Quinoprotein D-Glucose Dehydrogenase of the *Acinetobacter calcoaceticus* Respiratory Chain: Membrane-Bound and Soluble Forms Are Different Molecular Species†

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ABSTRACT: *Acinetobacter calcoaceticus* is known to contain soluble and membrane-bound quinoprotein D-glucose dehydrogenases, while other oxidative bacteria contain the membrane-bound enzyme exclusively. The two forms of glucose dehydrogenase were believed to be the same enzyme or interconvertible forms. Previously, Matsushita et al. [(1988) *FEMS Microbiol. Lett 55*, 53–58] showed that the two enzymes are different with respect to enzymatic and immunological properties, as well as molecular weight. In the present study, we purified both enzymes and compared their kinetics, reactivity with ubiquinone homologues, and immunological properties in detail. The purified membrane-bound enzyme had a molecular weight of 83 000, while the soluble form was 55 000. The purified enzymes exhibited totally different enzymatic properties, particularly with respect to reactivity toward ubiquinone homologues. The soluble enzyme reacted with short-chain homologues only, whereas the membrane-bound enzyme reacted with long-chain homologues including ubiquinone 9, the native ubiquinone of the *A. calcoaceticus*. Furthermore, the two enzymes were distinguished immunochemically; the membrane-bound enzyme did not cross-react with antibody raised against the soluble enzyme, nor did the soluble enzyme cross-react with antibody against the membrane-bound enzyme. Thus, each glucose dehydrogenase is a molecularly distinct entity, and the membrane-bound enzyme only is coupled to the respiratory chain via ubiquinone.

Bacterial D-glucose dehydrogenase (GDH)[1] is a respiratory-chain linked quinoprotein with pyrroloquinoline quinone as the prosthetic group. GDH is found in a wide variety of bacteria, where the enzyme is usually tightly bound to the outer surface of the cytoplasmic membrane (Matsushita et al., 1986). Unlike other bacteria, however, *Acinetobacter calcoaceticus* contains a soluble form of GDH in addition to the membrane-bound form (Hauge, 1960a; Duine et al., 1982). Soluble GDH was originally purified by Hauge (1960a, 1964) and recently has been purified by two other groups (Dokter et al., 1986; Geiger & Görisch, 1986). It has been shown that the soluble enzyme is a dimer of identical subunits, each of which has a molecular weight of 48 000–54 000, and the enzyme is able to oxidize disaccharides as well as monosaccharides. Furthermore, it has been reported that membrane-bound GDH from *A. calcoaceticus* is the same as soluble GDH or interconvertible with the soluble enzyme (Hauge & Hallberg, 1964; Dokter et al., 1987). However, immunoblotting experiments with an antibody prepared against *Pseudomonas* membrane-bound GDH showed that the membrane of *A. calcoaceticus* contained GDH polypeptide of higher molecular weight (83 000) than the soluble enzyme (Matsushita et al., 1986). In addition, Cleton-Jansen et al. (1988) showed that a *gdh* gene cloned from *A. calcoaceticus* encoded a protein of 83 000 and it was demonstrated recently (Matsushita et al., 1988) that there were two different forms of GDH in the soluble and membrane-bound fractions of *A. calcoaceticus* LMD 79.41.

There is controversy regarding the natural electron acceptor of GDH. Hauge (1961) and Dokter et al. (1988) have suggested that a soluble cytochrome *b* may be an electron acceptor for GDH from *A. calcoaceticus*; however, Beardmore-Gray and Anthony (1986) have reported that GDH is linked to the respiratory chain via ubiquinone. We have shown that GDH purified from *Pseudomonas fluorescens* or *Gluconobacter suboxydans* reacts with ubiquinone homologues in vitro (Matsushita et al., 1982, 1989) and that GDH from *Escherichia coli* or *G. suboxydans* reacts with the native ubiquinone in reconstituted proteoliposomes (Matsushita et al., 1987, 1989).

In this study, thus, the soluble and membrane-bound GDHs from *A. calcoaceticus* LMD 79.41 were purified, and their properties were compared. The results show clearly that the GDHs are distinct enzymes on the basis of several criteria. In addition, the results suggest that only membrane-bound GDH is coupled to the respiratory chain via ubiquinone in this bacterium.

