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D-*erythro*-Neopterin Biosynthesis in the Methanogenic Archaea *Methanococcus thermophila* and *Methanobacterium thermoautotrophicum* ΔH

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The steps in the biosynthetic transformation of GTP to 7,8-dihydro-D-*erythro*-neopterin (H_2neopterin), the precursor to the modified folates found in the methanogenic archaea, has been elucidated for the first time in two members of the domain *Archaea*. In *Methanococcus thermophila* and *Methanobacterium thermoautotrophicum* ΔH, it has been demonstrated that H_2neopterin 2′:3′-cyclic phosphate is an intermediate in this conversion. In addition, the formation of the pterin ring of the H_2neopterin 2′:3′-cyclic phosphate is catalyzed not by a single enzyme, as is known to occur with GTP cyclohydrolase I in the *Eucarya* and *Bacteria*, but rather by two or more enzymes. A 2,4,5-triamino-4(3H)-pyrimidinone-containing molecule, most likely 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-triphosphate, has been identified as an intermediate in the formation of the H_2neopterin 2′:3′-cyclic phosphate. Synthetic H_2neopterin 2′:3′-cyclic phosphate was found to be readily hydrolyzed by cell extracts of *M. thermophila* via the H_2neopterin 3′-phosphate to H_2neopterin, a known precursor to the pterin portion of methanopterin.

GTP cyclohydrolase I is recognized as the enzyme that catalyzes the first committed step in the biosynthesis of the pterin ring of folic acid, biopterin, and sepiapterin (2). In this reaction, the C-8 carbon of the GTP is removed as formate and the resulting enzyme-bound intermediate is converted to 7,8-dihydro-D-*erythro*-neopterin (H_2neopterin) 3′-triphosphate (12). In the case of *E. coli*, the required four steps of this transformation are carried out by the *folE* gene product (6). Genes encoding highly conserved sequences homologous to the sequence of this enzyme have been identified from a wide range of different *Eucarya* and *Bacteria* (12), and in a few cases, the proteins have been cloned, sequenced, overexpressed, and shown to catalyze the expected reaction (8, 25). Early labeling experiments using archaea indicated that GTP cyclohydrolase I also catalyzed the first committed step in the biosynthesis of the pterin portion of the modified folate, methanopterin (9, 20). Recent results from the analysis of the archaeal genome of *Methanococcus jannaschii*, however, show no genes with sequences identifiable as GTP cyclohydrolase I (3). This brought into question the nature of the enzyme(s) responsible for the generation of the pterin portion of the modified folates, such as methanopterin, which occur in the archaea (23). In this report, we demonstrate the absence of an *Escherichia coli*-like GTP cyclohydrolase I in the archaea *Methanococcus thermophila* and *Methanobacterium thermoautotrophicum* ΔH and show that in these archaea, GTP is transformed via a multistep process into H_2neopterin 2′:3′-cyclic phosphate. The H_2neopterin 2′:3′-cyclic phosphate is then hydrolyzed to H_2neopterin via H_2neopterin 3′-phosphate. The H_2neopterin is then converted to D-7,8-dihydro-6-hydroxymethylpterin, a known precursor to the pterin portion of methanopterin and sarcinopterin (22).

MATERIALS AND METHODS

Materials. Chromatographically purified *E. coli* alkaline phosphatase (type III, *E. coli*; catalog no. P 4252) and GTP were obtained from Sigma Chemical Co. D-(+)-Neopterin (D-*erythro*-neopterin) was obtained from Fluka Chemical Corp., and D-neopterin 3′-monophosphate was obtained from Schircks Laboratories, Jona, Switzerland. 2,3-Butanedione was obtained from Aldrich Chemical Co.

