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Partial Purification and Characterization of Two Hydrogenases from the Extreme Thermophile *Methanococcus jannaschii*

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F_{420}-nonreactive and F_{420}-reactive hydrogenases have been partially purified from *Methanococcus jannaschii*, an extremely thermophilic methanogen isolated from a submarine hydrothermal vent. The molecular weights of both hydrogenases were determined by native gradient electrophoresis in 5 to 27% polyacrylamide gels. The F_{420}-nonreactive hydrogenase produced one major band (475 kilodaltons), whereas the F_{420}-reactive hydrogenase produced two major bands (990 and 115 kilodaltons). The F_{420}-nonreactive hydrogenase consisted of two subunits (43 and 31 kilodaltons), and the F_{420}-reactive hydrogenase contained three subunits (48, 32, and 25 kilodaltons). Each hydrogenase was active at very high temperatures. Methyl viologen-reducing activity of the F_{420}-nonreactive hydrogenase was maximal at 80°C but was still detectable at 103°C. The maximum activities of F_{420}-reactive hydrogenase for F_{420} and methyl viologen were measured at 80 and 90°C, respectively. Low but measureable activity toward methyl viologen was repeatedly observed at 103°C. Moreover, the half-life of the F_{420}-nonreactive hydrogenase at 70°C was over 9 h, and that of the F_{420}-reactive enzyme was over 3 h.

Hydrogenases catalyze the oxidation of hydrogen (H_2) and thus play an important role in the production of methane by methanogens. Hydrogenases from methanogens can generally be distinguished on the basis of their activities. So-called F_{420}-reactive hydrogenases reduce the methanogen redox coenzyme F_{420} and artificial dyes such as methyl viologen (MV), whereas F_{420}-nonreactive hydrogenases are inactive toward F_{420} and its derivatives. Some methanogens contain only one such enzyme, for example, *Methanococcus voltae* (21), *M. vannielii* (27), and *Methanospirillum hungatei* (26) (F_{420} reactive in all three cases) and *Methanobacterium* sp. strain G2R (F_{420} nonreactive) (19). However, many other methanogens contain both types of hydrogenase (2, 6, 9, 10, 22). The reduction of F_{420} by F_{420}-reactive hydrogenase generates reducing equivalents for the redox metabolism of methanogens; the primary function of F_{420}-nonreactive hydrogenase in methanogens is less clear, although possible roles have been proposed (6).

Using a specialized bioreactor designed for high temperatures and pressures, we recently showed that the overall hydrogenase activity in cell extracts of *Methanococcus jannaschii* is substantially increased by increased hyperbaric pressure at the same partial pressure of H_2 (19a). *M. jannaschii* is an extremely thermophilic methanogen isolated from a submarine hydrothermal vent (11). In similar studies, the growth rate of *M. jannaschii* was accelerated by pressure up to 750 atm (ca. 76 MPa) (20). These parallel results suggest that the response to pressure of cellular hydrogenase(s) may be an important factor in the behavior of *M. jannaschii* in its natural, high-pressure habitat. A close correspondence between pressure effects on hydrogenase activity and methanogenesis would be consistent with the integral participation of hydrogenases in the biogenesis of methane.

Hydrogenases have also attracted considerable attention for their potential applications in vitro (15), which include cofactor regeneration in immobilized oxidoreductase systems (18), photochemical production of H_2 (23), detritiation of contaminated water (14), and the production of organic chemicals from H_2 and carbon dioxide (CO_2) (13). With regard to these possible applications, it is noteworthy that a few hydrogenases have shown activity at very high temperatures. Hydrogenase in cell extracts of *Clostridium thermoaceticum*, for example, exhibited maximum activity at 95°C (5), but the half-life of the enzyme at 70°C was only about 8 min. In addition, hydrogenase activity was measured at 90°C for crude extracts of the hyperthermophile *Pyrodictium brockii* (25); however, no thermostability results were reported. In this paper, we describe the partial purification and characterization of F_{420}-reactive and F_{420}-nonreactive hydrogenases from *M. jannaschii*. Both enzymes exhibited activity above 100°C. Moreover, the half-life of the F_{420}-reactive hydrogenase at 70°C was over 3 h, and that of the F_{420}-nonreactive enzyme was over 9 h.

