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Distribution of Coenzyme F_{420} and Properties of Its Hydrolytic Fragments

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The ability of hydrolytic products of coenzyme F_{420} to substitute for F_{420} in the hydrogenase and nicotinamide adenine dinucleotide phosphate-linked hydrogenase systems of *Methanobacterium* strain M.o.H. was kinetically determined. The nicotinamide adenine dinucleotide phosphate-linked hydrogenase system was employed to quantitate the levels of F_{420} in a number of methanogenic bacteria as well as in some nonmethanogens. *Methanobacterium ruminantium* and *Methanosarcina barkeri* contained low levels of F_{420}, whereas other methanogens tested contained high levels (100 to 400 mg/kg of cells). F_{420} from six of the seven methanogens was tested by thin-layer electrophoresis and was found to be electrophoretically identical to that purified from *Methanobacterium* strain M.o.H. The only exception was *M. barkeri*, which contained a more electronegative derivative of F_{420}. *Acetobacterium woodii, Escherichia coli*, and yeast extract contained no compounds able to substitute for F_{420} in the nicotinamide adenine dinucleotide phosphate-linked hydrogenase system.

Structures have been proposed recently for the unique electron carrier coenzyme F_{420} and some of its derivatives (9). These structures are shown in Fig. 1. F_{420}, when acid hydrolyzed, loses *N*-(*N*-L-lactyl-γ-L-glutamyl)-L-glutamic acid (lactyl-γLGLG) to form a deaza-flavin mononucleotide (a flavin mononucleotide [FMN] analog), 5-(8-hydroxy-5-deazaisoalloxazin-10-yl)-2,3,4-trihydroxypentyl phosphate (F+). The dephosphorylated derivative of F+, 8-hydroxy-10-(2,3,4,5-tetrahydroxypentyl)-5-deazaisoalloxazine (FO), also is formed during hydrolysis. Another derivative of F_{420}, 8-hydroxy-10-formylmethyl-5-deazaisoalloxazine (PA), is formed during the periodate oxidation of F_{420}.

F_{420} was first purified from *Methanobacterium* strain M.o.H. and was partially characterized by Cheeseman et al. (6). It now has been purified from *Methanobacterium ruminantium* strain PS (19), *Methanobacterium thermoautotrophicum* (22), and *Methanospirillum hungatii* (11). Tzeng et al. (19) reported the presence of an F_{420}-dependent NADP-linked hydrogenase system (19) and an F_{420}-dependent formate hydrogenlyase system (18) in cell extracts of *M. ruminantium* PS. *Methanospirillum hungatii* was found by Ferry and Wolfe (11) to possess a similar, if not identical, formate hydrogenlyase system. Both colonies (8) and cells (7, 15) of methanogens exhibit a bright blue-green fluorescence when illuminated with long-wavelength UV light. The fluorescence is partially due to high levels of oxidized F_{420} formed during active metabolism (8) and has been employed as a preliminary screening technique for the isolation and identification of methanogenic organisms.

We report here results of studies in which we have compared the biological activities of F_{420}, its derivatives, and its structural analogues, FMN and flavin adenine dinucleotide (FAD), by employing the hydrogenase and NADP-linked hydrogenase system of *Methanobacterium* strain M.o.H. A number of different organisms also have been tested, both qualitatively and quantitatively, for the presence of F_{420}.

MATERIALS AND METHODS

Cultivation of organisms. Unless stated otherwise, all the organisms listed below were mass cultured by the general procedure described previously (3). *Methanobacterium* strain M.o.H. was grown as described by Taylor and Wolfe (17). *Methanobacterium thermoautotrophicum* was grown at 60°C by the procedures of Zeikus and Wolfe (22). *Methanobacterium* strain M.o.H.G., which appears to be similar to strain M.o.H. in morphology and in lack of formate catabolism, was isolated by S. Schoberth from sludge; it was grown by methods described for *M. thermoautotrophicum* except that a temperature of 40°C was used. *Methanospirillum hungatii* was grown as described by Ferry et al. (10). *Methanobacterium formicicum* and *Methanobacterium* strain AZ were both grown in