Methods. D-*erythro*-Neopterin 2′:3′-cyclic phosphate was prepared as described by Sugiura et al. (17) and also from the reaction between neopterin and $POCl_3$ as described for the preparation of riboflavin 2′:3′-cyclic phosphate (6). Samples were converted into the 7,8-dihydropterins by reduction with sodium dithionate (23). Circular dichromism (CD) spectra were recorded at room temperature, using a Jasco J-720 spectropolarimeter mounted with a 1-cm cylindrical cell.

Bacterial strains and growth conditions. *M. thermophila* TM-1 (28) was grown in the departmental laboratory of James G. Ferry as previously described (16). Frozen cells of *M. thermoautotrophicum* ΔH were kindly supplied by Ralph S. Wolfe (Urbana, Ill.).

Preparation of cell extracts. Cell extracts of *M. thermophila* were prepared by French press lysis (13), and cell extracts of *M. thermoautotrophicum* ΔH were prepared by sonication under argon as previously described (21). Protein concentrations of the cell extracts typically ranged from 7 to 26 mg/ml, and they were stored frozen at −78°C until needed.

Separation of proteins in cell extracts. An *M. thermophila* cell extract (1.75 ml) was applied under anaerobic conditions to a fast protein liquid chromatography (FPLC) Mono Q HR 10/10 (Pharmacia) column preequilibrated in 50 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (pH 6.8) containing 2 mM dithiothreitol and 10% ethylene glycol. By using a flow rate of 1 ml/min and a 0 to 1 M linear KCl gradient in the same pH 6.8 buffer, the separated cell extract was collected, under argon, in 12 4-ml fractions. The fractions were stored frozen under argon at −78°C until needed.

Incubation of cell extracts with precursors and separation of pterin products. To 100 μl of a cell extract or 100 μl of a Mono Q cell extract fraction, contained in a septated bottle flushed with argon, was added 10 μl of a 0.1 M anaerobic solution of GTP in water. The mixture was then incubated at 50°C for 2 h (*M. thermophila*) and 60°C for 2 h (*M. thermoautotrophicum* ΔH), after which time the vial was cooled to room temperature and opened, and 50 μl of a solution (0.033 M iodine, 0.1 M KI, 1 M HCl) was added. After 10 min at room temperature, the sample was centrifuged (16,000 × *g*) to remove the precipitate, and the resulting clear iodine-colored aqueous layer was removed. This aqueous layer was mixed with 50 μl of 1 M NaOH and a few microliters of a dilute solution of sodium bisulfite to remove the iodine color.

Because of the instability of D-neopterin 2′:3′-cyclic phosphate to the strong acid conditions of this sample workup procedure, incubations conducted with this substrate were modified as follows. The incubation mixture was oxidized with 50 μl of a solution (0.033 M iodine, 0.1 M KI) for 10 min at room temperature, followed by reduction of the iodine with a few microliters of a dilute solution of sodium bisulfite. The proteins were precipitated by the addition of 0.5 ml of 100% ethanol and separated by centrifugation, and the ethanol was evaporated from the supernatant with a stream of nitrogen gas at room temperature. The samples were then processed in the same manner as the acid-treated samples.

If phosphatase cleavage of a sample was desired, 100 μl of 0.1 M glycine buffer

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(pH 10.4) containing 1 mM $ZnCl_2$ and 1 mM $MgCl_2$ was added, the mixture was adjusted to a final pH of 10.4 with 1 M NaOH, if required, and 0.8 U of alkaline phosphatase was added. After 2 h at 37°C, 100 μl of 1 M sodium acetate buffer (pH 5.5) was added, and the pterins present in the mixture were separated by absorption chromatography on a column of Sephadex G-25 superfine (1 by 46 cm, prequilibrated in water), using water as the eluting solvent and collecting 0.9-ml fractions. The presence and quantitation of pterins in the fractions were established by their fluorescence intensities (excitation λ_{max} = 360 nm; emission λ_{max} = 445 nm) compared with known standards. The relative fluorescent yields for D-neopterin 2′:3′-cyclic phosphate, D-neopterin 3′-phosphate, and D-neopterin were all found to be identical (±5%). Relative to 7-methylpterin, the pterins eluted from the Sephadex column with the following elution volumes: methanopterin, 0.55; D-neopterin 2′:3′-cyclic phosphate, 0.65; D-neopterin 3′-phosphate, 0.67; D-neopterin, 0.86; pterin, 0.97; 6-hydroxymethylpterin, 0.99; 7-methylpterin, 1.00; 6,7-dimethylpterin, 1.06; and guanosine, 1.06. Salts eluted from the column, relative to 7-methylpterin, at 0.63.