MATERIALS AND METHODS

Flavine adenine dinucleotide, DNase I, 2,3,5-triphenyltetrazolium chloride, 5-sulfosalicylic acid, and L-asparagine were from Sigma Chemical Co., St. Louis, Mo. Protein assay solution, chemicals for silver staining, ampholytes, and isoelectric focusing standards were obtained from Bio-Rad Laboratories, Richmond, Calif. DEAE-Sepharose CL-6B, Sephadex G-10, and protein molecular weight standards were purchased from Pharmacia LKB Biotechnology Inc., Piscataway, N.J. All reagents used for polyacrylamide gel electrophoresis (PAGE) were from Bio-Rad or from Boehringer Mannheim Biochemicals, Indianapolis, Ind. MV was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis. Yeast extract was a product of Difco Laboratories, Detroit, Mich. A gas mixture containing H_2, CO_2, and H_2S was purchased from Matheson Gas Products, Newark, Calif. All other chemicals were of reagent grade.

Growth of *M. jannaschii*. *M. jannaschii* was grown at 85°C in a 7-liter fermentor (Chemapec, Inc., Woodbury, N.Y.). The high temperature was maintained by using heating tape (Cole-Parmer, Chicago, Ill.) coupled to a proportional controller (Oven Industries, Inc., Mechanicsburg, Pa.). The growth medium was as described previously (20), except

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that 5 mM instead of 50 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) was used and 0.3 g of yeast extract per liter was added to the medium. A mixture of 79% H_2, 20% CO_2, and 1% H_2S was bubbled through the medium at a rate of 0.5 liter/min. The pH was adjusted to 6.8 with NaOH at room temperature before the addition of sodium thiosulfate, the gases, and 2.4 ml of β-mercaptoethanol to scavenge oxygen. *M. jannaschii* growing on a similar defined growth medium at 85°C in shaken hungate bottles was used to inoculate the fermentor (approximately 1%, vol/vol). Cells were cooled to 50°C and flushed with CO_2 for 1 h before harvesting. Cell pellets were obtained by centrifugation at 10,000 × *g* for 20 min at 4°C in a Sorvall Superspeed RC-2 automatic refrigerated centrifuge. The pellets were suspended in 10 mM sodium phosphate buffer (pH 7.2) and stored aerobically at −20°C.

Enzyme purification. All steps were performed at 4°C unless otherwise stated, and all centrifugation steps were carried out at 10,000 × *g* for 20 min. The cell pellet was thawed in 100 ml of sodium phosphate buffer (50 mM, pH 7.2) containing 10 mM $MgCl_2 \cdot 6H_2O$ and 3 mg of DNase I. Cells were lysed with 1% Triton X-100, and the cell debris was removed by centrifugation. The supernatant was filtered through a 0.45-μm-pore size filter and loaded onto a DEAE-Sepharose CL-6B column (5 by 7 cm) equilibrated with 50 mM sodium phosphate buffer (pH 7.2). A gradient from 0 to 600 mM KCl in 50 mM sodium phosphate buffer (650 ml) was used for protein elution. Fractions containing MV-reducing hydrogenase activity were pooled, and the hydrogenases were precipitated by adding ammonium sulfate to 70% saturation. After centrifugation, the sedimented proteins were dissolved in 10 mM Tris buffer (pH 7.5) and desalted by ultrafiltration at 0°C. The protein solution was diluted to 1 mg/ml by adding 34 mM asparagine–Tris buffer (pH 7.3) containing 2.5% (vol/vol) glycerol and 2.5 μM flavine adenine dinucleotide. Preparative electrophoresis in a 5% polyacrylamide slab gel (3 mm thick, 60-ml separating gel, 8-ml stacking gel, 10-mg protein load) was used to separate the two hydrogenases. The electrophoresis was carried out at 30 mA per gel for 10 h in the asparagine-Tris buffer system (8). Two major MV-reducing hydrogenase activity bands were obtained by activating a small strip of the gel. Each band was then cut out of the gel (using the activated strip as the marker), and the enzymes were eluted by using a Bio-Rad model 422 electro-eluter. Electroelution was carried out in Tris-glycine buffer (pH 8.3) for 3 h at 60 mA. The eluted enzyme solutions were concentrated in an Amicon miniconcentrator cell and stored in 10 mM Tris buffer (pH 7.5) at 4°C.

