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 quantify 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 electron-negative 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-[(L-lactyl)-γ-L-glutamyl]-L-glutamic acid (lactyl-γLGL) to form a deaza-flavin mononucleotide (a flavin mononucleotide [FMN] analog), 5'-(8-hydroxy-5-deazaalloxazin-10-yl)-2,3,4-trihydroxypentyl phosphate (F+). The dephosphorylated derivative of F+, 8-hydroxy-10-[(2,3,4,5-tetrahydroxypentyl)-5-deazaalloxazine (FO), also is formed during hydrolysis. Another derivative of F$_{420}$, 8-hydroxy-10-formyl-methyl-5-deazaalloxazine (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 Methanobacterium 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 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 Zekus 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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the medium described for *M. hungatii* except that NaHCO₃ (6 g/liter) was substituted for Na₂CO₃. 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; NaHCO₃, 1.7 g; NH₄Cl, 0.33 g; 0.1% resazurin, 1 ml; 1% ferric ammonium citrate, 0.25 ml; cysteine-HCl-H₂O, 1.25 g; and Na₂S·9H₂O, 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 H₂-CO₂ (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/in². 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 x 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. F₄₂₀ 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 F₄₂₀-dependent NADP-linked hydrogenase activity upon storage for 5 months at −20°C.

**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.1.), 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 (F₄₂₀, 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-Phadex-treated extract from * Methanobacterium strain M.O.H.1.), 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 F₄₂₀ concentration was linear from 0 to 5 μg of F₄₂₀ per 3 ml of reaction mixture. A double-reciprocal plot of initial velocity versus F₄₂₀ concentration was linear over concentrations of F₄₂₀ from 0.7 to 20 μg/3 ml of reaction mixture. The detection limit for F₄₂₀ 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 hungatei), 1.5 mg; and F₄₂₀, 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 spectrophotometric analysis of F₄₂₀ and its derivatives. F₄₂₀, F⁺, FO, and PA were purified, thin-layer electrophoretic analyses were performed, and UV-visible spectra and pKₐ values (determined spectrophotometrically) were obtained as described by Eirich et al. (9). Fluorescent spectra were obtained with an Amino-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 F₄₂₀ and its derivatives.** Even though structurally different, F₄₂₀ and its hydrolysis products, F⁺ and FO, demonstrated spectral properties which were similar with respect to pKₐ 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 F₄₂₀, but the extinction coefficients were 15% lower, probably as a result of an interference of the lactyl-yLGLG 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 F₄₂₀, PA, however, was found to differ in many of its spectral properties and exhibited only two pKₐ values. Reduced F₄₂₀ exhibited a UV-visible absorption spectrum very similar to that of 1,5-dihydro-5-deazairboflavin (9). The extinction coefficient of reduced F₄₂₀ at 320 nm amounted to about 18% of the value of oxidized F₄₂₀ 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-H₂ system was not readily oxidized by oxygen in the absence of enzymatically active cell extract.

**Hydrogenase assay.** To compare the biological activities of F₄₂₀, 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 Km for F₄₂₀ 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 Km (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

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$pK_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F+ and FO</td>
<td>0.1</td>
<td>375 (25.1); 267 (17.2); 250 (18.5); 230 (31.9)</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>395 (23.2); 385 (22.0); 267 (22.3); 250 (21.6); 234 (35.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.9</td>
<td>420 (39.3); 295 (11.0); 267 (22.2); 247 (32.5)</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>420 (41.1); 290 (9.9); 245 (45.8)</td>
<td>11.7 ± 0.2</td>
</tr>
<tr>
<td>PA</td>
<td>2.0</td>
<td>380 (23.4); 267 (13.3); 247 (18.1); 230 (27.2)</td>
<td>4.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>418 (22.8); 292 (13.4); 240 (24.6)</td>
<td>8.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>12.9</td>
<td>417 (27.7); 286 (12.8); 239 (34.3)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ $\epsilon$ values (millimolar$^{-1}$ centimeter$^{-1}$) are given in parentheses.

$^b$ sh, Shoulder.

Table 2. Fluorescence spectral properties of $F_{420}$ and its derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>pH</th>
<th>Maximum wavelength (nm)</th>
<th>Relative intensity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_{420}$, $F^+$, and FO</td>
<td>0.7</td>
<td>495 (375)$^b$</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>450 (395)</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>9.2</td>
<td>485 (420)</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>475 (420)</td>
<td>90</td>
</tr>
<tr>
<td>Reduced F+</td>
<td>6.8</td>
<td>390 (320)</td>
<td>0.8</td>
</tr>
<tr>
<td>PA</td>
<td>0.7</td>
<td>480 (380)</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>485 (418)</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>13.0</td>
<td>480 (418)</td>
<td>64</td>
</tr>
</tbody>
</table>

$^a$ 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.

The 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 F420 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 K2HPO4-KH2PO4 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 Km values for F420, F+, and FO were found to be 27, 110, and 44 μM, respectively (Fig. 4D). Vmax 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 F420, NADP, or hydrogen was omitted from the reaction mixture.

