydrogenase has a low specific activity compared to that of the periplasmic hydrogenase, under the condi-

tion used, and this is possibly why it has not been detected as a separate enzyme previously. It differs markedly from the periplasmic enzyme (hydrogenase I) which has the following distinguishing features:

(i) the R_f value found on polyacrylamide gel electrophoresis is 0.45;
(ii) it is reversibly inhibited by CO;
(iii) hydrogenase activity is not susceptible to digestion by pancreatin;
(iv) it reacts with anti-'periplasmic hydrogenase' serum to form a single-activity-staining arc;
(v) it does not form an activity-staining arc with anti-'whole cell' serum.

One of the two bands of hydrogenase activity visible on polyacrylamide gel electrophoresis of solubilized membranes possesses the five properties described above and appears to be identical to periplasmic hydrogenase and is designated hydrogenase I. A second activity (hydrogenase II) with a lower R_f value (0.12) does not possess any of the five properties of hydrogenase I. It is biochemically and immunologically distinct, thus it does appear to represent another hydrogenase activity not previously described.

Acknowledgements

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References

1 Odom, J.M. and Peck, H.D., Jr. (1984) Annu. Rev. Microbiol. 38, 551–592
2 Lalla-Maharajh, W.V., Hall, D.O., Cammack, R. and Rao, K.K. (1983) Biochem. J. 209, 445–454
3 Yagi, T., Kimura, K., Daidoji, H., Sakai, F., Tamura, S. and Inokuchi, H. (1976) J. Biochem. (Tokyo) 79, 661–671
4 LeGall, J., Dervartanian, D.V., Spilker, E., Lee, J.P. and Peck, H.D. Jr. (1971) Biochim. Biophys. Acta 234, 525–530
5 Hatchikian, E.C., Bruschi, M. and LeGall, J. (1978) Biochem. Biophys. Res. Commun. 82, 451–461
6 Glick, B.R., Martin, W.G. and Martin, S.M. (1980) Can. J. Microbiol. 26, 1214–1223
7 Odom, J.M. and Peck, H.D. Jr. (1981) FEMS Microbiol. Lett. 12, 47–50
8 Lupton, F.S., Conrad, R. and Zeikus, J.G. (1984) J. Bacteriol. 159, 843–849
9 Lupton, F.S., Conrad, R. and Zeikus, J.G. (1984) FEMS Microbiol. Lett. 23, 263–268
10 Adams, M.W.W., Mortenson, L.E. and Chen, J.S. (1981) Biochim. Biophys. Acta 594, 105–176
11 Schneider, K., Cammack, R., Schlegel, H.G. and Hall, D.O. (1979) Biochim. Biophys. Acta 578, 445–561
12 Chua, H.B. (1983) Ph.D. Thesis, University of London, U.K.
13 Pankhania, I.P., Gow, L.A. and Hamilton, W.A. (1986) FEMS Microbiol. Lett. (In press)
14 Martin, S.M., Glick, B.R. and Martin, W.G. (1980) Can. J. Microbiol. 26, 1209–1223
15 Ballantine, S.P. and Boxer, D.H. (1985) J. Bacteriol. 163, 454–459
16 Yagi, T., Endo, A. and Tsuji, K. (1978) in Hydrogenases: Their Catalytic Activity, Structure and Function (Schlegel, H.-G. & Schelegel, K., eds.) pp. 107–124, E. Goltzem, Gottingen
17 Rieder, R., Cammack, R. and Hall, D.,O. (1984) Eur. J. Biochem. 145, 637–643
18 Saunders, G.F., Campbell, L.L. and Postgate, J.R. (1964) J. Bacteriol. 87, 1073–1078
19 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–27
20 Van der Westen, H.M., Mayhew, S.G. and Weever, C. (1978) FEBS Lett. 86, 122–126
21 Laemmli, U.K. (1970) Nature (Lond.) 227, 680–685
22 Sawyers, R.G., Ballantine, S.P. and Boxer, D.H. (1985) J. Bacteriol. 164, 1324–1331
23 Voordouw, G., Walker, J.E. and Brenner, S. (1985) Eur. J. Biochem. 148, 509–514