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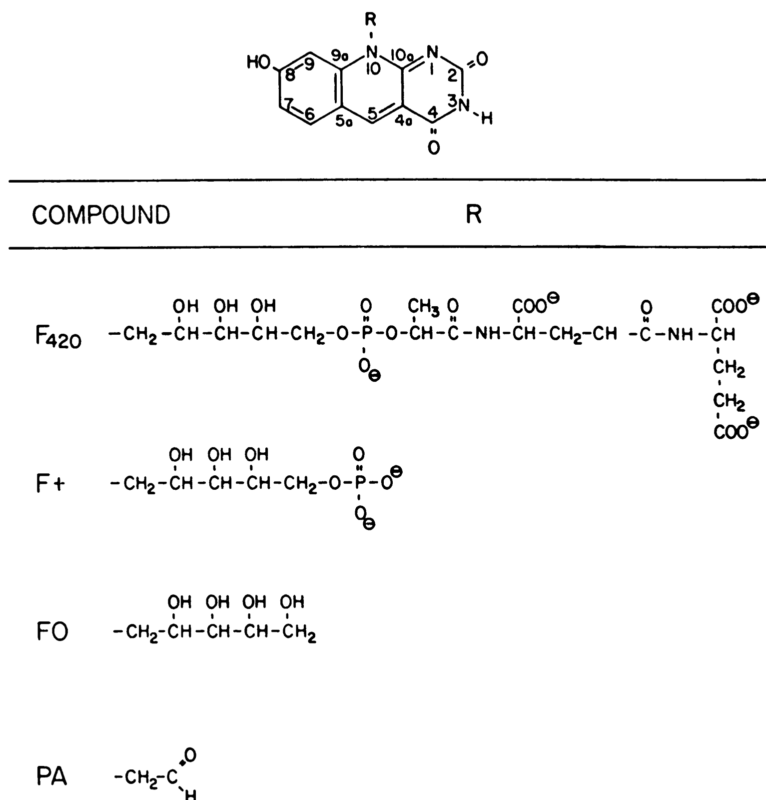

FIG. 1. *Structures of F_{420}, F+, FO, and PA.*

the medium described for *M. hungatii* except that $NaHCO_3$ (6 g/liter) was substituted for Na_2CO_3. Cells of *Methanobacterium ruminantium* strain M-1 were grown as described by Balch and Wolfe (3). Cultures of *M. formicicum*, *M. hungatii*, *Methanobacterium* strain AZ, and *M. ruminantium* M-1 were maintained and kindly supplied by W. E. Balch. Cells of *Methanosarcina barkeri* MS were kindly supplied by S. Shapiro, who grew the cells at 37°C on a medium consisting of the following constituents (per liter): mineral solution no. 1 (5), 37.5 ml; mineral solution no. 2 (5), 37.5 ml; vitamin solution (20), 10 ml; trace mineral solution (20), 10 ml; yeast extract, 2 g; Trypticase (BBL Microbiology Systems), 2 g; sodium acetate, 1.5 g; $NaHCO_3$, 1.7 g; NH_4Cl, 0.33 g; 0.1% resazurin, 1 ml; 1% ferric ammonium citrate, 0.25 ml; cysteine-$HCl \cdot H_2O$, 1.25 g; and $Na_2S \cdot 9H_2O$, 1.25 g. The pH was adjusted to 6.5. Cells of *Methanogenium marisnigri* were kindly supplied by J. A. Romesser and were grown as described by Romesser and Wolfe (16). Cells of *Acetobacterium woodii* were kindly supplied by R. Tanner. The cells were grown as described by Balch et al. (2). All of the organisms listed above were grown on H_2-CO_2 (80:20, vol/vol) as the substrate (3-5). *Escherichia coli* strain JK-1 was obtained from J. Konisky and was grown aerobically in nutrient broth at 37°C.