Materials and Methods

Materials. Ubiquinone 1 (Q_1), Q_2, Q_6, and Q_9 were kindly supplied by Eizai Co. Octyl β-D-glucoside (octyl glucoside) was purchased from Calbiochem-Behring. DEAE- or CM-Toyopearl, which was used as a medium-performance ion exchanger, was from Toyo Soda Co. All other materials were of reagent grade and obtained from commercial sources.

Bacterial Strain and Growth Conditions. A. calcoaceticus LMD 79.41 was kindly provided by Dr. J. Duine and grown as described (Matsushita et al., 1988).

Preparation of Soluble and Membrane-Bound Fractions. The cells were collected by centrifugation, washed with 50 mM potassium phosphate, pH 7.5, and suspended in the same

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[1] Abbreviations: GDH, D-glucose dehydrogenase (EC 1.1.99.17); PMS, phenazine methosulfate; DCIP, 2,6-dichlorophenolindophenol; Q_1, Q_2, Q_6, or Q_9, ubiquinone 1, 2, 6, or 9; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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buffer. The suspension was passed twice through a French pressure cell press at 16000 psi and then centrifuged at 12000*g* for 20 min to remove intact cells and cell debris. The supernatant was separated into crude soluble and membrane-bound fractions by centrifugation at 120000*g* for 90 min. Centrifugation was repeated to obtain soluble and membrane-bound fractions from the crude counterparts.

Purification of Soluble GDH. Soluble GDH was purified from the soluble fraction prepared as described above instead of treating cells with 1% Triton X-100, which was used previously (Dokter et al., 1986; Geiger & Görisch, 1986). The soluble fraction was dialyzed extensively against 20 mM potassium phosphate, pH 7.0, and centrifuged at 120000*g* for 90 min to remove the membrane-bound fraction contaminated. The supernatant was applied to a DEAE-Toyopearl column equilibrated with 20 mM potassium phosphate, pH 7.0. After the column was washed with the same buffer, the enzyme was eluted by a linear gradient up to 100 mM potassium phosphate, pH 7.0. The enzyme was further purified by CM-Toyopearl, hydroxyapatite, and phenyl-Sepharose column chromatography essentially as described by Geiger and Görisch (1986).

Purification of Membrane-Bound GDH. All steps were performed at 0–10 °C unless noted otherwise. Membrane-bound fraction prepared as described above was suspended to a final protein concentration of 10 mg/mL in 10 mM potassium phosphate, pH 6.0, and Triton X-100 was added to a final concentration of 0.2%. The suspension was incubated on ice for 30 min and centrifuged at 120000*g* for 90 min, and the supernatant was discarded. The pellet was homogenized to a final protein concentration of 10 mg/mL in 50 mM potassium phosphate, pH 7.5, and Triton X-114 and KCl were added to final concentrations of 1% and 0.3 M, respectively. The suspension was incubated on ice for 30 min and centrifuged at 120000*g* for 90 min. The supernatant was warmed to 30 °C to obtain phase separation and centrifuged at 1500*g* for 3 min at room temperature. The resultant lower phase was collected and dialyzed against 50-fold excess of 0.1% Triton X-114 for 5 h twice. The dialysate was centrifuged at 120000*g* for 90 min to remove the membranous aggregates. The supernatant was applied on a DEAE-Toyopearl column (about 1 mL bed volume per 1–2 mg of protein applied), which had been equilibrated with 50 mM potassium phosphate, pH 7.5, and washed with 20 bed volumes of distilled water and then with 2 bed volumes of 0.1% Triton X-114. After application of the sample, the column was washed with 2 bed volumes of 0.1% Triton X-114, and the enzyme was eluted by a linear gradient consisting of 6 bed volumes each of 0.1% Triton X-114 and 50 mM potassium phosphate, pH 7.5, containing 0.1% Triton X-114. The enzyme eluted at about 18 mM potassium phosphate. Active fractions were pooled and applied on a CM-Toyopearl column (about 1 mL bed volume per 1–2 mg of protein applied), which was equilibrated with 20 mM potassium phosphate, pH 7.5, and washed with 2 bed volumes of the same buffer containing 0.1% Triton X-114. After the column was washed with 3 bed volumes of 20 mM potassium phosphate, pH 7.5, containing 0.1% Triton X-114, the enzyme was eluted with 50 mM potassium phosphate, pH 7.5, containing 0.1% Triton X-114.