F_{420} purification. Fractions eluted from the ion-exchange column at around 310 mM KCl showed high A_{420}. These fractions were combined and purified further to obtain cofactor F_{420}. The pooled samples were loaded onto a DEAE-Sepharose CL-6B column (5 by 7 cm) equilibrated with 900 ml of distilled water at 4°C. The cofactor was eluted from the column in the dark by using a gradient of 0 to 1 M Na_2CO_3. F_{420}-containing fractions with an A_{420}/A_{250} ratio of greater than 0.9 were combined. These fractions were rotoevaporated, dissolved in a minimal amount of water, and then desalted by passage through a column of Sephadex G-10 (1.5 by 23 cm). The final, desalted F_{420}-containing solution was shielded from light and stored at 4°C.

Reactivation of hydrogenases. Aerobically purified hydrogenases were reversibly inactivated and required reactivation for activity measurements. The enzyme was typically reactivated in an anaerobic glove box (gas phase of N_2-H_2, 95:5) in a solution containing 50 μM MV, 10 mM β-mercaptoethanol, and 50 mM *N*-(2-hydroxyethyl)-piperazine-*N*′-3-propanesulfonic acid (EPPS) (pH 7.5). Development of a light blue color at room temperature indicated active enzyme. For F_{420} assays, MV was replaced by 2 μM F_{420} in the reactivation solution; the disappearance of yellow color indicated active enzyme.

Hydrogenase assays. The reduction of MV was monitored by A_{578} (ε = 9.7 mM cm^{-1}), and F_{420} reduction was followed by A_{420} (ε = 45.5 mM cm^{-1}) with a Beckman DU-6 spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.). The reactions were carried out in test tubes (1.3 by 10 cm) capped with rubber stoppers. The assay mixture contained 2 ml of 50 mM EPPS buffer (pH 7.5), 2 mM MV, and 2 mM β-mercaptoethanol. The headspace was flushed with H_2 (2 atm [ca. 202.5 kPa]), and the test tubes were incubated at the assay temperature for at least 30 min. The reaction was initiated by adding 100 μl of the reactivated enzyme solution to the tube and mixing the contents by shaking. For reduction of F_{420}, MV was replaced by 9 μM F_{420}. Assay conditions were varied for specific experiments as described below.

Protein determination. Protein concentrations were determined by the Bradford Bio-Rad protein assay (4) with bovine serum albumin (BSA) as the standard.

Gel electrophoresis. Gel electrophoresis was performed using a Bio-Rad Protean II slab gel apparatus. Native gel electrophoresis was carried out with 5% polyacrylamide gels by the method of Hedrick et al. (8). Stacking was carried out at 25 mA, and the separation was at 35 mA. The gels were stained for protein with Coomassie brilliant blue R-250. MV-reducing hydrogenase activity was located by immersing the gel in a solution of 2 mM MV–10 mM β-mercaptoethanol–50 mM Tris (pH 7.5) and incubating under hydrogen at room temperature. The reduced MV bands were fixed by adding 1% (wt/vol) tetrazolium chloride solution.

Molecular weight estimation. Native gradient gel electrophoresis (5 to 27% polyacrylamide) was run as described by O'Farrell (24) with the omission of sodium dodecyl sulfate (SDS) and β-mercaptoethanol. The electrophoresis was performed at 200 V for 10 h. A plot of log molecular weight of standard proteins versus mobility was used for estimating the molecular weights of hydrogenases.

The molecular weights of hydrogenase subunits were estimated by using two-dimensional electrophoresis. The lanes were cut from the native gradient gel and incubated in the sample buffer (24) for 1 h at room temperature and for 5 min at 90°C. These gel pieces were then annealed to the top of the SDS-polyacrylamide gels (10% acrylamide, 2.5% methylenebisacrylamide) (3). The electrophoresis was run at 25 mA constant current.

Isoelectric focusing. A Bio-Rad model 111 mini-IEF cell was used for isoelectric focusing as recommended by the manufacturer. Ampholytes covering a pH range of 3 to 10 provided the pH gradient. Protein bands were silver stained as recommended by Bio-Rad.

pH optima and stability. The effect of pH on hydrogenase activity was measured over a pH range of 5 to 11 in an EPPS-cyclohexylaminopropanesulfonic acid (CAPS)-morpholineethanesulfonic acid solution (each buffer at 50 mM) at 70°C. The different pK_a values of the component buffers maintained the buffering capacity over the entire pH range. The pH of each assay solution was adjusted with 4 M NaOH at 70°C.