Preparation of extracts. Cells were harvested and cell extracts were prepared as described previously (12). Deproteinated extracts were prepared in the following manner. The cells were broken by means of a French pressure cell at a pressure of 20,000 lb/in^2. The broken-cell preparation (cell extract in the case of *M. hungatii*) from each organism was placed in a boiling-water bath for 15 min, cooled, and centrifuged at 40,000 × *g*. The supernatant solution was collected. The pellet was suspended in an amount of water equal to its volume and recentrifuged. The supernatant solution from each organism was pooled. All steps were performed aerobically. F_{420} and other anionic compounds were removed from the cell extracts by means of anion-exchange chromatography, using a method similar to that of Tzeng et al. (18). Cell extract (10 ml) was applied to a DEAE-Sephadex A-25 column (10 by 1 cm) which had been equilibrated with 50 mM potassium phosphate buffer, pH 7.0. The extract was eluted at 4°C by use of the same buffer at a maximal flow rate. The dark-brown band which passed directly through the column was collected and immediately placed under an atmosphere of hydrogen. This extract, which will be referred to as DEAE-Sephadex-treated extract, lost no F_{420}-dependent NADP-linked hydrogenase activity upon storage for 5 months at −20°C. The protein content in extracts was estimated by the method of Lowry et al. (14), with bovine serum albumin as a standard.

Enzyme assays. The reaction mixture for the hydrogenase assay (3.0 ml) contained 270 μmol of potassium phosphate buffer (pH 7.0), 30 to 40 μmol of 2-

mercaptoethanol, 5 μg of protein (cell extract from *Methanobacterium* strain M.o.H.), and electron acceptor as desired. The hydrogenase was extremely sensitive to oxygen, and care was taken to maintain anaerobic conditions during all manipulations. The buffer was degassed and sparged for 1 h with oxygen-free hydrogen. 2-Mercaptoethanol was added by syringe, and the buffer was dispensed into assay tubes under a nitrogen atmosphere. Each tube was sealed with a rubber serum stopper and flushed with hydrogen before the addition of the electron acceptor. The reaction solution was saturated with hydrogen by periodic mixing throughout the 20-min gassing period. The enzyme was added to start the reaction. Reduction rates were determined at 40°C by spectrophotometrically following the decrease in absorbance at 420 nm (F_{420}, F+, FO, and PA) or at 445 nm (FMN and FAD) with a modified Bausch & Lomb Spectronic 20 spectrophotometer (containing a jacketed cuvette holder) with an attached recorder. The reaction mixture for the NADP-linked hydrogenase assay (3 ml) contained: potassium phosphate buffer (pH 8.0), 290 μmol; NADP, 250 nmol; protein (DEAE-Sephadex-treated extract from *Methanobacterium* strain M.o.H.), 1.2 mg; and electron carrier as indicated. The addition of 10 μmol of 2-mercaptoethanol, though not required, aided in maintaining anaerobic conditions. The experimental procedure followed was similar to that of the hydrogenase assay except that NADP was added to start the reaction. The increase in absorbance at 340 nm was followed at 40°C. The recorder was adjusted so that a change in absorbance of 0.05 would cause a full-scale pen deflection. Initial velocities were determined by adjusting the chart speed to give a full-scale pen deflection within 12.5 to 25 cm of chart paper. Typical chart speeds varied from 2.5 cm/10 s to 2.5 cm/2 min. The initial rates were linear over the absorbance range used. A plot of initial velocity versus F_{420} concentration was linear from 0 to 5 μg of F_{420} per 3 ml of reaction mixture. A double-reciprocal plot of initial velocity versus F_{420} concentration was linear over concentrations of F_{420} from 0.7 to 20 μg/3 ml of reaction mixture. The detection limit for F_{420} in the assay was 0.1 μg. In both assay systems described, the replacement of hydrogen with argon was used as a control, and the results from duplicate reactions were averaged.

