Malate Dehydrogenase in Phototrophic Purple Bacteria: Purification, Molecular Weight, and Quaternary Structure

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The citric acid cycle enzyme malate dehydrogenase was purified to homogeneity from the nonsulfur purple bacteria *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodomicrobium vannielii*, and *Rhodocyclus purpureus*. Malate dehydrogenase was purified from each species by either a single- or a two-step protocol; triazine dye affinity chromatography was the key step in purification of malate dehydrogenase in all cases. Purification of malate dehydrogenase resulted in a 130- to 240-fold increase in malate dehydrogenase specific activity, depending on the species, with recoveries ranging from 30 to 70%. Homogeneity of malate dehydrogenase preparations from the four organisms was determined by sodium dodecyl sulfate and nondenaturing polyacrylamide gel electrophoresis; a single protein band was observed in purified preparations by both techniques. The molecular weight of native malate dehydrogenases was determined by four independent methods and estimated to be in the range of 130,000 to 140,000 for the enzyme from *R. capsulatus*, *R. rubrum*, and *R. vannielii* and 57,000 for that from *R. purpureus*. It is concluded that malate dehydrogenase from *R. capsulatus*, *R. rubrum*, and *R. vannielii* is a tetramer composed of four identical subunits, while the enzyme from *R. purpureus* is a dimer composed of two identical subunits.

Nonsulfur purple phototrophic bacteria can grow photosynthetically (anaerobically in the light) or heterotrophically (aerobically in the dark) on citric acid cycle intermediates, especially malate, under proper nutritional conditions (24, 27). Although most species are very nutritionally versatile, malate is the only organic carbon source that is universally catabolized by nonsulfur purple bacteria (27), and it is oxidized to oxalacetate through the action of NAD-specific malate dehydrogenases (MDHs) (24). In contrast, purple sulfur bacteria such as *Chromatium* species lack MDH, but nevertheless convert malate to oxalacetate through the combined activities of malic enzyme and pyruvate carboxylase (20). In addition, a few species of phototrophic bacteria catabolize malate via a reversal of the glyoxylate cycle, forming glyoxylate and acetyl coenzyme A (28).

MDH has been purified from animal, plant, and bacterial sources by conventional (2, 15) and affinity chromatographic techniques (1, 10, 22, 32). Based on molecular size, bacterial MDHs are divided into two groups: small and large (2, 16, 26). The molecular weight of small MDHs ranges from 60,000 to 65,000 (mammalian and other eucaryotic MDHs are in this category), and that of large MDHs is in the range of 117,000 to 146,000. However, the subunit molecular weight for both classes of enzymes is in the region of 30,000 to 35,000 (2, 15, 16, 26). The low-molecular-weight enzymes are therefore dimers and the high-molecular-weight enzymes are tetramers of identical subunits.

Here we report the purification and quaternary structure of MDH from several species of nonsulfur purple photosynthetic bacteria. The species chosen, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodomicrobium vannielii*, and *Rhodocyclus purpureus*, are representative species of each of the four phylogenetic subgroups of nonsulfur purple bacteria previously defined by 16S rRNA sequencing (30, 31). Our results indicate that *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodomicrobium vannielii* MDHs belong to the large molecular weight class (tetramers) characteristic of MDH from most gram-positive bacteria, whereas MDH from *Rhodocyclus purpureus* is a low-molecular-weight (dimeric) enzyme, typical of MDH from eucaryotic sources and most gram-negative bacteria.

**MATERIALS AND METHODS**

**Bacterial strains.** *Rhodobacter* (*Rhodopsseudomonas*) *capsulatus* B100, *Rhodospirillum rubrum* 1.1.1, *Rhodomicrobium vannielii* EE33, and *Rhodocyclus purpureus* 6770 were obtained from our culture collection.