The effect of pH on enzyme stability was determined by incubating reactivated enzyme in an assay tube at the desired pH for 1 h at 70°C before adding substrate. The assay tubes contained 10 μg of BSA per ml for the F_{420}-reactive

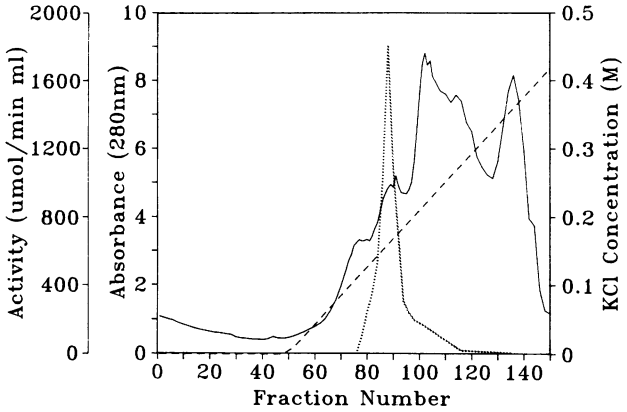

FIG. 1. Ion-exchange chromatography on DEAE-Sepharose of an aerobic crude extract from *M. jannaschii*. Fractions (5 ml each) were collected during a gradient of 0 to 0.6 M KCl (– – –) in sodium phosphate buffer and were assayed for MV-reducing activity (····) and protein content (——), determined by A_{280}.

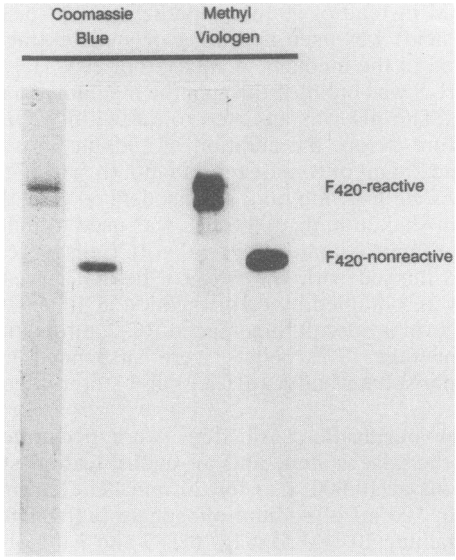

FIG. 2. Coomassie blue staining (left lanes) and MV-reducing activity staining (right lanes) of hydrogenases from *M. jannaschii* in 5% native polyacrylamide gel. Samples of 5 μg of F_{420}-reactive hydrogenase and 8 μg of F_{420}-nonreactive hydrogenase were loaded in the appropriate lanes.

enzyme and 2.5 μg of BSA per ml for the F_{420}-nonreactive enzyme. BSA had a substantial stabilizing effect on both hydrogenases over the pH range studied.

Temperature optima and thermostability. The temperature optimum of each hydrogenase was determined by measuring activities over a temperature range of 25 to 110°C. Assays were performed in 50 mM EPPS buffer (pH 7.5), and buffers of various pHs at room temperature were used to maintain a constant pH at the assay temperatures. Assays up to 90°C were carried out directly in the spectrophotometer equipped with a water-heated sample cell. At higher temperatures, the tubes were heated in a heating block. The reaction was followed by measuring the absorbance of the tube transferred every 15 s to the spectrophotometer maintained at 90°C. Transferring the tubes between the block and the spectrophotometer decreased the temperature of the tube by less than 2°C, as determined by measuring the temperature in the tube with a thermocouple.

Thermostability was examined by incubating the reactivated enzyme at various temperatures. Samples were removed at different times, and the activity was measured at 70°C in 50 mM EPPS buffer (pH 7.5). The assay tubes contained 10 and 2.5 μg of BSA per ml for F_{420}-reactive and F_{420}-nonreactive hydrogenases, respectively.

RESULTS

Enzyme purification. Ion-exchange chromatography of the crude extract is illustrated in Fig. 1. The F_{420}-nonreactive hydrogenase was present primarily in fractions 80 through 98, whereas the F_{420}-reactive hydrogenase was found mostly in fractions 92 through 104. The activity peak corresponding to F_{420}-reactive hydrogenase is not shown in Fig. 1. Fractions containing the two hydrogenases were pooled and further purified by ammonium sulfate precipitation and preparative electrophoresis. Several buffers were tried in efforts to elute hydrogenases from the preparative gel. The asparagine-Tris buffer could not be used because the higher voltage required led to system failure within an hour. Shut-down of the electroeluter also occured when 10 mM Tris buffer (without glycine) was used over a pH range of 7.3 to 8.3. The best elution results were obtained in the Tris-glycine buffer at pH 8.3.