The reaction mixture for the formate hydrogenlyase assay (3 ml) contained: *N*-tris(hydroxymethyl)methyl-2-aminoethane-sulfonic acid buffer (pH 7.1), 20 μmol; sodium formate, 10 μmol; protein (DEAE-cellulose-treated extract from *Methanospirillum hungatii*), 1.5 mg; and F_{420}, 0 to 25 μg. A Warburg flask (approximate volume, 7.0 ml), fitted with rubber serum caps at all openings, was used as a reaction vessel. All additions (except formate) were placed in the main compartment; 50 μl of 6 M KOH was placed in the center well. After gassing the vessel and contents with argon for 10 min and preincubating for 5 min at 40°C, an oxygen-free solution of sodium formate was added to start the reaction. The incubation temperature was 40°C. The evolution of hydrogen gas was followed by assaying 50-μl gas samples at various intervals in a Packard 871 gas chromatograph which contained a silica gel column connected to an electron capture detector.

Purification, electrophoresis, and spectroscopic analysis of F_{420} and its derivatives. F_{420}, F+, FO, and PA were purified, thin-layer electrophoretic analyses were performed, and UV-visible spectra and pK_a values (determined spectrophotometrically) were obtained as described by Eirich et al. (9). Fluorescent spectra were obtained with an Aminco-Bowman spectrophotofluorometer employing dilute solutions of the test compounds dissolved in 0.1 M sulfuric acid, 0.1 M sodium acetate buffer (pH 3.6) and/or 0.1 M potassium phosphate buffer (pH 6.7), and 0.1 M sodium hydroxide.

RESULTS

Spectrophotometric comparisons between F_{420} and its derivatives. Even though structurally different, F_{420} and its hydrolysis products, F+ and FO, demonstrated spectral properties which were similar with respect to pK_a values, absorption maxima, extinction coefficients, and fluorescence activation and emission maxima and intensities (Tables 1 and 2). The absorption maxima of F+ and FO were similar to those reported (9) for F_{420}, but the extinction coefficients were 15% lower, probably as a result of an interference of the lactyl-γLGLG moiety with the ring system. The fluorescence spectra of the three compounds were similar within the limits of the accuracy of the measurements (Table 2 and Fig. 2). The periodate oxidation product of F_{420}, PA, however, was found to differ in many of its spectral properties and exhibited only two pK_a values. Reduced F_{420} exhibited a UV-visible absorption spectrum very similar to that of 1,5-dihydro-5-deazariboflavin (9). The extinction coefficient of reduced F_{420} at 320 nm amounted to about 18% of the value of oxidized F_{420} at 420 nm, but the fluorescence (measured with reduced F+) was only 0.75% (Table 2 and Fig. 3). The fluorescence of the reduced F+ disappeared almost completely on acidification of this compound. The reduced product formed by the hydrogenase-H_2 system was not readily oxidized by oxygen in the absence of enzymatically active cell extract.

Hydrogenase assay. To compare the biological activities of F_{420}, its derivatives, and its analogs, FMN and FAD, we used a spectrophotometric hydrogenase assay. The assay took advantage of the fact that these compounds lose their long-wavelength absorption maxima upon reduction. The hydrogenase was found to have an apparent K_m for F_{420} of 25 μM but exhibited a much higher value of 100 μM for F+ and FO (Fig. 4A). V_{max} amounted to 8 μmol of electron acceptor reduced per min per mg of protein. An eightfold-greater apparent K_m (200 μM) was observed for FMN (Fig. 4B). A stimulation of the rate of reduction of FAD was observed when the

TABLE 1. *UV-visible spectral properties and pK_a values of F+, FO, and PA*

Compound	pH	λ_{max} (nm)	pK_a
F+ and FO	0.1	375 (25.1)[a]; 267 (17.2); 250 (18.5); 230 (31.9)	
	4.0	395 (23.2); 385sh[b] (22.0); 267 (22.3); 250 (21.6); 234 (35.7)	1.8 ± 0.1
	9.9	420 (39.3); 295 (11.0); 267 (22.2); 247 (32.5)	6.1 ± 0.2
	13.0	420 (44.1); 290 (9.9); 245 (45.8)	11.7 ± 0.2
PA	2.0	380 (23.4); 267 (13.3); 247 (18.1); 230 (27.2)	4.4 ± 0.2
	7.0	418 (22.8); 292 (13.4); 240 (24.6)	8.6 ± 0.3
	12.9	417 (27.7); 286 (12.8); 239 (34.3)	

[a] ϵ values (millimolar^{-1} centimeter^{-1}) are given in parentheses.
[b] sh, Shoulder.