**Growth media and growth-conditions.** *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodomicrobium vannielii* were grown in minimal synthetic media (designated RCVB) containing the following per liter of deionized water: disodium EDTA, 20 mg; (NH₄)₂SO₄, 1.0 g; dl-malic acid, 4.0 g; MgSO₄·7H₂O, 200 mg; CaCl₂·2H₂O, 75 mg; thiamine hydrochloride, 1.0 mg; biotin, 15 μg; KH₂PO₄, 0.6 g; K₂HPO₄, 0.9 g; and trace elements solution (9), 1 ml. The medium was adjusted to pH 6.8 before autoclaving. For growth of *Rhodocyclus purpureus*, RCVB medium was supplemented with 20 μg of vitamin B₁₂ per liter and 0.01% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.), and the phosphate content of the medium was doubled.

Cultures were grown photosynthetically in screw-cap tubes (17 ml) or bottles (1 liter); large volumes were grown in 20-liter glass carboys sealed with rubber stoppers. Carboys were inoculated with 1.5% (vol/vol) of an overnight culture and sealed under an atmosphere of N₂-CO₂ (99:1). Following inoculation, all cultures were kept in the dark for 2 h and then incubated at 30°C at a light intensity of 5,400 1x (incandescent illumination). *Escherichia coli* and *Bacillus megaterium* were grown aerobically in shake flasks at 37°C in tryptic soy broth (Difco).

**Preparation of cell extracts.** Exponentially growing cells were harvested by a high-pressure ultrafiltration system (Millipore Corp., Bedford, Mass.) and washed once with 50 mM potassium phosphate buffer (pH 7). The pellets were
either used immediately or frozen at −20°C. For cell breakage, cell paste suspended in 2 volumes of 0.1 M potassium phosphate buffer (pH 7.5) was passed once through a precooled (4°C) French pressure cell operating at 110 MPa. Intact cells and cell debris were removed by centrifugation (27,000 × g, 20 min), and the resulting supernatant fluid was further centrifuged at 144,000 × g (4°C) for 90 min. The clear supernatant fluid was saved for enzyme assay or was frozen at −20°C.

**Enzyme assays.** MDH (l-malate:NAD⁺ oxidoreductase; EC 1.1.1.37) activity was assayed in either the forward (6) or reverse direction (21) by measuring the initial rates of malate oxidation (NAD⁺ reduction) or oxalacetate reduction (NADH oxidation), respectively, at 340 nm in a spectrophotometer (DU-50; Beckman Instruments, Inc., Fullerton, Calif.). The standard assay (NADH oxidation) mixture contained 100 mM Tris hydrochloride (pH 8.4), 0.25 mM NADH, 0.5 mM neutralized oxalacetate, and enzyme to a final volume of 3 ml. The reaction mixture for malate oxidation contained 100 mM glycine-NaOH (pH 9.6), 120 mM neutralized dl-malate, 0.67 mM NAD⁺, and enzyme in a final volume of 5 ml. All reactions were carried out at 25°C. One unit of MDH activity is defined as the amount of enzyme that catalyzes the oxidation-reduction of 1 μmol of NADH/NAD⁺ per min at 25°C.

**Purification of Rhodobacter capsulatus and Rhodospirillum rubrum MDHs.** Crude extracts were applied to a column (2.5 by 20 cm) of Mâtrex Gel Red A (Amicon Corp., Lexington, Mass.) previously equilibrated with 10 mM potassium phosphate buffer (pH 7.5; hereafter referred to as buffer) at 4°C. Following washing with buffer, the column was washed successively with separate fractions of buffer (5 to 10 column volumes each) containing 0.1, 0.25, 0.5, 0.75, and 1 M KCl. MDH was then eluted with 150 to 200 ml of buffer containing 5 mM NAD⁺ and 10 mM malate. Active fractions were pooled and concentrated by ultrafiltration with immersible ultrafilters (CX; Millipore).

**Purification of Rhodococcus vannielii MDH.** Solid ammonium sulfate was added slowly to stirred crude extracts (25°C) to bring the solution to 40% (wt/vol) saturation. Stirring was continued for 30 min, after which the suspension was centrifuged at 27,000 × g, and the precipitate was discarded. The clear supernatant was brought to 60% saturation with ammonium sulfate, and the suspension was centrifuged as described above. The precipitate was dissolved in a small volume of buffer and desalted on a column (2.6 by 60 cm) of Sephadex G-25. Active fractions were pooled and applied to the Mâtrex Gel Red A column equilibrated with buffer. The column was washed with buffer and with 0.1, 0.25, and 0.5 M KCl in buffer until no protein appeared in the eluate. MDH was eluted with 120 ml of buffer containing 10 mM NAD⁺ and 50 mM malate (pH 7.5). Active fractions were pooled and concentrated by ultrafiltration.