Enzyme Assays. GDH activity was measured spectrophotometrically by using phenazine methosulfate (PMS) and 2,6-dichlorophenolindophenol (DCIP) as electron acceptors as described (Matsushita et al., 1980). The assay for soluble GDH was performed at 50 mM potassium phosphate, pH 6.5, and activity was calculated by using a millimolar extinction coefficient of 13.2. Membrane-bound GDH activity was measured at 50 mM Tris-HCl, pH 8.5, by using a millimolar extinction coefficient of 15.6. Quinone reductase activity was also measured spectrophotometrically with Q_1 or Q_2 by using a millimolar extinction coefficient of 12.25 (Matsushita et al., 1982). For the measurement of Q_6 or Q_9 reductase activity, a dual-wavelength spectrophotometer was used to compensate for the partial insolubility of quinone. Quinone reductase activities were measured at a wavelength pair of 273 and 298 nm by using a millimolar extinction coefficient of 13. The assays for soluble and membrane-bound GDHs were performed at 50 mM Tris-HCl, pH 8.0, and at 50 mM sodium acetate, pH 4.8, respectively, in the presence of 0.25% octyl glucoside. In the case of Q_6 or Q_9, instead of the quinones being added directly into the reaction buffer containing 0.25% octyl glucoside, the quinones were initially solubilized in 10% octyl glucoside and then diluted into the reaction buffer.

Other Analytical Procedures. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), protein determinations, preparation of antibodies, and immunoblotting analysis were performed as described (Matsushita et al., 1986, 1988).

Table I: Purification of Soluble GDH from the Soluble Fraction of *A. calcoaceticus*

fraction	protein (mg)	GDH activity[a] units	GDH activity[a] units/mg	recovery (%)
soluble fraction	2132	12726	6.0	100
dialysis and ultracentrifugation	1061	8490	8.0	66.7
DEAE-Toyopearl	56.2	7609	135	59.8
CM-Toyopearl	5.6	6682	1193	52.5
hydroxyapatite	2.3	5011	2178	39.4
phenyl-Sepharose	1.6	3534	2209	27.8

[a] GDH activity was measured with PMS/DCIP at pH 6.5 as described under Materials and Methods.

Results

Purification of Soluble and Membrane-Bound GDHs. Soluble GDH of *A. calcoaceticus* has been purified to homogeneity by several groups (Hauge, 1960a,b, 1964; Dokter et al., 1986; Geiger & Görisch, 1986). In this study, the soluble fraction was used as starting material, and ultracentrifugation was repeated after dialysis of the soluble fraction such that contamination with the membrane-bound enzyme was excluded. After dialysis and ultracentrifugation, the enzyme was adsorbed to a DEAE column, contrary to the previous results that the enzyme did not bind to the column (Hauge, 1960a; Duine et al., 1979; Geiger & Görisch, 1986). After elution from DEAE, the enzyme was purified further by several column chromatographies as summarized in Table I. As shown, the enzyme was purified about 350-fold from the soluble fraction, and the purified enzyme showed a specific activity of 2200 μmol of glucose oxidized min^{-1} (mg of protein)$^{-1}$. The membrane-bound GDH of *A. calcoaceticus* was purified for the first time in this study. Since the enzyme is tightly bound to the membrane, 0.2% Triton X-100 scarcely solubilizes the enzyme, and the detergent was used therefore as a negative purification step. After the membranes were washed with 0.2% Triton X-100, the enzyme was solubilized with 1% Triton X-114 in the presence of 0.3 M potassium chloride. Since the enzyme scarcely bound to a DEAE column under normal ionic strength (10–50 mM), the enzyme was adsorbed to the column that had been washed with distilled water to decrease the ionic strength and then eluted by a linear gradient of potassium phosphate in the presence of Triton X-114. Afterward, the enzyme was further purified by a CM

Table II: Purification of Membrane-Bound GDH from the Membrane Fraction of *A. calcoaceticus*

fraction	protein (mg)	GDH activity[a] units	GDH activity[a] units/mg	recovery (%)
membrane fraction	458	4895	10.7	100
washed membrane with Triton X-100	384	4693	12.2	95.9
extract with Triton X-114	74.1	3981	53.8	81.3
phase separation	40.2	3200	80.0	65.4
dialysis and ultracentrifugation	20.0	1829	91.6	37.4
DEAE-Toyopearl	3.5	1337	382	27.3
CM-Toyopearl	1.3	742	571	15.2

[a] GDH activity was measured with PMS/DCIP at pH 8.5 as described under Materials and Methods.