TLC identification of pterins. The identities of the pterins purified by the Sephadex chromatography were further confirmed by thin-layer chromatography (TLC), using comparison to known compounds in two different solvent systems. In the solvent system *n*-butanol–acetic acid–water (12:3:5, vol/vol/vol), the pterins had the following indicated R_f values: D-neopterin 3′-phosphate, 0.02; neopterin-2′:3′-cyclic phosphate, 0.05; D-neopterin, 0.19; 6-hydroxymethylpterin, 0.30; 7-methylpterin, 0.310; 6-methylpterin, 0.345; 6,7-dimethylpterin, 0.380; 6-ethylpterin, 0.458; and 6-ethyl-7-methylpterin, 0.494. In the solvent system acetonitrile-water-formic acid (88%) (40:10:5, vol/vol/vol), the pterins had the following indicated R_f values: D-neopterin 3′-phosphate, 0.33; neopterin 2′:3′-cyclic phosphate, 0.31; D-neopterin, 0.41; 6-hydroxmethylpterin, 0.51; 7-methylpterin, 0.52; 6-methylpterin, 0.55; 6,7-dimethylpterin, 0.60; 6-ethylpterin, 0.64; and 6-ethyl-7-methylpterin, 0.66.

Samples containing neopterin 2′:3′-cyclic phosphate were identified by first hydrolyzing the samples to neopterin 3′-phosphate and/or to neopterin 2′-phosphate followed by phosphatase cleavage of the phosphate monoester bond. The first step in the hydrolysis of the cyclic phosphate was accomplished either by heating a small portion of the sample (0.1 nmol) for 10 min at 100°C in 100 μl of 0.1 M HCl or by incubating the sample for 1 h at 37°C in 100 μl of 1 M NaOH. After neutralization of the acid or base present in the sample, the sample was cleaved by the alkaline phosphatase method described above.

Identification of the triaminopyrimidine-containing intermediates. To 100 μl of a cell extract of *M. thermophila* sealed in a septated bottle flushed with argon was added 10 μl of an 0.1 M anaerobic solution of GTP in water. After incubation at 50°C for 2 h, 20 μl of 0.058 M 2,3-butanedione in methanol and 100 μl of an anaerobic 0.2 M Tris hydrochloride buffer (pH 8.5) were added, and the sample was heated at 100°C for 30 min. The sample was then centrifuged (16,000 × *g*) for 2 to 3 min, and the pellet was washed two times with 100 μl of water. The combined supernatants were then mixed with 100 μl of 1 M sodium acetate buffer (pH 5.5), and the mixture was separated on a column of Sephadex G-25 superfine (1 by 46 cm) as described above. We observed a broad peak of fluorescence that eluted between fractions 53 and 60, overlapping the positions of elution of 7-methylpterin (fraction 54) and 6,7-dimethylpterin (fraction 57). Fractions 53 to 56 and fractions 57 to 60 were combined and separately concentrated, and each was analyzed by TLC, using the two different solvent systems. Fractions 53 to 56 were found to contain mostly 7-methylpterin, whereas fractions 57 to 60 were found to contain mostly 6,7-dimethylpterin.