TABLE 2. *Fluorescence spectral properties of F_{420} and its derivatives*

Compound	pH	Fluorescence emission[a]: Maximum wavelength (nm)	Fluorescence emission[a]: Relative intensity (%)
F_{420}, F+, and FO	0.7	495 (375)[b]	42
	3.6	450 (395)	35
		470 (395)	36
	9.2	480 (420)	100
	13.0	475 (420)	90
Reduced F+	6.8	390 (320)	0.8
PA	0.7	480 (380)	36
	6.7	485 (418)	44
	13.0	480 (418)	64

[a] The wavelengths may deviate 5 nm and the intensities may deviate 10% of the actual values.
[b] Numbers given in parentheses are activation wavelengths given in nanometers.

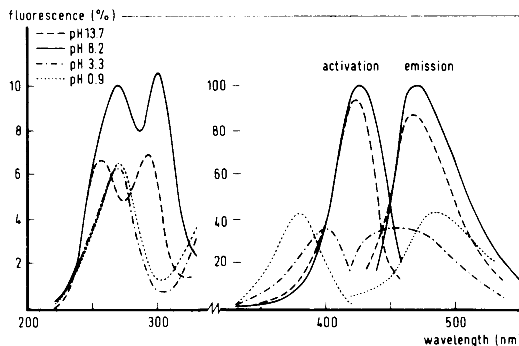

FIG. 2. *Fluorescence spectra of F+ obtained at the indicated pH values. The fluorescence intensities are compared with the fluorescence emission (which was assigned a value of 100%) of an equimolar concentration at pH 9.2. The emission wavelength (for the activation spectra) and the activation wavelength (for the emission spectra) were the same as those shown in Table 2. The spectra at the extreme left are activation spectra obtained at a higher sensitivity than those on the right.*

concentration of FAD was increased. This stimulation was greatest in the lower concentration ranges (less than 25 μM). For this reason an accurate value for the apparent K_m was not measurable. However, rates similar to those of FMN were observed within the concentration range employed for the assay. PA was not reduced under the given conditions. The artificial electron acceptors methylene blue and methyl viologen were reduced, although the kinetics of these reactions were not studied.

Figure 5 shows a pH titration of the long-wavelength absorption maxima of F_{420}. This figure indicates that, when the pH was changed from 8.5 to 5.0, an absorption peak at 395 nm appeared concomitantly with the loss of the 420-nm absorption maximum. An apparent isosbestic point was found at 401 nm. For spectrophotometric comparison of the hydrogenase activities at various pH values, it would therefore be best to follow the loss in absorbance of F_{420} at 401 nm, rather than at 420 nm.

NADP-linked hydrogenase assay. Tzeng et al. (19) developed a spectrophotometric assay for the NADP-linked hydrogenase system which involved the F_{420}-mediated reduction of NADP by hydrogen, resulting in an increase in the absorbance at 340 nm. A similar spectrophotometric assay was employed to study some features of the NADP-linked hydrogenase system from *Methanobacterium* strain M.o.H.

A temperature optimum of 40°C was determined for this system. However, 85% of the maximal activity was found at 28°C and 68%

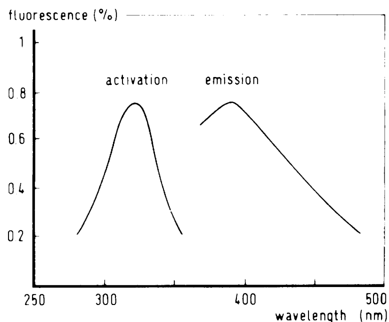

FIG. 3. *Fluorescence spectrum of reduced F+. The fluorescence intensities are compared with the values of the emission of oxidized F_{420} at pH 9.2. F+ was reduced under an atmosphere of hydrogen by the hydrogenase of Methanobacterium strain M.o.H. at pH 6.8. Conditions for obtaining spectral data were the same as noted in Fig. 2 and Table 2.*

was at 60°C. A pH optimum of about 8 was found by use of K_2HPO_4-KH_2PO_4 buffers between pH 5.8 and 8.5. The effect of varying the pH was similar to that found by Tzeng et al. (19) for the same system in *M. ruminantium* PS.