Table III: Kinetics for D-Glucose or Lactose of Purified Soluble and Membrane-Bound GDHs[a]

GDH	sugar	K_m (mM)	V_{max} (units/mg)	turnover number at V_{max}[b] ($e^- s^{-1} mol^{-1}$)
soluble GDH	D-glucose	24.5	3205	5870
	lactose	26.7	1789	3277
membrane-bound GDH	D-glucose	4.2	614	1706

[a] Activities were measured with PMS/DCIP at pH 6.5 for soluble GDH and at pH 8.5 for membrane-bound GDH. [b] The values were calculated from each V_{max} by using the molecular weights of 55 000 and 83 000 for soluble and membrane-bound GDHs, respectively.

column chromatography. The enzyme was purified about 50-fold from the membrane fraction, and the purified enzyme showed a specific activity of 570 μmol of glucose oxidized min^{-1} (mg of protein)$^{-1}$ (Table II). Purified soluble and membrane-bound GDHs showed a single polypeptide band on SDS–PAGE (Figure 1), and the molecular weights were estimated to be 55 000 and 83 000, respectively.

Kinetic Properties of Soluble and Membrane-Bound GDHs. The kinetics of D-glucose oxidation catalyzed by the purified enzymes was considerably different, an observation that is consistent with the previous results obtained by using soluble and membrane-bound fractions (Matsushita et al., 1988). Soluble GDH exhibited a biphasic function in reciprocal plots, while membrane-bound GDH did not (data not shown), and the K_m for membrane-bound GDH was much lower than that of the soluble enzyme (Table III). In addition, the purified membrane-bound enzyme exhibited no activity toward lactose, in contrast with the purified soluble enzyme (Table III), and pH optima for soluble and membrane-bound GDHs with PMS/DCIP as electron acceptors were at neutral pH around 6.5 and at pH 8.5, respectively (Figure 2).

Reactivity of Purified Soluble and Membrane-Bound GDHs with Ubiquinone Homologues. Although purified soluble and membrane-bound GDHs react with short-chain ubiquinone homologues, Q_1 or Q_2 (see Table IV), the kinetics and pH optima were considerably different. As a significant aut-

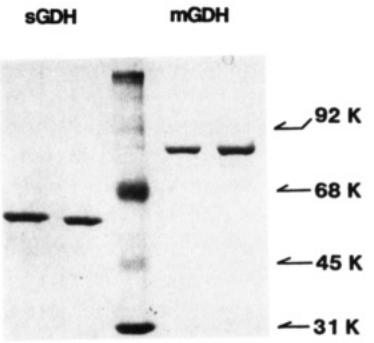

FIGURE 1: SDS–PAGE of soluble and membrane-bound GDHs purified from *A. calcoaceticus* LMD 79.41. Purified soluble GDH (sGDH, 5.4 μg of protein) and membrane-bound GDH (mGDH, 3.8 μg of protein) were treated with 3% SDS and 100 mM DTT (left side) and with only 3% SDS (right side) and then electrophoresed in 10% polyacrylamide gel containing 0.1% SDS. The gel was stained with Coomassie blue. The middle lane between lanes of sGDH and mGDH contained molecular weight markers including phosphorylase *b*, bovine serum albumin, ovalbumin, and carbonic anhydrase.