**Purification of Rhodococcus purpureus MDH.** Crude extracts were applied to the Mâtrex Gel Red A column equilibrated with buffer. The column was washed with 2 liters (each) of buffer and of 0.1 M KCl in buffer, respectively. The column was then washed with 150 ml of buffer containing 20 mM NAD⁺, and MDH activity was eluted with 100 to 150 ml of a buffer solution containing 20 mM NAD⁺ plus 100 mM malate. Fractions containing >10 U of MDH activity were pooled. The Mâtrex Gel Red A eluate was desalted on a column of Sephadex G-25, and the active fractions were applied to a column (1.5 by 40 cm) of Bio-Gel HTP (Bio-Rad Laboratories, Richmond, Calif.) equilibrated with 10 mM potassium phosphate buffer (pH 6.8). The column was washed with 100 ml of buffer, and MDH was eluted with 400 ml of a linear gradient of 10 to 200 mM potassium phosphate (pH 6.8). Active fractions were passed through Sephadex G-25 and concentrated by ultrafiltration.

**Gel filtration.** Native molecular weights of MDHs were determined by gel filtration as described by Andrews (1). A column (2.6 by 60 cm) of Sephacryl S-300 (Pharmacia, Inc., Piscataway, N.J.) equilibrated with 50 mM potassium phosphate buffer (pH 7.5) and 0.1 M NaCl and 0.02% (wt/vol) azide was used. The elution volumes of protein markers were measured by determining protein (optical density at 280 nm) and of MDH by determining enzyme activity in the direction of oxalacetate reduction.

**Sucrose density gradient centrifugation.** Linear gradients (total volume 4.6 ml) of 5 to 20% (wt/vol) sucrose were prepared in 0.1 M potassium phosphate (pH 7.5). Purified MDHs, which were mixed with protein markers (catalase, alcohol dehydrogenase, and hemoglobin) in a total volume of 0.1 ml, were layered on the top of the gradient and centrifuged in a rotor (SW50.1; Beckman for 16 h at 39,000 rpm (3°C). Fractions (0.12 ml) were collected from the bottom of each tube with a 25-gauge needle. Catalase and alcohol dehydrogenase were assayed from fractions by standard procedures (13), and hemoglobin was determined spectrophotometrically at 405 nm by collecting fractions in a microtiter plate and reading the absorbance in an enzyme-linked immunosorbent assay reader. Native molecular weights of standards and MDHs were estimated from sedimentation coefficients by the procedure described by Martin and Ames (13).

**Polyacrylamide gel electrophoresis.** For molecular weight determination of native MDHs, electrophoresis under nondenaturing conditions was performed in the Tris-glycine buffer system described by Davis (5) at several gel concentrations, as described in Sigma Technical Bulletin No. M-137 (Sigma Chemical Co., St. Louis, Mo.). A continuous phosphate buffer system (29) was used for the detection of MDH activity on polyacrylamide gels; the separating gel was composed of 7.5% (wt/vol) acrylamide in 0.1 M sodium phosphate (pH 7.5). Electrophoresis was carried out at a constant current of 40 mA per gel (4°C). The positions of the enzymes on the gel were located by either immersing the gel in an activity staining solution containing the assay mixture (in the direction of malate oxidation), 0.08 mg of phenazine methosulfate per ml, and 0.2 mg of Nitro Blue Tetrazolium per ml (7), or by staining the gels with Coomassie blue R-250. Subunit molecular weights of malate dehydrogenases were determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) in vertical slab gels as described by Laemmli (12). Samples were denatured by boiling for 5 min in an equal volume of sample buffer consisting of 0.125 M Tris hydrochloride (pH 6.8), 2% (wt/vol) SDS, 2% (vol/vol) 2-mercaptoethanol, 20% (vol/vol) glycerol, and 0.001% (wt/vol) bromphenol blue. Electrophoresis was carried out for 4 h on 10% polyacrylamide slab gels containing 0.1% (wt/vol) SDS at a constant current of 30 mA per slab (25°C).