The apparent K_m values for F_{420}, F+, and FO were found to be 27, 110, and 44 μM, respectively (Fig. 4D). V_{max} amounted to 0.18 μmol of NADP reduced per min per mg of protein. PA was not reduced by hydrogenase and was found also to be inactive in the NADP-linked hydrogenase system. Neither FMN nor FAD was found to be active in this system. No activity was observed when F_{420}, NADP, or hydrogen was omitted from the reaction mixture.

Since the NADP-linked hydrogenase system was quite specific for F_{420} or its closely related derivatives, F+ and FO, it was employed to quantitate the amounts of F_{420} in various meth-

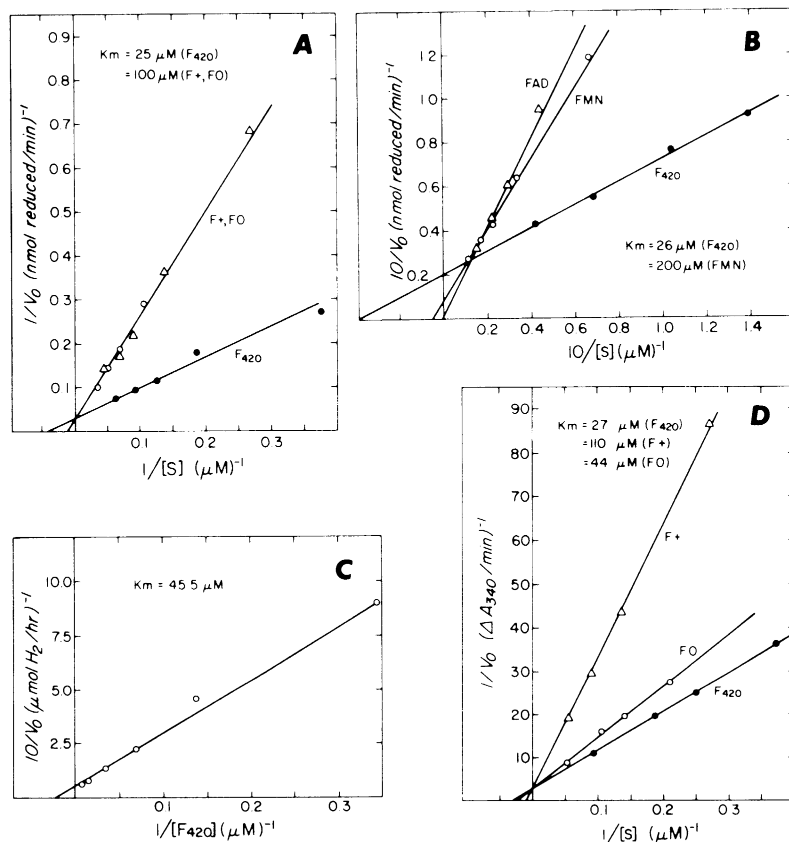

FIG. 4. *Kinetic studies with F_{420}, F+, FO, FMN, and FAD. (A) Comparison of the concentration-dependent rates of reduction of F_{420}, F+, and FO by hydrogenase. The assay for the hydrogenase was performed as described in Materials and Methods. The rate of reduction of F_{420}, F+, and FO was determined at 40°C by spectrophotometrically following the decrease in absorbance at 420 nm. (B) Comparison of the concentration-dependent rates of reduction of F_{420}, FMN, and FAD by hydrogenase. (C) Effect of F_{420} concentration on the rate of hydrogen evolution by the formate hydrogenlyase system. (D) Comparison of the rates of reduction of NADP by the NADP-linked hydrogenase system using F_{420}, F+, and FO as electron carrier. The assay was performed as described in Materials and Methods.*