Native PAGE of F_{420}-nonreactive hydrogenase eluted from the preparative gel yielded a single band upon treatment with silver stain, Coomassie blue, and MV (Fig. 2). Native PAGE of the eluted F_{420}-reactive hydrogenase reproducibly gave a double band (Fig. 2). Isoelectric focusing of each hydrogenase revealed a single protein band corresponding to a pI of 5.5 (data not shown). The molecular weights of both hydrogenases were determined by 5 to 27% polyacrylamide native gradient electrophoresis. The F_{420}-nonreactive hydrogenase produced one major band (475 kilodaltons [kDa]), whereas the F_{420}-reactive hydrogenase produced two major bands (990 and 115 kDa). In addition to the major bands, both hydrogenases showed a few minor bands.

The three-step procedure summarized in Table 1 provided about 1 mg of each purified hydrogenase from 775 mg of crude extract. The specific activities of the final enzyme preparations were quite low relative to those obtained after ion-exchange chromatography and salt precipitation. Substitution of preparative electrophoresis with chromatography on columns of Octyl-Sepharose and hydroxylapatite led to a 19-fold overall increase in MV-reducing activity but failed to separate the two hydrogenases from each other. Subsequent fast-protein liquid chromatography on a Mono Q (QAE monodisperse resin) column also failed to resolve the two enzymes.

TABLE 1. Typical purification of F_{420}-reactive and F_{420}-nonreactive hydrogenases from *M. jannaschii*

Prepn	Total protein (mg)	Sp act at 25°C (μmol of H_2 min^{-1} mg^{-1})	Purification (fold)
Crude extract	775	128[a]	
DEAE-Sepharose	262	361[a]	2.8
70% salt precip.	105	426[a]	3.3
Preparative PAGE			
F_{420}-nonreactive enzyme	1.1	7.9	
F_{420}-reactive enzyme	1.0	16 (MV), 5.2 (F_{420})	

[a] Combined MV-reducing activity of the two hydrogenases.

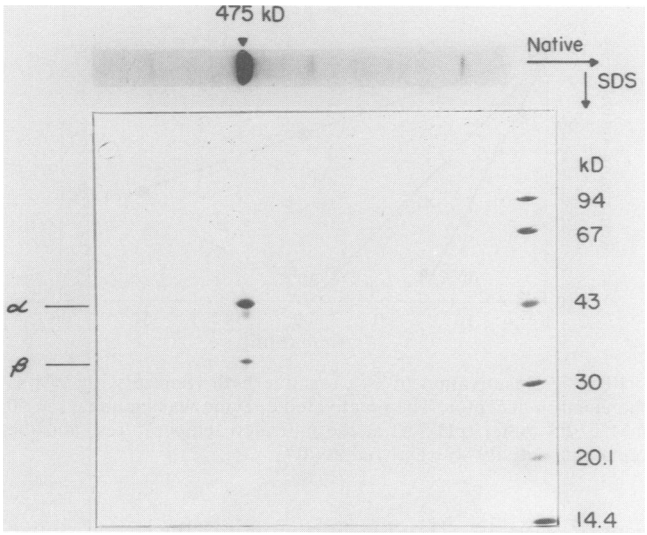

FIG. 3. Two-dimensional native SDS-PAGE of F_{420}-nonreactive hydrogenase. The first dimension was native gradient (5 to 27% polyacrylamide) PAGE, and the second dimension was SDS-PAGE (12.5% polyacrylamide), as described in the text. Samples of 165 μg of enzyme were loaded in each lane, and the gels were stained with Coomassie blue.

The subunit composition of each hydrogenase was examined by two-dimensional PAGE. The F_{420}-nonreactive hydrogenase produced two bands (43 and 31 kDa; Fig. 3), whereas the F_{420}-reactive hydrogenase produced three bands (48, 32, and 25 kDa; Fig. 4). The same bands were obtained repeatedly upon treatment with either Coomassie blue or silver stain.

