Characterization of an Iron-Sulfur Flavoprotein from Methanosarcina thermophila*

Matthew T. Latimer†, Michael H. Painter, and James G. Ferry§

From the Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania 16802-4500 and the Department of Biochemistry and Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0305

A gene (isf) encoding an iron-sulfur flavoprotein (Isf) from Methanosarcina thermophila was cloned and sequenced. The gene was located directly upstream of the genes (pta and ack) encoding phosphotransacetylase and acetate kinase and is transcribed in the opposite direction. The amino acid sequence deduced from isf contained a cluster of cysteine residues reminiscent of proteins that accommodate either a [4Fe-4S] or [3Fe-4S] center. The protein was heterologously produced in Escherichia coli and purified to apparent homogeneity. The 29-kDa subunit molecular mass of heterologously produced Isf (determined by SDS-polyacrylamide gel electrophoresis) corresponded to the molecular mass of 30,451 Da calculated from the amino acid composition deduced from isf. Gel filtration estimated a molecular mass of 65 kDa for the native Isf indicating an α2 homodimer. The UV-visible absorption spectrum was characteristic of iron-sulfur flavoproteins with maxima at 484, 452, 430, 378, and 280 nm. Analyses identified 2 FMN, 7–8 non-heme iron atoms, and 6–7 acid-labile sulfur atoms per α2 homodimer. Comparisons of the deduced Isf sequence with sequences in available protein data bases suggested Isf is a novel iron-sulfur-flavoprotein. Western blot analysis indicated the presence of Isf in extracts of acetate-grown M. thermophila. Ferredoxin stimulated the CO-dependent reduction of Isf by the CO dehydrogenase-acetyl-CoA synthase complex that suggested ferredoxin is a physiological electron donor to Isf.

Biologically produced CH4 derives from either the reduction of CO2 or the methyl group of acetate by two separate pathways present in the methanooarchaeon. The latter accounts for most of the CH4 produced in nature. Elucidation of a pathway for CO2 reduction to CH4, the first to be investigated, has yielded several novel enzymes and cofactors (1). Methanosarcina thermophila is a moderately thermophilic methanooarchaeon capable of growth with acetate, methanol, and methylated amines (2). The pathway for the fermentation of acetate to CH4 and CO2 in M. thermophila (3, 4) is now understood on a biochemical and genetic level comparable with understanding of the CO2-reducing pathway (14, 15). Here we report on a novel FMN-binding iron-sulfur protein (Isf) in acetate-grown M. thermophila. The results suggest Isf functions as an electron carrier in the pathway for the fermentation of acetate.

The terms methanooarchaeon and methanooarchaeon, first suggested by Ralph S. Wolfe, are used here to indicate the phylogenetic placement of methane-producing microbes in the Archaea domain.

EXPERIMENTAL PROCEDURES

DNA Analysis—DNA analyses were performed using the Pustell/IBI DNA Analysis software (Riverside Scientific, Seattle, WA). Amino acid alignments were performed using the default parameters of the Version 8, BestFit program (Genetics Software Group, Madison, WI). The DNA and deduced amino acid sequences of isf were compared with the non-redundant updated nucleotide and protein data bases at the National Center for Biotechnology Information using the BLAST program (16). The isf sequence (Fig. 2) was submitted to GenBank and assigned the accession number U50189.

Heterologous Production and Purification of Isf—The coding region of isf was amplified using polymerase chain reaction. The sequence of the coding region was submitted to GenBank with accession number U50189.

© 1996 by The American Society for Biochemistry and Molecular Biology, Inc.
the upstream primer was 5’-GAGCGATCCATATGAAAATAA-
CAGGA-3’ that corresponds, in part, to nucleotides 1–15 in Fig. 2 and
contains the recognition sequence for NdeI. The sequence of the down-
stream primer was 5’-GCTGTATTGGATCCTGCGATCATAAAC-
9 that corresponds, in part, to nucleotides 890–902 in Fig. 2 and contains
the recognition sequence for BamHI. The resulting DNA was cloned into the BamHI and NdeI sites of the
pT7–7 overexpression vector of Tabor and Richardson (17). The pT7–7
derivative carrying the isf gene (designated pML701) was transformed
into Escherichia coli strain BL21(DE3) (18) that was grown at 37 °C in
Luria-Bertani broth (containing 100 µg ml−1 ampicillin) to an A600 nm
of 1.6, at which point the culture was induced to produce Isf by the
addition of 1% (final concentration, w/v) Bacto-lactose. After 2 h growth
at 30 °C, the cells were harvested by centrifugation at 11,800 × g for 10
min at 4 °C.