Since the NADP-linked hydrogenase system was quite specific for F420 or its closely related derivatives, F+ and FO, it was employed to quantitate the amounts of F420 in various meth-
anogens (Table 3). The results demonstrated that F₄₂₀ 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 Acetobacter woodii, a gram-positive anaerobe, was found to contain measurable levels of F₄₂₀. Yeast extract contained nothing which would substitute for F₄₂₀ in this system (results not shown).

By employing thin-layer electrophoresis at pH 4.4, F₄₂₀, 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 F₄₂₀ or to one of its derivatives. Preparations of F₄₂₀ 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 F₄₂₀ 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 F₄₂₀ 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 F₄₂₀-dependent formate hydrogenlyase activity. The electrons from reduced F₄₂₀ that were formed in this reaction could also be used to reduce NADP. Ferry and Wolfe (11) have reported that Methanospirillum hungatii also exhibited F₄₂₀-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 Kₘ of 45.5 μM for F₄₂₀. Vₘₐₓ amounted to 0.2 μmol of H₂ produced per min per mg of protein. No activity was observed if F₄₂₀ or formate was removed from the reaction mixture.

**DISCUSSION**

Since the lactyl-γLGLG moiety of F₄₂₀ probably plays a role when the coenzyme interacts with the enzyme, it was not surprising to find that the apparent Kₘ in the hydrogenase assay for F⁺ and FO was about fourfold higher than the value for F₄₂₀ of 25 μM. The NADP-linked hydrogenase system also showed a fourfold-higher apparent Kₘ for F⁺ but only a slightly higher apparent Kₘ for FO than for F₄₂₀. The two negative charges on the phosphate group of F⁺ appear to have a negative effect on the

**TABLE 3. Content of F₁₃₀ in different species**

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Cell mass tested (mg)</th>
<th>Amt of F₁₃₀ found (μg)</th>
<th>Amt of F₁₃₀/kg of cell mass (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanobacterium strain M.o.H.</td>
<td>40.6</td>
<td>16.7</td>
<td>410</td>
</tr>
<tr>
<td>Methanobacterium strain M.o.H.G.</td>
<td>37.5</td>
<td>8.5</td>
<td>226</td>
</tr>
<tr>
<td>Methanobacterium thermoautotrophicum</td>
<td>47.6</td>
<td>15.4</td>
<td>324</td>
</tr>
<tr>
<td>Methanobacterium formicicum</td>
<td>32.1</td>
<td>6.6</td>
<td>206</td>
</tr>
<tr>
<td>Methanospirillum hungatii</td>
<td>41.7</td>
<td>13.3</td>
<td>319</td>
</tr>
<tr>
<td>Methanogenium marisnigeri</td>
<td>54.0</td>
<td>6.4</td>
<td>120</td>
</tr>
<tr>
<td>Methanobacterium strain AZ</td>
<td>38.6</td>
<td>11.8</td>
<td>306</td>
</tr>
<tr>
<td>Methanobacterium ruminantium M-1</td>
<td>109.0</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>Methanosarcina Barkeri</td>
<td>70.0</td>
<td>1.1</td>
<td>16</td>
</tr>
<tr>
<td>Acetobacter woodii</td>
<td>54.1</td>
<td>&lt;0.1</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Escherichia coli JK-1</td>
<td>36.1</td>
<td>&lt;0.1</td>
<td>&lt;3</td>
</tr>
</tbody>
</table>

*Deproteinated extracts were prepared as described in Materials and Methods and were assayed for the presence of F₁₃₀ by use of the NADP-linked hydrogenase system of Methanobacterium strain M.o.H.; cell masses were wet-weight determinations. The detection limit for F₁₃₀ in this assay is 100 ng.
interaction of F+ with the F420:NADP oxidoreductase. FO, which has lost this highly charged group, seems to react with the enzyme nearly as well as F420 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 F420 in the hydrogenase and NADP-linked hydrogenase assays of strain M.o.H., a similar observation was made. The apparent _K_m_ for hydrogenase for FMN was approximately eight times larger than that for F420. Both FAD and FMN were unable to substitute for F420 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 F420 (9). The formate hydrogenlyase system of _M. hungatii_ was also found to have a low apparent _K_m_ for F420.

Since the UV-visible and fluorescent spectral properties of F420, 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 F420 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 F420 from strain M.o.H. _M. barkeri_ was found to contain a similar compound that had a significantly different electrophoretic mobility from that of F420. This compound, when partially purified, was active in the NADP-linked hydrogenase system, indicating its close relationship to F420. Since it was found to be more negatively charged than F420 at pH 4.4, it may be a polyglutamate derivative of F420, 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 F420, as determined by the NADP-linked hydrogenase system. The levels of F420 in these two species were from 20 to 40 times lower than for other methanogens. Since the different electrophoretic properties of F420, from _M. barkeri_ probably reflect a structural difference in the molecule, it is possible that F430 from this organism may not be as reactive in the enzymatic assay; the levels of F420 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 F420. 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 F420, present observations of F420 support this concept; detectable levels of F420 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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The assistance of Victor Gabriel in the mass culture of _Methanobacterium_ is gratefully acknowledged. We thank R. P. Gunsalus for providing the results presented in Fig. 5.

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LITERATURE CITED