Properties of the purified hydrogenases. The purified hydrogenases were stored at 4°C and used within a week for activity measurements. Once the enzymes were reactivated at room temperature, there was no loss of activity over a period of 24 h. The activity decreased by about 5% per day upon storage at 4°C, however, and storage for a few days at −70°C resulted in the loss of high-temperature (above 80°C) activity for both hydrogenases. In addition, the extent of reactivation was dependent on the buffer. The purified enzymes reactivated and assayed in biological buffers (Tris, EPPS) at pH 7.5 exhibited twice as much activity as those reactivated in 50 mM sodium phosphate buffer at the same pH.

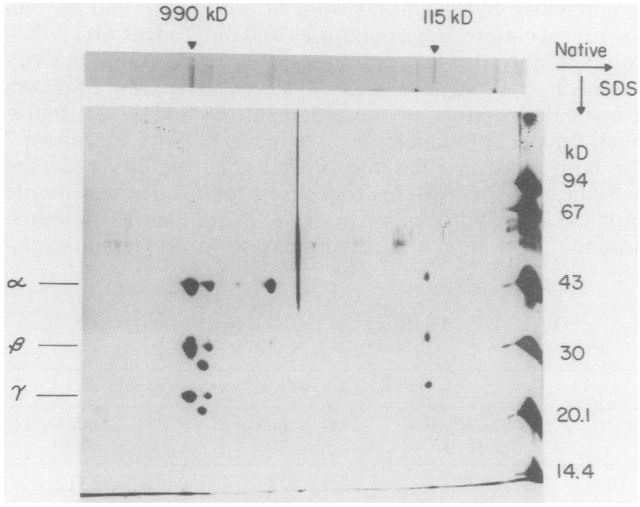

FIG. 4. Two-dimensional native SDS-PAGE of 45 μg of F_{420}-reactive hydrogenase. The conditions were as described in the legend to Fig. 3. The gradient gel was stained with Coomassie blue, and the SDS-PAGE gel was silver stained.

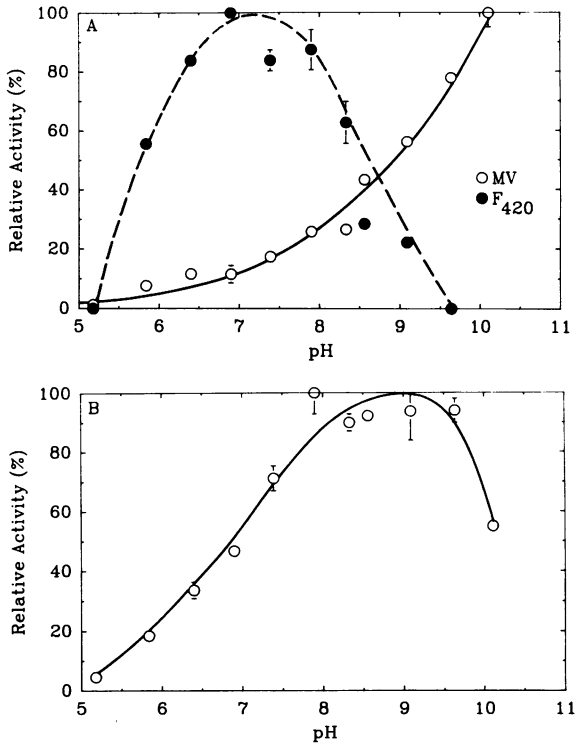

FIG. 5. (A) pH profiles of F_{420} and MV reduction by F_{420}-reactive hydrogenase. (B) pH profile of MV reduction by F_{420}-nonreactive hydrogenase. Assays were performed in EPPS-CAPS-MES solution (each buffer at 50 mM) at 70°C. All values shown are averages of duplicate measurements, and the absence of error bars indicates that the mean deviation was within the limits denoted by the size of the symbol.

The optimum pH of F_{420}-reactive hydrogenase differed greatly for the two substrates, F_{420} and MV (Fig. 5A). The optimum pH for F_{420} was about 7, whereas the activity toward MV continued to increase up to pH 10, beyond which the enzyme was unstable. Similar results were observed for F_{420}-reactive hydrogenase from *Methanobacterium thermoautotrophicum* ΔH (6). The pH dependence of the MV-reducing activity of F_{420}-nonreactive hydrogenase was markedly different. The activity-pH curve of F_{420}-nonreactive hydrogenase was relatively broad and displayed an optimum at about pH 9 (Fig. 5B). Table 2 summarizes the specific activities measured at the optimum pH of each hydrogenase.