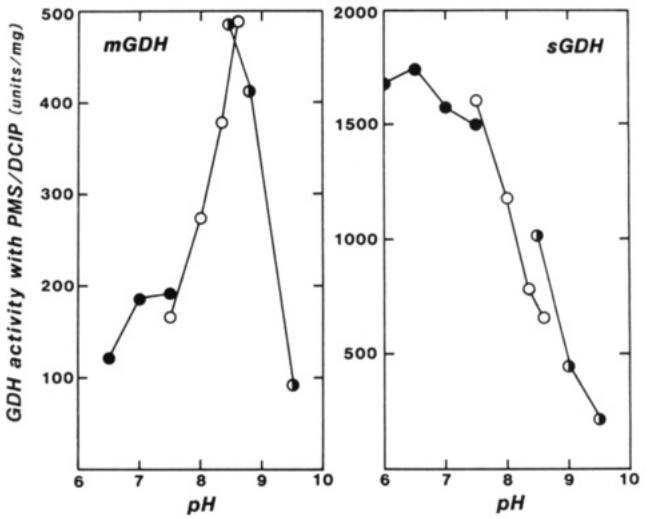

FIGURE 2: pH profiles for PMS/DCIP reductase activities of purified soluble and membrane-bound GDHs. Enzyme activity was measured with soluble GDH (sGDH) or membrane-bound GDH (mGDH) in 50 mM potassium phosphate, pH 6.0–7.5 (●), 50 mM Tris-HCl, pH 7.5–8.6 (○), or 50 mM glycine–NaOH, pH 8.45–9.5 (◑).

oxidation of ubiquinol was observed at pH higher than 8.5 at least under the assay conditions at which reactivity with ubiquinone was examined (data not shown), quinone reductase activity of GDH may be underestimated at alkaline pH region. Although the exact pH optimum is not determined at alkaline pH, it is possible to compare what pH region both enzymes work at. Thus, membrane-bound GDH had an Q_1 or Q_2 reductase activity at both acidic and alkaline pH regions, while soluble GDH exhibited Q_1 or Q_2 reductase activity only at alkaline pH, as shown in Figure 3 for Q_2 reductase activity (data not shown for Q_1 reductase activity). The difference between soluble and membrane-bound GDHs was more critical

Table IV: Kinetics for Ubiquinone Homologues and PMS of Purified Soluble and Membrane-Bound GDHs

electron acceptor	soluble GDH activity[a] (units/mg)	soluble GDH K_m (μM)	soluble GDH V_{max} (units/mg)	membrane-bound GDH activity[a] (units/mg)	membrane-bound GDH K_m (μM)	membrane-bound GDH V_{max} (units/mg)
PMS		1900	4098		74	1098
Q_1	55	213	667	51	148	348
Q_2[b]	35	85	200	138	12	208
Q_6[b]	0			43	4.4	46
Q_9[b]	0			35	3.4	41

[a] The activities were measured with 20 μM concentrations of each ubiquinone homologue at pH 8.0 for soluble GDH and at pH 4.8 for membrane-bound GDH. [b] The activities for these ubiquinone homologues were measured in the presence of 0.25% octyl glucoside.

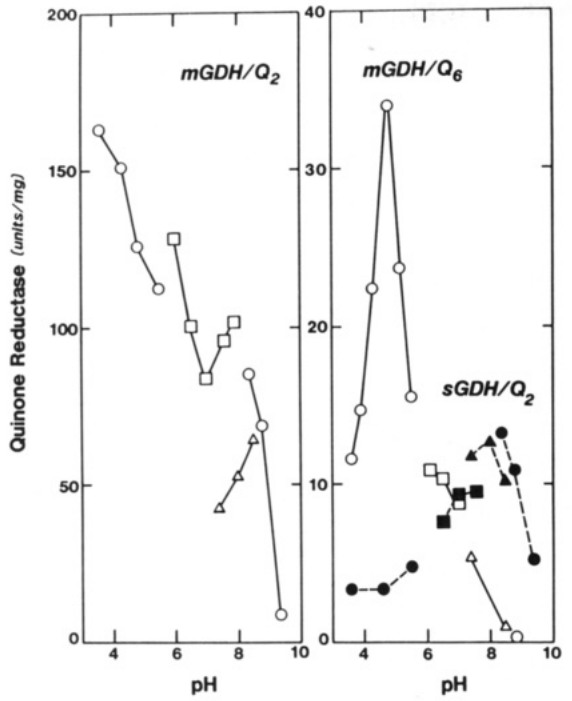

FIGURE 3: pH profiles for Q_2 or Q_6 reductase activities of purified soluble and membrane-bound GDHs. Q_2 reductase activity was measured with soluble GDH, sGDH (solid symbol), or membrane-bound GDH, mGDH (open symbol), in 50 mM sodium acetate, pH 3.6–5.5 (circle), 50 mM potassium phosphate, pH 6.0–7.5 (square), 50 mM Tris-HCl, pH 7.4–8.5 (triangle), or 50 mM glycine–NaOH, pH 8.35–9.35 (circle). Likewise, Q_6 reductase activity was measured with mGDH.