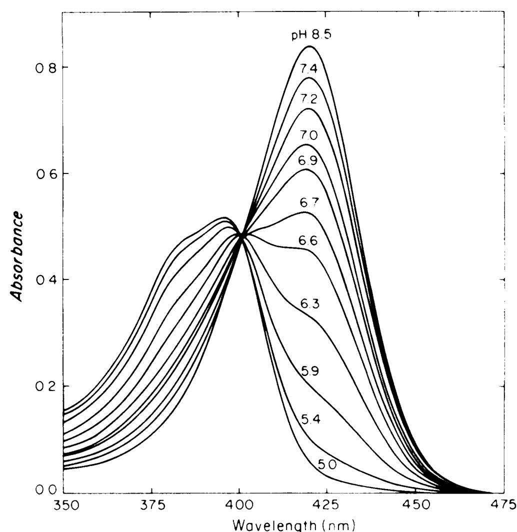

FIG. 5. *Effect of pH on the absorbance of oxidized F_{420}. Absorption spectra of F_{420} at the indicated pH values were obtained on a Cary 14 recording spectrophotometer by R. P. Gunsalus. A potassium phosphate buffer (100 mM) was employed.*

anogens (Table 3). The results demonstrated that F_{420} was present in all the methanogenic organisms tested. Most of the organisms contained relatively high levels, i.e., greater than 150 mg/kg of cells (wet weight). However, two species, *Methanosarcina barkeri* and *Methanobacterium ruminantium* M-1, were found to have very low amounts (less than 20 mg/kg of cells). Neither *Escherichia coli* nor *Acetobacterium woodii*, a gram-positive anaerobe, was found to contain measurable levels of F_{420}. Yeast extract contained nothing which would substitute for F_{420} in this system (results not shown).

By employing thin-layer electrophoresis at pH 4.4, F_{420}, F+, FO, and PA were separated and identified easily (9). This method was chosen to determine whether the activity that one sees with crude preparations is due to F_{420} or to one of its derivatives. Preparations of F_{420} from *Methanobacterium* strain M.o.H. and strain M.o.H.G., *M. ruminantium* M-1, *M. thermoautotrophicum*, *M. hungatii*, and *M. formicicum* exhibited a similar electrophoretic mobility (5.9 cm to the anode), whereas F_{420} from *M. barkeri* was more negatively charged at pH 4.4 (7.5 cm to the anode). This compound was not F+, FO, or PA, and a partially purified preparation was found to substitute for F_{420} in the NADP-linked hydrogenase system; no further studies were made to elucidate its structure.

Formate hydrogenlyase. Tzeng et al. (18) reported that extracts of *M. ruminantium* PS exhibited F_{420}-dependent formate hydrogenlyase activity. The electrons from reduced F_{420} that were formed in this reaction could also be used to reduce NADP. Ferry and Wolfe (11) have reported that *Methanospirillum hungatii* also exhibited F_{420}-dependent formate hydrogenlyase activity. A gas chromatographic assay was employed to measure the formate hydrogenlyase activity of DEAE-cellulose-treated extracts of *M. hungatii*. The results presented in Fig. 4C indicate that this system has an apparent K_m of 45.5 μM for F_{420}. V_{max} amounted to 0.2 μmol of H_2 produced per min per mg of protein. No activity was observed if F_{420} or formate was removed from the reaction mixture.

DISCUSSION

Since the lactyl-γLGLG moiety of F_{420} probably plays a role when the coenzyme interacts with the enzyme, it was not surprising to find that the apparent K_m in the hydrogenase assay for F+ and FO was about fourfold higher than the value for F_{420} of 25 μM. The NADP-linked hydrogenase system also showed a fourfold-higher apparent K_m for F+ but only a slightly higher apparent K_m for FO than for F_{420}. The two negative charges on the phosphate group of F+ appear to have a negative effect on the

TABLE 3. *Content of F_{420} in different species*[a]