Each hydrogenase was active at very high temperatures. The maximum activities of F_{420}-reactive hydrogenase for F_{420} and MV were measured at 80 and 90°C, respectively (Fig. 6A). Low but measurable activity toward MV was repeatedly observed at 103°C. The MV-reducing activity of F_{420}-nonreactive hydrogenase was maximal at 80°C but was still detectable at 103°C (Fig. 6B). The specific activities at the optimum temperatures are listed in Table 2.

In addition to their activities at high temperatures, both hydrogenases displayed good thermostability. The thermostability of each enzyme was evaluated by measuring the activity remaining after incubation at a given temperature for

TABLE 2. Temperature and pH optima of F_{420}-reactive and F_{420}-nonreactive hydrogenases

Hydrogenase (substrate)	70°C		pH 7.5	
	Optimum pH	Sp act (μmol of H_2 min^{-1} mg^{-1})	Optimum temp (°C)	Sp act (μmol of H_2 min^{-1} mg^{-1})
F_{420} reactive (F_{420})	7	13.9	80	12.9
F_{420} reactive (MV)	10	581	90	467
F_{420} nonreactive (MV)	9	40.0	80	46.6

a fixed period of time. Thermal inactivation was studied at pH 7.5 because at 70°C both hydrogenases displayed maximal pH stability at this pH (data not shown). Representative results, obtained with F_{420}-reactive hydrogenase and MV, are shown in Fig. 7. All deactivation trajectories obeyed first-order kinetics, and the half-lives of both enzymes are presented in Table 3.

DISCUSSION

Two types of hydrogenase, F_{420}-reactive and F_{420}-nonreactive, were partially purified from the extreme thermophile *M. jannaschii*. Each hydrogenase was present as a high-molecular-mass aggregate (ca. 990 kDa for the F_{420}-reactive hydrogenase and 475 kDa for the F_{420}-nonreactive hydrogenase), which is common for hydrogenases from methanogens (6, 10, 19, 21, 26). Furthermore, the F_{420}-reactive hydrogenase was also present as a low-molecular-mass species (ca. 115 kDa). It is interesting that an αβγ complex with a molecular mass of 115 kDa was also the minimum structure of F_{420}-reducing activity in *M. thermoautotrophicum* (6).

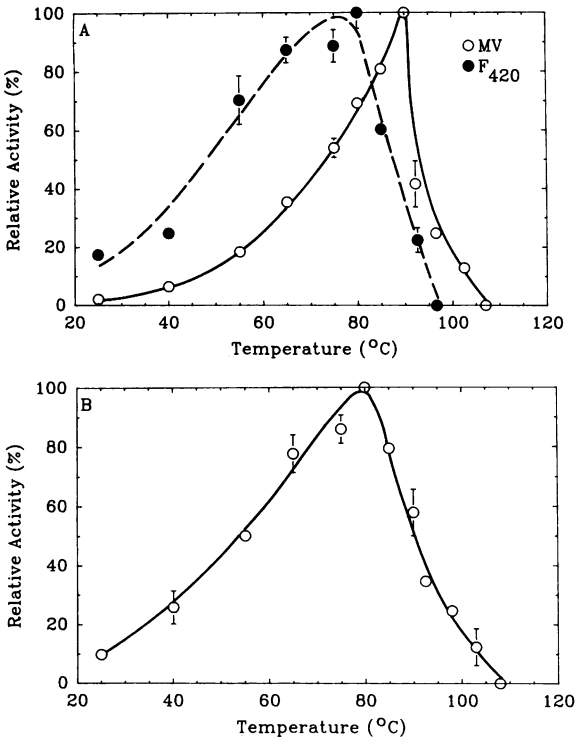

FIG. 6. (A) Temperature profiles of F_{420}- and MV-reducing activity of F_{420}-reactive hydrogenase. (B) Temperature profile of F_{420}-nonreactive hydrogenase activity. All rates were measured in 50 mM EPPS buffer adjusted to a pH of 7.5 at the assay temperature. All values shown are averages of duplicate measurements, and the absence of error bars indicates that the mean deviation was within the limits denoted by the size of the symbol.