The identities of the pterins in these combined sets of fractions were further confirmed by the following procedure. A portion of the pterins contained in each set of fractions was recovered by evaporation of the samples, dissolved in 100 μl of 50 mM HCl, and absorbed on a Dowex 50W 8X H^+ column (1 by 3 mm); the column was then washed with water and eluted with 3 M aqueous ammonia. After the samples were evaporated to dryness with a stream of nitrogen gas and dissolution in 5 to 10 μl of water, TLC analysis, using the two different solvent systems, again confirmed the presence of 6,7-dimethylpterin in the ammonia-eluted material.

To establish the anionic charge, and thus the number of phosphates on the triaminopyrimidine-containing intermediates, a cell extract of *M. thermophila* was incubated with GTP as described above, and at the end of the incubation, the incubation mixture was separated on a DEAE-Sephadex column (1.5 by 25 cm), preequilibrated with 0.04 M sodium phosphate buffer (pH 7.0), using a linear salt gradient from 0 to 1 M NaCl in the pH 7.0 buffer. (These elution conditions used in this separation were developed because they were found to separate nucleotide mono-, di- and triphosphates.) Fractions (5.5 ml) were collected, and 1-ml portions of the individual fractions were reacted with 20 μl of 0.058 M 2,3-butanedione in methanol and 100 μl of an anaerobic 0.2 M Tris hydrochloride buffer (pH 8.5) and heated at 100°C for 30 min. The amount of 6,7-dimethylpterin produced was directly measured by recording the fluorescent intensities of the reacted fractions. Fractions producing fluorescent material contained within single peaks were combined, concentrated, and mixed with 100 μl of 1 M sodium acetate buffer (pH 5.5), and the presence of 6,7-dimethylpterin was confirmed by Sephadex G-25 chromatography and TLC as described above.

Synthesis of H_2neopterin-P_3 and neopterin-P_3 by using the *E. coli* GTP cyclohydrolase I. A cell extract of *E. coli* (0.5 ml; 20 mg of protein per ml) obtained by sonication of cells suspended in 50 mM TES buffer (pH 7.5) containing 20 mM $MgCl_2$ and 10 mM mercaptoethanol was heated at 90°C for 10 min and centrifuged (16,000 × *g*). The resulting clear supernatant was made 5 mM in GTP and 20 mM in dithiothreitol and incubated under argon for 2 h at 70°C. The sample was then separated on a DEAE-Sephadex column (1.5 by 25 cm), using a linear salt gradient from 0 to 1 M NaCl in 0.04 M sodium phosphate (pH 7.0) buffer as described above. After a few hours at room temperature, the H_2neopterin-P_3 contained in the fractions was air oxidized to neopterin-P_3, which was identified in the fractions by fluorescence (excitation λ_{max} = 360 nm; emission λ_{max} = 445 nm). To remove inorganic phosphate from the sample, the fractions containing the neopterin-P_3 peak were combined, adjusted to pH 7 with 1 M NaOH, and absorbed on a second DEAE-Sephadex column (1.5 by 25 cm) preequilibrated in water. After being washed with water, the neopterin-P_3 was eluted by using a linear salt gradient from 0 to 1 M NH_4HCO_3. The neopterin-P_3 eluted at about 0.7 M and was recovered by evaporation of the salts, using a stream of nitrogen gas at 100°C.