All steps in the purification of Isf were performed anaerobically, and
all buffers contained 2 mM dithiothreitol. Where applicable, a Coy
anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) was
employed. All steps were performed at 21 °C except where indicated.

Fast protein liquid chromatography (FPLC) columns and Q-Sepharose
Fast anionic exchange resin were obtained from Pharmacia Biotech Inc.
Oxygen-impermeable Saran (Pyramid Plastics, Hope, AK) tubing was
used with the Pharmacia FPLC system. Protein concentrations in
column elution samples were routinely determined using the Bio-Rad
microassay (19) with bovine serum albumin as the standard.

Cells (approximately 45 g, wet weight) were suspended in a total
volume of 130 ml of 25 mM Tris (pH 7.4) and lysed by French pressure
cell disruption at 20,000 p.s.i. (138 megapascals). The lysate was con-
centrifuged at 78,400 × g for 20 min at 4 °C. Streptomycin sulfate (final
concentration, 1% w/v) was added to the supernatant solution and
centrifuged as above. The soluble fraction was applied to a Q-Sepharose
Fast Flow column (5 × 10 cm) previously equilibrated with 2 column
volumes of 25 mM Tris (pH 7.4). A 500-ml 0–1.0 M NaCl linear gradient
was applied at 6 ml min−1. Fractions containing Isf were determined by

---

FIG. 1. Physical map of M. thermophila DNA containing isf, pta, and ack. Arrows indicate orientation and relative sizes of the
genes. Positions of selected restriction endonuclease cleavage sites are
indicated above the line. kb, kilobase pair.

---

FIG. 2. Nucleotide sequence of isf and flanking DNA from
M. thermophila. The sequence of one strand is presented 5′ to 3′, and
numbering is relative to the putative translational start for isf. The N-terminal amino acid sequence deduced from pto is shown below the first base
of each codon. The determined (11) box A and box B promoter sequences upstream of pta are single and double overlined, respectively.
The amino acid sequence deduced from isf is shown above the first base of each codon, and the stop codon is indicated by end. Residues postulated
to be involved in iron-sulfur center ligation are boxed. A potential ribosome binding site for isf is indicated by asterisks. Potential box A and
box B promoter sequences upstream of isf are single and double underlined, respectively. A potential transcription termination sequence is indicated (#).
Iron-Sulfur Flavoprotein from M. thermophila

SDS-PAGE (see below). An equal volume of 1.2 × (NH₄)₂SO₄ in 25 mM Tris (pH 7.4) was added to the pooled fractions containing Isf. The solution was passed over a phenyl-Sepharose Hi-Load 26/10 FPLC column preequilibrated with 600 mM (NH₄)₂SO₄ in 25 mM Tris (pH 7.4). The eluate containing Isf was diluted 1:10 with 50 mM Tris (pH 7.6) and divided into four equal aliquots of approximately 25 mg of protein each. Each of the four aliquots was separately applied to a Mono-Q 10/10 FPLC column preequilibrated with 50 mM Tris (pH 7.6). The column was developed with 50 ml of a 0–1.0 M NaCl linear gradient applied at 2 ml min⁻¹. Pure Isf was recovered in a single symmetrical peak eluting between 220 and 240 mM NaCl.

Preparation and Fractionation of Extracts from M. thermophila—M. thermophila was grown on acetate to midlog phase and harvested as a visitecon (1 ml) with an atmosphere of 100% N₂; the stopped cuvette (1 ml) was injected into a mobile phase consisting of 80% water (containing 5 mM ammonium acetate (pH 6.0)) and 20% methanol running at 2 ml min⁻¹ at 21°C. A Hewlett-Packard model 1050 HPLC system in conjunction with a Hewlett-Packard LDCosorb C-18 reversed phase column was used to identify the flavin extracted from Isf. Neutralized samples were injected into a mobile phase consisting of 80% water (containing 5 mM ammonium acetate (pH 6.0)) and 20% methanol running at 2 ml min⁻¹ (22). Emission of flavins was monitored at 450 nm. The amount of FNN released from the protein and purified by HPLC was determined spectrophotometrically using an extinction coefficient for FNN of 12.2 × 10⁻³ cm⁻¹ at 450 nm (23). The concentration of Isf in the original sample (before flavin extraction) was determined using the biuret assay (24) with bovine serum albumin as the standard.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