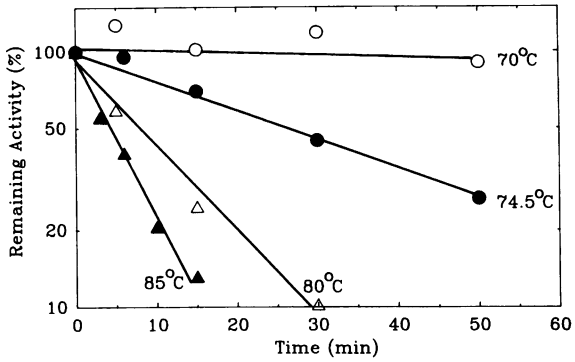

FIG. 7. Deactivation of F_{420}-reactive hydrogenase with MV as the electron acceptor. The reactivated enzyme was incubated in 50 mM EPPS buffer (pH 7.5) at the indicated temperatures, and the remaining activity was assayed at 70°C.

In general, the two types of hydrogenase from methanogens contain two to four subunits but differ in their subunit compositions. The purified F_{420}-nonreactive hydrogenase from *M. jannaschii* consisted of two subunits, as did F_{420}-nonreactive hydrogenases from *M. thermoautotrophicum* (16) and *Methanobacterium formicicum* (10). However, the estimated molecular masses of the subunits from *M. jannaschii* were lower than those from *M. thermoautotrophicum* (57 and 42 kDa) and from *M. formicicum* (48 and 38 kDa). Like many other F_{420}-reactive hydrogenases (6, 10, 21, 27), the F_{420}-reactive hydrogenase from *M. jannaschii* contained three subunits. The molecular masses of these subunits were similar to those of the F_{420}-reactive hydrogenase from *M. thermoautotrophicum* (47, 31, and 26 kDa), determined by two-dimensional native SDS-PAGE (6).

Other *Methanococcus* species from which a hydrogenase has been isolated and characterized to date are *M. voltae* (21) and *M. vannielii* (27), both mesophiles. Comparing F_{420}-reactive hydrogenases from *M. jannaschii* and *M. voltae* reveals interesting similarities and differences (*M. voltae* contains only the F_{420}-reactive enzyme). Maximal activity for each hydrogenase was observed near the optimum growth temperature of the organism (85 and 37°C); moreover, the pH optimum for F_{420}-reducing activity was about 7 for both enzymes. On the other hand, the MV-reducing activities of the two hydrogenases followed different pH profiles. The hydrogenase from *M. voltae* had an optimum around 7.5, whereas the hydrogenase from *M. jannaschii*

TABLE 3. Half-lives of purified hydrogenases at different temperatures

Temp (°C)	Half-life (min) of hydrogenase (substrate)		
	F_{420} reactive (F_{420})	F_{420} reactive (MV)	F_{420} nonreactive (MV)
70	72	222	551
75	16	25	107
80	3.9	9.2	37
85	2.7	5.2	6.2

exhibited increasing activity up to pH 10. Neither the pH nor temperature dependence of the enzyme activity was measured for hydrogenase from *M. vannielii*; hence, a comparison of these properties with hydrogenases from *M. jannaschii* is not yet possible.

Reports on the effects of temperature on hydrogenase activity and stability are rare. However, the limited data available indicate that the thermostability of a hydrogenase does not necessarily correlate with its optimum temperature. For example, hydrogenases purified from *Rhodospirillum rubrum* (1) and *M. voltae* (21) have temperature optima of 55 and 37°C, respectively, and show no loss of activity after 30 min at 80 and 65°C, respectively. In contrast, hydrogenases from *Thiocapsa roseopersicina* (7) and in cell extracts of *C. thermoaceticum* (5) have temperature optima of 75 and 95°C, respectively, but retain full activity after 30 min up to only 70 and 55°C, respectively. Hence, stability and activity data are both needed for hydrogenase characterization. The data presented here for partially purified hydrogenases from *M. jannaschii* illustrate that the F_{420}-nonreactive and F_{420}-reactive hydrogenases have half-lives of 37 and 1.2 min, respectively, at their optimum temperatures.

A common strategy for increasing enzyme stability is to immobilize the enzyme on an insoluble support (12), and improvements in hydrogenase stability upon immobilization have been reported (17, 23). For example, Nosaka et al. (23) prepared an artificial photosystem by immobilizing hydrogenase from *Desulfovibrio vulgaris* in nylon gel containing polymer viologen. The immobilized enzyme had a storage half-life of 20 days under air at 4°C, compared with 7 days for the soluble enzyme. Furthermore, the immobilized hydrogenase retained 75% activity after being used eight times, whereas the native enzyme lost 90% of its activity after being used only three times. Likewise, immobilizing hydrogenases from *M. jannaschii* may provide very stable catalysts. Immobilized hydrogenases will be the focus of future research in this laboratory.

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