The molecular weight of cross-linked malate dehydrogenase (see below) was estimated by SDS-PAGE by the procedure described by Davies and Stark (4). Proteins were subjected to electrophoresis on 5% acrylamide in the presence of 0.1% (wt/vol) SDS. The gel and electrode buffer was 0.1 M sodium acetate-0.1 M boric acid containing 0.1% (wt/vol) SDS.

**Cross-linking of Rhodospirillum rubrum MDH.** Cross-
linking of proteins with dimethylsuberimidate was performed by the procedure described by Carpenter and Harrington (3). Solutions of dimethylsuberimidate were prepared in 0.2 M triethanolamine hydrochloride (pH 8.5) immediately before treatment. Protein solutions (55 μg each) were mixed with dimethylsuberimidate to final concentrations of 13 and 26 mM (of the cross-linker) and incubated for 90 min at room temperature (40-μl volume). The mixture was then treated with 40 μl of 2% (wt/vol) SDS and 2% (vol/vol) 2-mercaptoethanol in 0.2 M triethanolamine hydrochloride (pH 8.5), incubated for 2 h at 37°C, immediately frozen in dry ice-isopropanol, and stored overnight at -10°C. Bromphenol blue in 50% (vol/vol) glycerol was added to protein samples, and electrophoresis was carried out as described above.

**Protein determination.** Protein in crude extracts and purified fractions were determined by using a dye binding assay (Bulletin 1069; Bio-Rad Laboratories, Richmond, Calif.); bovine serum albumin was used as a standard. Proteins in gel filtration fractions were monitored as the A280.

**Chemicals.** Alcohol dehydrogenase, pig heart mitochondrial malate dehydrogenase, bovine serum albumin, cytochrome c, hemoglobin, dl-malic acid, oxalacetate, dimethylsuberimidate, and protein standards for nondenaturing PAGE were purchased from Sigma. NAD, NADH, phenazine methosulfate, and Nitro Blue Tetrasonolum were obtained from U.S. Biochemicals. Catalase, ferritin, and ovalbumin were obtained from Pharmacia; and alphachymotrypsin was obtained from Worthington Diagnostics, Freehold, N.J.

**RESULTS**

**Purification of MDH from nonsulfur purple bacteria.** The purification schemes for MDHs from the four nonsulfur purple bacteria studied are summarized in Table 1. MDH was obtained from *Rhodobacter capsulatus* in high yield by a single purification step by using affinity chromatography on a triazine dye. This purification procedure was highly efficient because the *Rhodobacter capsulatus* enzyme bound tightly to the affinity column and was not removed by KCl, even after extensive washing; specific elution with KCl; *Rhodobacter capsulatus* MDH required 2 mM NADH or a combination of 5 mM NAD⁺ and 10 mM malate. Recoveries of *Rhodobacter capsulatus* MDH were nearly 70% (Table 1). The purification protocol for *Rhodospirillum rubrum* MDH was similar to that for *Rhodobacter capsulatus*, except the former enzyme could be partially eluted with 1 M KCl; *Rhodospirillum rubrum* MDH was thus eluted with NADH or NAD⁺-malate following only a brief wash with 1 M KCl. MDH from *Rhodobacter capsulatus* was released from the affinity column at lower ionic strengths (0.6 M KCl), and thus a modified procedure was necessary to purify MDH from this organism. *Rhodobacter capsulatus* MDH was obtained by a combination of ammonium sulfate precipitation and affinity chromatography (Table 1). Alternatively, affinity chromatography and rechromatography could be used to purify *Rhodobacter capsulatus* MDH, but lower recoveries were obtained by this method than by salting out followed by a single affinity step.