The flavin cofactor was extracted from purified Isf by acidification of 531 μg of protein with 0.5% (final concentration, v/v) trifluoroacetic acid at 21 °C. A Hewlett-Packard model 1050 HPLC system in conjunction with a Hewlett-Packard LDCosorb C-18 reversed phase column was used to identify the flavin extracted from Isf. Neutralized samples were injected into a mobile phase consisting of 80% water (containing 5 mM ammonium acetate (pH 6.0)) and 20% methanol running at 2 ml min⁻¹ (22). Emission of flavins was monitored at 450 nm. The amount of FNN released from the protein and purified by HPLC was determined spectrophotometrically using an extinction coefficient for FNN of 12.2 × 10⁻³ cm⁻¹ at 450 nm (23). The concentration of Isf in the original sample (before flavin extraction) was determined using the biuret assay (24) with bovine serum albumin as the standard.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

The flavin cofactor was extracted from purified Isf by acidification of 531 μg of protein with 0.5% (final concentration, v/v) trifluoroacetic acid at 21 °C. A Hewlett-Packard model 1050 HPLC system in conjunction with a Hewlett-Packard LDCosorb C-18 reversed phase column was used to identify the flavin extracted from Isf. Neutralized samples were injected into a mobile phase consisting of 80% water (containing 5 mM ammonium acetate (pH 6.0)) and 20% methanol running at 2 ml min⁻¹ (22). Emission of flavins was monitored at 450 nm. The amount of FNN released from the protein and purified by HPLC was determined spectrophotometrically using an extinction coefficient for FNN of 12.2 × 10⁻³ cm⁻¹ at 450 nm (23). The concentration of Isf in the original sample (before flavin extraction) was determined using the biuret assay (24) with bovine serum albumin as the standard.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

The flavin cofactor was extracted from purified Isf by acidification of 531 μg of protein with 0.5% (final concentration, v/v) trifluoroacetic acid at 21 °C. A Hewlett-Packard model 1050 HPLC system in conjunction with a Hewlett-Packard LDCosorb C-18 reversed phase column was used to identify the flavin extracted from Isf. Neutralized samples were injected into a mobile phase consisting of 80% water (containing 5 mM ammonium acetate (pH 6.0)) and 20% methanol running at 2 ml min⁻¹ (22). Emission of flavins was monitored at 450 nm. The amount of FNN released from the protein and purified by HPLC was determined spectrophotometrically using an extinction coefficient for FNN of 12.2 × 10⁻³ cm⁻¹ at 450 nm (23). The concentration of Isf in the original sample (before flavin extraction) was determined using the biuret assay (24) with bovine serum albumin as the standard.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.

Iron (25) and acid-labile sulfur (26) were determined as described. The subunit molecular mass of Isf was estimated by 12% SDS-PAGE (21) using low molecular weight protein standards from Bio-Rad. Native molecular mass estimates were based on elution from a Superose-12 gel filtration FPLC column calibrated with the following proteins of known molecular masses: bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (36 kDa), chymotrypsinogen (25 kDa), and RNase A (13.7 kDa). The buffer was used at 50 mM Tris (pH 7.6) containing 150 mM NaCl. A flow rate of 0.5 ml min⁻¹ was used.
Iron-Sulfur Flavoprotein from M. thermophila

RESULTS AND DISCUSSION

Cloning, Sequencing, and Analysis of isf—Sequence analysis of the previously reported 3.9-kilobase pair EcoRI fragment of M. thermophila genomic DNA containing pta and ack (7) identified a third open reading frame (Figs. 1 and 2) designated isf (iron-sulfur flavoprotein) for reasons described below. The isf gene was located 265 base pairs upstream of pta and contained several sequences with identity to the consensus box A archaeal promoter sequence (5'-TTTA(T/A)ATA-3') (29). The transcription of archaeal genes typically initiates 22–27 base pairs downstream of box A at a purine-pyrimidine dinucleotide located in a box B sequence (consensus 5'-ATGC-3') (29). Three potential box A sequences were located 22–27 base pairs upstream of sequences with identity to the consensus box B (Fig. 2). One of the three potential box A sequences coincided with the putative N-terminal methionine codon of the putative box A sequence previously determined for the pta-ack operon by primer extension analysis (11). A potential ribosome binding site was identified 10 bases upstream of the putative N-terminal methionine codon of isf. A stretch of thymine bases (Fig. 2) was identified 98 bases downstream. Thymine-rich regions have been shown to function as transcriptional terminators in the Archaea (29). Additional experimentation is needed to determine if isf is monocistronic or part of a larger operon.