Test organism	Cell mass tested (mg)	Amt of F_{420} found (μg)	Amt of F_{420}/kg of cell mass (mg)
Methanobacterium strain M.o.H.	40.6	16.7	410
Methanobacterium strain M.o.H.G.	37.5	8.5	226
Methanobacterium thermoautotrophicum	47.6	15.4	324
Methanobacterium formicicum	32.1	6.6	206
Methanospirillum hungatii	41.7	13.3	319
Methanogenium marisnigri	54.0	6.4	120
Methanobacterium strain AZ	38.6	11.8	306
Methanobacterium ruminantium M-1	109.0	0.6	6
Methanosarcina barkeri	70.0	1.1	16
Acetobacterium woodii	54.1	<0.1	<2
Escherichia coli JK-1	36.1	<0.1	<3

[a] Deproteinated extracts were prepared as described in Materials and Methods and were assayed for the presence of F_{420} by use of the NADP-linked hydrogenase system of *Methanobacterium* strain M.o.H.; cell masses were wet-weight determinations. The detection limit for F_{420} in this assay is 100 ng.

interaction of F+ with the F_{420}:NADP oxidoreductase. FO, which has lost this highly charged group, seems to react with the enzyme nearly as well as F_{420} does. The fact that PA did not act as an electron acceptor from hydrogenase is of interest. Perhaps the carbonyl group on the side chain prevents this compound from interacting properly with the active site of the enzyme.

Tzeng et al. (19) found that FMN and FAD were reduced by the hydrogenase of *M. ruminantium* PS but were unable to donate electrons for the reduction of NADP. When FMN and FAD were tested for their ability to substitute for F_{420} in the hydrogenase and NADP-linked hydrogenase assays of strain M.o.H., a similar observation was made. The apparent K_m of hydrogenase for FMN was approximately eight times larger than that for F_{420}. Both FAD and FMN were unable to substitute for F_{420} in the NADP-linked hydrogenase system. In addition to structural differences, perhaps there is relevance in the fact that the redox potential for FMN and FAD (13) is much different than that for F_{420} (9). The formate hydrogenlyase system of *M. hungatii* also was found to have a low apparent K_m for F_{420}.

Since the UV-visible and fluorescent spectral properties of F_{420}, F+, and FO are almost identical, spectral properties alone are insufficient to distinguish the compounds. For this reason we employed thin-layer electrophoresis to distinguish F_{420} from its derivatives. It was found that *M. thermoautotrophicum*, *M. formicicum*, *M. ruminantium* M-1, *Methanobacterium* strain M.o.H.G., and *M. hungatii* all contain a blue fluorescent compound that is electrophoretically identical to F_{420} from strain M.o.H. *M. barkeri* was found to contain a similar compound that had a significantly different electrophoretic mobility from that of F_{420}. This compound, when partially purified, was active in the NADP-linked hydrogenase system, indicating its close relationship to F_{420}. Since it was found to be more negatively charged than F_{420} at pH 4.4, it may be a polyglutamate derivative of F_{420}, as is found with some natural folic acid derivatives.

With the exception of *M. ruminantium* M-1 and *M. barkeri*, all the methanogens tested were found to contain high levels of F_{420}, as determined by the NADP-linked hydrogenase system. The levels of F_{420} in these two species were from 20 to 40 times lower than for other methanogens. Since the different electrophoretic properties of F_{420} from *M. barkeri* probably reflect a structural difference in the molecule, it is possible that F_{420} from this organism may not be as reactive in the enzymatic assay; the levels of F_{420} detected in *M. barkeri* could be artificially low. According to the results of Tzeng et al. (19), *M. ruminantium* PS also contains low levels of F_{420}. We have no explanation for this wide variability.

Balch et al. (1) have shown by means of 16S rRNA oligonucleotide catalogue comparisons that the methanogens are phylogenetically distant from typical procaryotes. Although a large number of organisms have not as yet been screened for the presence of F_{420}, present observations of F_{420} support this concept; detectable levels of F_{420} have not been found in *Clostridium pasteurianum* (19), *E. coli*, *Acetobacterium woodii*, or in yeast extract. In addition, no compound with similar properties has been reported for any organism except the methanogens.

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