MDH from *Rhodocyclus purpureus* bound less tightly to *Matrex Gel Red A*, since it was released with as low as 0.15 M KCl. *Rhodobacter capsulatus* MDH was therefore obtained in two purification steps, affinity chromatography followed by hydroxyapatite chromatography (Table 1). Affinity chromatography was still the major technique for purification of *Rhodocyclus purpureus* MDH, however, because the majority of contaminating proteins were removed at this step. Hydroxyapatite chromatography of *Rhodobacter capsulatus* MDH on Bio-Gel HTP resulted in the removal of the few remaining contaminating proteins and was performed with minimal losses of MDH activity (Table 1).

Specific activities of purified MDHs from nonsulfur purple bacteria varied considerably (Table 1). The MDH from *Rhodobacter capsulatus* had a substantially lower specific activity than that from *Rhodospirillum rubrum*, which had the highest; the specific activity of the purified MDH from *Rhodospirillum rubrum* was almost three times that of *Rhodobacter capsulatus* MDH (Table 1). Purified *Rhodobacter capsulatus* and *Rhodocyclus purpureus* MDHs showed specific activities intermediate between those of *Rhodospirillum rubrum* and *Rhodobacter capsulatus* (Table 1).

**Criteria of enzyme purity.** Purified MDHs from nonsulfur purple bacteria were adjudged to be homogeneous since they migrated as single bands on SDS-PAGE (Fig. 1A) and on nondenaturing PAGE (Fig. 1B). In addition, activity staining of nondenaturing gels (data not shown) yielded one active band for each MDH, the location of which on gels coincided with the band stained for protein (Fig. 1B).

**Molecular weight.** Three criteria of molecular weight were used to confirm the native molecular weights of MDHs from nonsulfur purple bacteria: gel filtration through a calibrated Sephacryl S-300 column (Fig. 2), sucrose density gradient centrifugation (Fig. 3), and nondenaturing PAGE at various gel concentrations (Fig. 4). The values obtained by each method were in excellent agreement and are summarized in Table 2. In all cases, the data indicate that the molecular weight of *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodobacter capsulatus* MDH is within the range of $130 \times 10^3$ to $140 \times 10^3$, and that the molecular weight of the enzyme from *R. purpureus* is about half that size, $(60 \times 10^3)$. The subunit molecular weights of *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodobacter capsulatus* MDH.
The chromatography. calculated by the method described by Reiland (19).

FIG. 1. Gel electrophoresis of phototrophic purple bacterial MDHs. (A) SDS-PAGE. Lane 1, *Rhodospirillum rubrum* crude extract (100 µg of protein); lanes 2 to 5, purified MDH from *Rhodospirillum rubrum* (20 µg; lane 2), *Rhodobacter capsulatus* (16 µg; lane 3), *Rhodomicrobium vannielii* (16 µg; lane 4) *Rhodocyclus purpureus* (10 µg; lane 5); lane 6, molecular weight standards. (B) PAGE (7.5% acrylamide) of native MDHs from phototrophic purple bacteria. Lane 1, *Rhodospirillum rubrum* (20 µg of protein); lane 2, *Rhodobacter capsulatus* (20 µg); lane 3, *Rhodomicrobium vannielii* (15 µg); lane 4, *Rhodocyclus purpureus* (15 µg).

vannielii MDH were all in the range of $35 \times 10^3$ to $37 \times 10^3$, whereas the subunit of *Rhodococcus purpureus* MDH was substantially smaller, about $26 \times 10^3$ to $28 \times 10^3$ (Fig. 1A and Table 2).

**Quaternary structure.** Since the results of SDS-PAGE experiments (Fig. 1A) show that MDH from phototrophic purple bacteria consists of only a single subunit, we propose that the quaternary structure of MDH from *Rhodospirillum rubrum*, *Rhodobacter capsulatus*, and *Rhodomicrobium vannielii* is tetrameric, consisting of four identical subunits, and that MDH of *Rhodocyclus purpureus* is dimeric, consisting of two identical subunits. To confirm these predictions, the elution volumes of MDH from *E. coli*, *B. megaterium*, and pig heart mitochondria were determined by gel filtration. The results (Fig. 2) showed that *E. coli* and pig heart mitochondrial MDHs (which are dimers [16]) coeluted with *Rhodococcus purpureus* MDH, while *B. megaterium* MDH (which is a tetramer [16]) coeluted with MDH from the other phototrophic species.