The putative polypeptide deduced from isf (Isf) was 273 amino acids in length and had a calculated anhydrous molecular mass of 30,451 Da (Fig. 2). Analysis of the deduced protein sequence revealed a cluster of cysteine residues (Fig. 2) with a spacing (CXXCXXC) reminiscent of proteins that accommodate either a [4Fe-4S] or [3Fe-4S] center (30). Iron-sulfur proteins with [4Fe-4S] centers utilize, in addition to the CXXCXXC motif, a Cys-Pro pair to provide the fourth acid-stable cysteine sulfur ligand to the iron atoms (30). Two Cys-Pro pairs are located in the C-terminal portion of Isf (Fig. 2).

Heterologous Production, Purification, and Biochemical Characterization of Isf—The heterologously produced Isf was purified to apparent homogeneity as determined by SDS-PAGE. N-terminal sequencing of the first five residues confirmed that the protein purified from E. coli was encoded by isf. SDS-PAGE indicated a subunit molecular mass of approximately 29 kDa that is in close agreement with the molecular mass predicted from the amino acid sequence deduced from isf (30.4 kDa). Native gel filtration chromatography using a calibrated Superose-12 column yielded a single peak corresponding to a molecular mass of 65 ± 7 kDa, indicating that Isf was purified as an α2 homodimer.
The UV-visible spectrum of purified Isf showed absorbance maxima at 484, 452, 430, 378, and 280 nm, typical of iron-sulfur flavoproteins (Fig. 3). No absorbance was observed at wavelengths greater than 550 nm (data not shown). Addition of dithionite decreased absorbance in the 350–550 nm range (Fig. 3). Exposure to air reoxidized the dithionite-reduced Isf yielding the same spectrum (data not shown) obtained for the purified protein (Fig. 3). These results indicate that Isf is a redox-active protein. Although purified anaerobically, exposure to air for 30 min at 21 °C did not change the spectrum (data not shown), suggesting that purified Isf was in the oxidized form. The molar absorption coefficients calculated for the $a_2$ homodimer at 272, 378, and 452 nm were 140,350, 35,087, and 57,894 m$^{-1}$ cm$^{-1}$. Mildly acidified Isf had a visible spectrum typical of flavins (Fig. 3). The flavin released from Isf by acidification was identified as FMN by HPLC. A total of 15 nmol of FMN was detected in whole extract and a Mono-Q fraction (Fig. 5) from acetate-grown cells amended with the recently described FMN-binding protein from *M. thermophila* (13). The results suggest that Isf is a novel iron-sulfur flavoprotein possessing a 2-electron carrier with the same spectrum (data not shown) obtained for the purified and partially purified components from acetate-grown *M. thermophila* (13). The results show that electron flow in *vivo* is: CO $\rightarrow$ CODH-ACS complex $\rightarrow$ ferredoxin $\rightarrow$ membranes $\rightarrow$ CoM-S-S-CoB reductase; however, the participation of other electron carriers was not excluded. Addition of Isf resulted in stimulation of CO-CoM-S-S-CoB oxidoreductase activity of the reconstituted system (Fig. 7), which is consistent with Isf functioning as an electron transfer component of the system. Since Isf was reduced by ferredoxin and contains FMN and Fe-S centers, it is possible that Isf functions as a 1-electron/2-electron switch reducing obligate electron carriers other than ferredoxin that couple with Isf and to identify whether Isf is a component of the in *vivo* electron transport chain linking the CODH-ACS complex with the CoM-S-CoB reductase in acetate-grown *M. thermophila*.

Acknowledgments—We thank John Peters for expert advice on heterologous production of proteins and Birgit Alber for critical reading of the manuscript.