in the reactivity for longer chain ubiquinone homologues, Q_6 or Q_9, which were solubilized with 10% octyl glucoside, and quinone reductase activity was measured at a final octyl glucoside concentration of 0.25%. The quinone reductase activity was maximal at an octyl glucoside concentration around 0.25%, while PMS/DCIP reductase activity of soluble or membrane-bound GDH was inhibited 10% or activated 17%, respectively, by the addition of the same concentration of octyl glucoside (data not shown). Thus, membrane-bound GDH reduces Q_6 and Q_9 at acidic pH, while soluble GDH had appreciably no activity for longer chain ubiquinone homologues at either acidic or alkaline pH (Table IV). Interestingly, the Q_6 reductase activity of membrane-bound GDH exhibited an optimum pH 4.8 only, unlike the Q_1 or Q_2 reductase activity (Figure 3). Kinetic parameters for the quinone reductase activity of soluble and membrane-bound GDHs are summarized in Table IV and compared with the kinetics of PMS reductase. Membrane-bound GDH reacts with ubiquinone homologues with long and short side chains at a relatively high rate and with a low K_m at acidic pH region. On the other hand, at alkaline pH only, both soluble and membrane-bound GDHs react with short-chain but not long-chain ubiquinone homologues.

Immunological Relationship between Soluble and Membrane-Bound GDHs. Antibody raised against GDH purified from the membrane of *P. fluorescens* cross-reacts with membrane-bound GDH from *A. calcoaceticus*, but not with soluble GDH (Matsushita et al., 1986, 1988). By use of the *Pseudomonas* antibody and an antibody newly prepared against soluble GDH purified from *A. calcoaceticus*, the cross-reactivity of soluble and membrane-bound GDHs was examined by an immunoblotting analysis (Figure 4). Antibody against *Pseudomonas* GDH did not cross with purified soluble GDH, but did so with purified membrane-bound GDH. Furthermore, the antibody for soluble GDH of *Acinetobacter* did not react with the purified membrane-bound GDH even at a relatively high concentration. The results clearly indicate that soluble and membrane-bound GDHs are different from each other structurally. Data also showed that the soluble fraction contained some GDH cross-reacted with the antibody for membrane-bound GDH besides soluble GDH. This may be due to a partial contamination of the membranes into the soluble fraction.

Discussion

Soluble GDH of *A. calcoaceticus* has been purified and characterized by several groups (Hauge, 1960a,b, 1964; Dokter et al., 1986; Geiger & Görisch, 1986), but the membrane-bound form of the enzyme has never been purified and characterized. Thus, as described in a previous paper (Matsushita et al., 1988), the membrane-bound GDH of *A. calcoaceticus* was confused with the soluble enzyme, so that both enzymes were believed to be the same or interconvertible in previous studies (Hauge & Hallberg, 1964; Dokter et al., 1987). The results presented here clearly demonstrate that membrane from *A. calcoaceticus* contained an enzyme that was distinct from soluble GDH in all aspects, i.e., optimum pH, kinetics, substrate specificity, ubiquinone reactivity, molecular size, and immunoreactivity. Purified membrane-bound GDH is a hydrophobic protein with a molecular weight of