Additional evidence of the tetrameric nature of the high-molecular-weight MDHs of *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodomicrobium vannielii* was obtained in protein cross-linking experiments with dimethylsuberimidate. Following treatment of purified MDH from *Rhodospirillum rubrum* with cross-linker, denaturation with SDS, and separation by SDS-PAGE, four protein species were observed (Fig. 5). The typical cascading appearance of a tetrameric protein was observed in cross-linking experiments, with the major protein band present as the monomer and decreasing amounts of protein in the dimeric, trimeric, and tetrameric forms of the enzyme (Fig. 5). In addition, the molecular weight of each multiple of *Rhodospirillum rubrum* MDH could be determined from the cross-linking experiment and was as follows: monomer, 37,000; dimer, 72,000; trimer, 110,000; and tetramer, 146,000. The last value is in excellent agreement with the other measurements of native molecular weight made in this study (Table 2). Similar

FIG. 2. Estimation of the molecular weight (MW) of MDH from nonsulfur purple phototrophic bacteria by S-300 Sephacryl column chromatography. The protein markers and their respective molecular weights were as follows: 1, ferritin (440,000); 2, catalase (235,000); 3, yeast alcohol dehydrogenase (150,000); 4, bovine serum albumin (67,000); 5, ovalbumin (43,000); 6, alpha-chymotrypsin (24,000); 7, cytochrome c (12,400). MDHs were as follows: a, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *B. megaterium*; b, *Rhodomicrobium vannielii*; c, *Rhodocyclus purpureus*, *E. coli*, and pig heart mitochondria. $K_v$ values were calculated by the method described by Reiland (19).

FIG. 3. Sucrose gradient centrifugation of phototrophic purple bacterial MDHs. The protein markers and their respective molecular weights (MW) were as follows: 1, bovine liver catalase (250,000); 2, yeast alcohol dehydrogenase (150,000); 3, bovine hemoglobin (64,000). MDHs were as follows: a, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *B. megaterium*; b, *Rhodomicrobium vannielii*; and c, *Rhodococcus purpureus*, *E. coli*, and pig heart mitochondria. Centrifugation was performed on a 5 to 20% linear gradient of sucrose, as described in the text.
results were obtained in cross-linking experiments with Rhodobacter capsulatus MDH (data not shown).

**DISCUSSION**

In this study the citric acid cycle enzyme MDH was purified to homogeneity from several species of nonsulfur purple bacteria in either a single purification step on a triazine dye affinity column or a two-step procedure with the affinity dye plus other biochemical techniques. Similar purification procedures have been employed previously for the isolation of MDH from a variety of gram-positive and gram-negative bacteria, including two *Rhodobacter* species (17, 21, 22). In general, triazine dye affinity columns bind dehydrogenases quite tightly, and enzyme inhibition studies have shown that the dye interacts with the substrate or cofactor binding sites of many dehydrogenases. In preliminary studies we found Matrex Gel Red A to be far superior to the blue, orange, or green matrices in effecting purification of phototrophic bacterial MDHs. The only drawback to using the Matrex Gel Red A matrix for purifying phototrophic bacterial MDHs was that relatively high concentrations (millimolar) of NAD+ or NADH were necessary to elute the enzyme from the column.

Because two size classes of MDH are known (2, 15, 16, 26) and the majority of gram-negative bacteria contain the smaller (dimeric) form of the enzyme (2), it was necessary to verify the molecular weight of MDH from the phototrophic species (which are all gram negative) by several methods. Measurements by four different techniques yielded essentially the same results, and comparisons of known dimeric and tetrameric MDHs with those of the phototrophic species serves as further proof that *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, and *Rhodobacter microbium vannieli* produce tetrameric MDHs, whereas *Rhodococcus purpureus* produces a dimeric MDH.