REFERENCES

1. Ferry, J. G. (1992) *Crit. Rev. Biochem. Mol. Biol.* 27, 473–503
2. Zinder, S. H., Sowers, K. R., and Ferry, J. G. (1985) *Int. J. Syst. Microbiol.* 35, 522–523
3. Ferry, J. G. (1993) in *Methanogenesis: Ecology, Physiology, Biochemistry, & Genetics* (Ferry, J. G., ed) pp. 304–334, Chapman and Hall, New York
4. Ferry, J. G. (1995) *Annu. Rev. Microbiol.* 49, 305–333
5. Alber, B. E., and Ferry, J. G. (1994) *Proc. Natl. Acad. Sci. U.S.A.* 91, 6809–6813
6. Clements, A. P., and Ferry, J. G. (1994) *J. Bacteriol.* 176, 6822–6829
7. Latimer, M. T., and Ferry, J. G. (1993) *J. Bacteriol.* 175, 6822–6829
8. Maupin-Furlow, J. A., and Ferry, J. G. (1996) *J. Biol. Chem.* 271, 28617–28622
9. Ferry, J. G., and Maupin-Furlow, J. A. (1996) in *Microbial Growth on C1 Compounds* (Lidstrom, M., and Tabita, R., eds) pp. 64–71, Kluwer Academic, Dordrecht
10. Maupin-Furlow, J. A., and Ferry, J. G. (1996) *J. Bacteriol.* 178, 340–346
11. Singh-Wissmann, K., and Ferry, J. G. (1995) *J. Bacteriol.* 177, 1699–1702
12. Sowers, R. K., Thai, T. T., and Gunosalus R. P. (1993) *J. Biol. Chem.* 268, 23172–23178
13. Peer, C. W., Painter, M. H., Rasche, M. E., and Ferry, J. G. (1994) *J. Bacteriol.* 176, 6974–6979
14. Wasserfallen, A., Huber, K., and Leisinger, T. (1995) *J. Bacteriol.* 177, 2436–2441
15. Nolling, J., Ishii, M., Koch, J., Phil, T. D., Reeve, J. N., Thauer, R. K., and Hedderich, R. (1985) *Eur. J. Biochem.* 151, 628–638
16. Alischul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) *J. Mol. Biol.* 215, 403–410
17. Tabor, S., and Richardson, C. C. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82, 1074–1078
Iron-Sulfur Flavoprotein from M. thermophila

18. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) Methods Enzymol. 185, 60–89
19. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
20. Sowers, K. R., Nelson, M. J. K., and Ferry, J. G. (1984) Curr. Microbiol. 11, 227–230
21. Laemmli, U. K. (1970) Nature 227, 680–685
22. Light, D. R., Walsh, C., and Marletta, M. A. (1986) Anal. Biochem. 160, 87–93
23. Dawson, R. M. C., Elliott, D. C., Elliot, W. H., and Jones, K. M. (1989) in Data for Biochemical Research, pp. 126–127, Oxford University Press, New York
24. Gornall, A. G., Bardawill, C. J., and David, M. M. (1949) J. Biol. Chem. 177, 751–766
25. Fortune, W. B., and Mellon, M. G. (1936) Ind. Eng. Chem. Anal. Ed. 10, 60–64
26. Singel, L. M. (1965) Anal. Biochem. 11, 126–132
27. Baron, S. F., and Ferry, J. G. (1989) J. Bacteriol. 171, 3846–3853
28. Timmons, T. M., and Dunbar, B. S. (1990) Methods Enzymol. 182, 679–688
29. Reeve, J. N. (1993) in Methanogenesis: Ecology, Physiology, Biochemistry & Genetics (Ferry, J. G., ed) pp. 493–527, Chapman and Hall, New York
30. Cammack, B. (1983) Chem. Scr. 21, 87–95
31. Santangelo, J. D., Jones, D. T., and Woods, D. R. (1991) J. Bacteriol. 173, 1088–1095
32. Terlesky, K. C., and Ferry, J. G. (1988) J. Biol. Chem. 263, 4080–4082
Characterization of an Iron-Sulfur Flavoprotein from *Methanosarcina thermophila*

Matthew T. Latimer, Michael H. Painter and James G. Ferry

*J. Biol. Chem.* 1996, 271:24023-24028.
doi: 10.1074/jbc.271.39.24023

Access the most updated version of this article at [http://www.jbc.org/content/271/39/24023](http://www.jbc.org/content/271/39/24023)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 14 of which can be accessed free at [http://www.jbc.org/content/271/39/24023.full.html#ref-list-1](http://www.jbc.org/content/271/39/24023.full.html#ref-list-1)