RESULTS AND DISCUSSION

The first approach to determining the metabolism of GTP into pterin-containing compound(s) was to incubate cell extracts of the methanogens with GTP and to look for the production of fluorescent, pterin-containing products. Incubation of a cell extract of *M. thermophila* with GTP, followed by treatment of the incubation mixture with alkaline phosphatase and separation of the reaction mixture on a Sephadex G-25 superfine column (1 by 46 cm), gave the elution profile shown in Fig. 1. (A control incubation without the addition of GTP produced only sarcinapterin [peak A] and 7-methylpterin [peak D], both of which resulted from the oxidation of the tetrahydrosarcinapterin present in the cell extract [19].) Five major fluorescent peaks, designated A, B, C, D, and E, were observed. The major pterin components in peaks A, B, C, and D were established to be sarcinapterin, D-*erythro*-neopterin 2′:3′-cyclic phosphate, D-*erythro*-neopterin, and 7-methylpterin, respectively, based on comparison of the pterin in each peak with known pterin samples, using the following rationale. Each of the four peaks eluted from the Sephadex G-25 column at the same position as the respective known compound. The fluorescence excitation and emission spectra (excitation λ_{max} = 360 nm; emission λ_{max} = 445 nm) as well as the absorbance spectra of each peak were the same as those of the known compound. Finally, the TLC R_fs of the fluorescent compounds recovered from each peak were the same as those of the known pterins, using the two different TLC solvent systems. In addition to these methods of identification, the sarcinapterin contained in peak A was confirmed by its mild acid cleavage to the same pterin-containing fragment as derived from the acid cleavage of methanopterin (23). The D-*erythro*-neopterin 2′:3′-cyclic phosphate contained in peak B was confirmed by its acid and base hydrolysis to either D-*erythro*-neopterin 3′-phosphate or D-*erythro*-neopterin 2′-phosphate followed by alkaline phosphatase cleavage to D-*erythro*-neopterin. Both the synthetic and biosynthetic D-*erythro*-neopterin 2′:3′-cyclic phosphate were found to be resistant to cleavage by alkaline phosphatase, as had been previously reported for this compound (10).

TLC analysis of portions of the individual fractions making up peak D showed that the beginning of this peak eluted at the same elution position as a known sample of 6-hydroxymethylpterin and was 6-hydroxymethylpterin, a known metabolite of D-*erythro*-neopterin in these cells (22). We were not able to identify peak E, since it had chromatographic characteristics different from those of any of the known pterins.

A similar elution profile was obtained by the separation of a cell extract of *M. thermoautotrophicum* ΔH incubated in the same manner with GTP. Chromatographic and chemical analyses of the fluorescent peaks, which were about 10 times less than those observed with *M. thermophila*, showed that they were identical to those observed for *M. thermophila* except that peak A was identified as methanopterin. Because of the low production of the desired products in cell extracts of *M. ther-*