Our results are not in agreement with previously published accounts of the molecular weight of MDH from certain phototrophic bacteria. Using a different strain of *Rhodobacter capsulatus* (strain 37b4) than that employed herein (strain B100), Ohshima and Sakuraba (17) reported the molecular weight of MDH from this organism to be 80,000, implying a dimeric quaternary structure. Similar results have been published concerning the molecular properties of MDH from *Rhodobacter sphaeroides* (21) and *Rhodopseudomonas palustris* (16). In each of these cases, however, the sole criterion of molecular weight was measurement of elution volumes of MDH from gel filtration columns. In the work of Ohshima and Sakuraba (17), for example, the size of the MDH from *Rhodobacter capsulatus* was determined on a Sephadex G-150 column. We found Sephadex G-150 to be unsatisfactory for resolving MDHs from phototrophic bacteria because this matrix yielded broad peaks with consider-

![FIG. 4. Estimation of native molecular weights (MW) of *Rhodospirillum rubrum* and *Rhodococcus purpureus* MDHs by PAGE at several gel concentrations. The protein markers and their respective molecular weights were as follows: 1, jack bean urease, dimer (240,000); 2, bovine serum albumin, dimer (132,000); 3, bovine serum albumin, monomer (66,000); 4, chicken egg albumin (45,000); 5, fumaric anhydride (29,000); 6, alpha-lactalbumin (14,200). MDHs were as follows: a, *Rhodospirillum rubrum*; b, *Rhodococcus purpureus*.](image1)

![FIG. 5. SDS-PAGE of purified MDH from *Rhodospirillum rubrum* cross-linked with dimethylsuberimidate. Cross-linking of MDH was performed as described in the text. Lane 1, high-molecular-weight standards (indicated in thousands) lanes 2 and 3, R. rubrum MDH cross-linked with 13 and 26 mM dimethylsuberimidate, respectively; lane 4, low-molecular-weight standards (indicated in thousands).](image2)
able tailing. By contrast, fractions collected from Sephacryl S-300 columns yielded sharp peaks which allowed easy separation of dimeric and tetrameric MDHs. In our hands, MDH from *Rhodobacter capsulatus* 376b (studied by Ohshima and Sakuraba [17]) showed a tetrameric structure identical to that of *Rhodobacter capsulatus* B100 (unpublished results). In addition, measurements by gel filtration of MDH from *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris* performed in our laboratory shows that the enzyme from both species was tetrameric (data not shown).

Therefore, a major implication from the results of our work is that tetrameric MDHs are widely distributed among phototrophic purple bacteria.

The occurrence of both low- and high-molecular-weight MDHs in different groups of gram-positive or gram-negative eubacteria is not uncommon, although most gram-negative eubacteria examined to date have dimeric MDHs (2, 16).

Murphy et al. (16) reported both small and large MDHs in different species of members of the families *Brevibacteriaceae* (all representatives are gram-negative) and *Corynebacteriaceae* (all representatives are gram-positive). MDH from *Bacillus* species are clearly tetramers (16, 26), with the exception of that from the extreme thermophile *Bacillus caldolyticus*, which is a dimer (11). MDH from *Thermoactinomyces* species (an endospore former which is phylogenetically highly related to *Bacillus* species) is also a tetramer (23). MDHs from other actinomycetes, however, are dimers (23).

MDHs have been studied from only a few archaeabacteria, but both large and small enzymes have been observed. Tetrameric malate dehydrogenases are found in the thermoacidophiles *Thermoplasm acidophilum* and *Sulfolobus acidocaldarius* (8), while dimeric malate dehydrogenases are found in *Methanobacterium hungatii* (25) and a *Halobacterium* species (14).

The presence of tetrameric MDHs in nonsulfur purple bacteria may have some physiological and biochemical significance. These organisms grow phototrophically (anaerobically) in nature and seem to specialize in the use of organic acids (such as malate) as their main carbon (but not energy) sources (18, 24). It is possible that tetrameric MDHs are better suited than dimeric enzymes to these physiological demands. In this connection, further study of the catalytic and regulatory properties of MDHs from nonsulfur purple bacteria may help explain the widespread distribution of tetrameric MDHs in these organisms.

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**LITERATURE CITED**