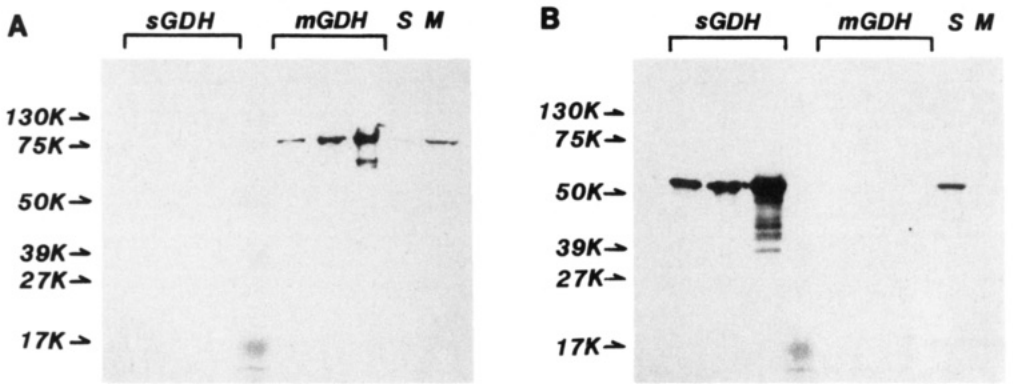

FIGURE 4: Immunoblotting analysis of soluble and membrane-bound GDHs and soluble and membrane fractions with an antibody directed for GDH purified from *Pseudomonas* and with an antibody for soluble GDH purified from *Acinetobacter*. Soluble GDH (sGDH) was applied on two separate 12.5% polyacrylamide gels containing 0.1% SDS with 0.1, 0.5, and 2.5 μg of proteins (from left to right) and membrane-bound GDH (mGDH) with 0.2, 1.0, and 5.0 μg of proteins (from left to right). Soluble (S) and membrane-bound (M) fractions were also applied on the same gels with each 20 μg of proteins. Between sGDH and mGDH were applied marker proteins. After electrophoresis, the two gels were blotted and analyzed separately with two different antibodies, one specific for *Pseudomonas* membrane-bound GDH (A) and the other specific for *Acinetobacter* soluble GDH (B).

83 000 and has an optimum pH of 8.5 for PMS/DCIP reductase activity, and it does not react with disaccharide. On the other hand, purified soluble GDH is a hydrophilic protein with a molecular weight of 55 000 and has a neutral optimum pH around 6.5 for the PMS/DCIP reductase activity, and it oxidizes lactose as well as D-glucose. Furthermore, the immunochemical evidence is inconsistent with the notion that soluble GDH is a degradation product or a precursor of the membrane-bound enzyme. The conclusion that soluble and membrane-bound GDHs are independent molecular species is consistent with the genetic evidence obtained recently by Cleton-Jansen et al. (unpublished data) demonstrating that *A. calcoaceticus* LMD 79.41 contains different *gdh* genes encoding proteins of 87 000 and 50 000 that have no sequence homology.

The results obtained here also showed that soluble and membrane-bound GDHs of *A. calcoaceticus* had different activities for ubiquinone homologues. Membrane-bound GDH reacts with long-chain ubiquinone homologues (Q_6 or Q_9), while soluble GDH has no activity for these ubiquinone homologues in spite of its ability to react with short-chain homologues (Q_1 or Q_2). Activity for Q_1 or Q_2 is observed with soluble GDH only at alkaline pH, but at both acidic and alkaline pH with membrane-bound GDH; membrane-bound GDH activity for long-chain ubiquinone is observed at acidic pH only. Therefore, it seems that the quinone reductase activity for short-chain ubiquinone homologues observed at the alkaline pH region does not reflect a physiological electron transfer but some kind of artificial reaction similar to the reaction with PMS. In other words, only the membrane-bound GDH may have an ability to transfer electrons from D-glucose to ubiquinone in the native system. To conclusively demonstrate the reactivity of the enzyme to ubiquinone in the membrane, however, the reaction has to be reproduced in a phospholipid bilayer. Such reconstitution has been achieved with GDH from *E. coli* or *G. suboxydans* (Matsushita et al., 1987, 1989), as well as the membrane-bound GDH from *A. calcoaceticus* (unpublished data). Therefore, it seems reasonable to conclude that membrane-bound GDH of *A. calcoaceticus* is coupled to the respiratory chain via ubiquinone.

The function of soluble GDH in the respiratory chain is unresolved. Recently, Dokter et al. (1988) concluded that soluble GDH donates electrons to the respiratory chain via a soluble cytochrome b_{562} that is reduced at a low rate. However, such a slow reduction of cytochrome may not necessarily mean an involvement of the cytochrome for the physiological electron transfer but only its physicochemical redox equilibrium. Furthermore, it has been reported that mutants deficient in glucose oxidation still exhibit a fairly high soluble GDH activity (Cleton-Jansen, 1988). Thus, soluble GDH might be nonfunctional in *A. calcoaceticus* cells grown under normal conditions, while the membrane-bound GDH is clearly involved in glucose oxidation and presumably generation of an electrochemical proton gradient via the membrane-bound respiratory chain.

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