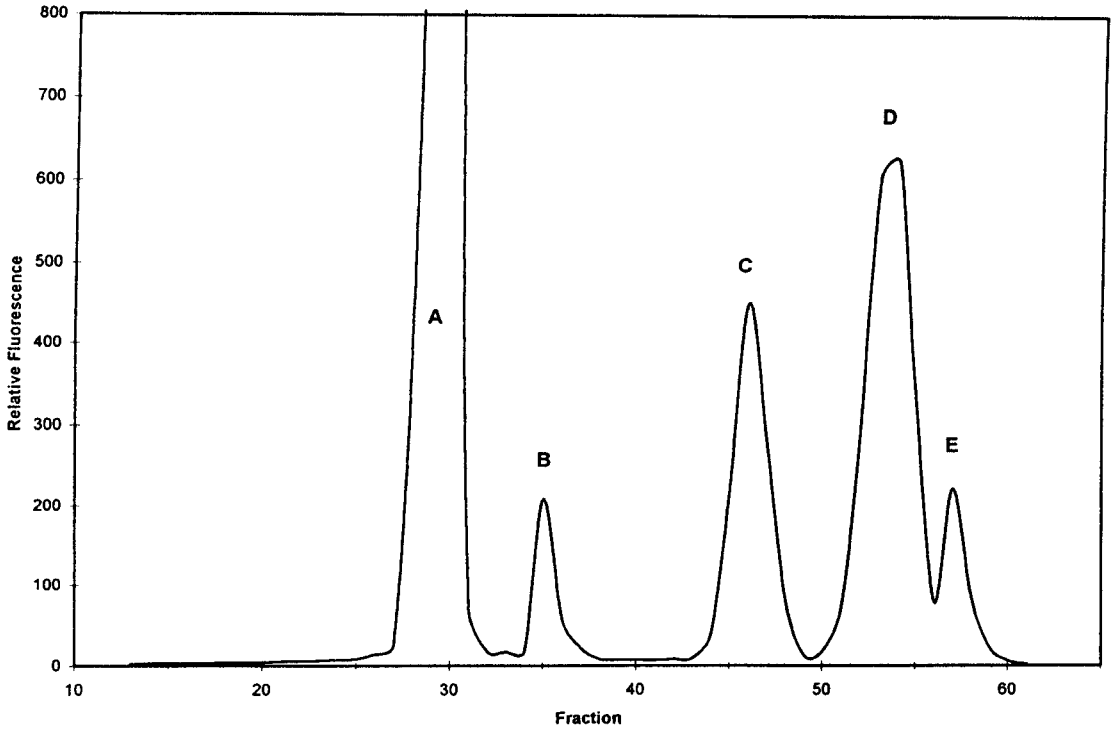

FIG. 1. Separation of the fluorescent products of an incubation of a cell extract of *M. thermophila* with GTP. The major pterin components in peaks A, B, C, and D were established to be sarcinapterin, D-*erythro*-neopterin 2′:3′-cyclic phosphate, D-*erythro*-neopterin, and 7-methylpterin, respectively.

moautotrophicum ΔH, the remaining experiments were conducted with cell extracts of *M. thermophila.*

Based on the observed fluorescence intensities of the *M. thermophila*-derived peaks, 10 nmol of D-neopterin and 2.7 nmol of D-neopterin 2′:3′-cyclic phosphate were recovered from the incubation mixture after cleavage of the phosphate monoesters with alkaline phosphatase. Based on the CD spectra (positive CD band at 247 nm) and the TLC R_f of the recovered neopterin peak, the neopterin isomer was identified as D-*erythro*-neopterin (14, 24). Treatment of the pterin contained in peak B with base followed by phosphatase cleavage led to the isolation of 2.1 nmol of D-*erythro*-neopterin, indicating that peak B was originally D-*erythro*-neopterin 2′:3′-cyclic phosphate. An identical incubation and Sephadex column separation of an incubation mixture without the phosphatase treatment gave only 2.5 nmol of neopterin, indicating that about 7.7 nmol of neopterin 3′-phosphate was generated during the incubation. Phosphatase treatment of pterin(s) contained in peak B, which eluted at the same position as neopterin-P_3, produced no neopterin, indicating that no neopterin-P_3 was present in the incubated sample. Incubation of H_2neopterin-P_3 and neopterin-P_3 under conditions even harsher than those used in the incubation (2 h at 70°C in 0.2 M Tris hydrochloride buffer [pH 8.5]) failed to produce any detectable neopterin cyclic phosphate.

Further proof of the involvement of H_2neopterin 2′:3′-cyclic phosphate in the pathway leading to H_2neopterin was confirmed by the observation that the cyclic phosphate was rapidly hydrolyzed first to H_2neopterin-3′-phosphate and then finally to neopterin by cell extracts of *M. thermophila*. These observations, along with previously published results showing that D-*erythro*-neopterin was readily converted into 6-hydroxymethylpterin by cell extracts of *M. thermophila* (22), indicate that H_2neopterin 2′:3′-cyclic phosphate is very likely involved in the sequence of reactions leading to the pterin portion of methanopterin and sarcinapterin.

The involvement of H_2neopterin 2′:3′-cyclic phosphate in the pathway to neopterin is in contrast to the established pathway of pterin formation where neopterin-P_3, generated by the action of GTP cyclohydrolase I on GTP, is an intermediate. Attempts to isolate GTP cyclohydrolase I from *M. thermophila* and *M. thermoautotrophicum* ΔH by the method of Yim and Brown (27) gave negative results; no enzyme producing a fluorescent neopterin-containing product could be found. These observations, along with the absence of a protein with a sequence homologous to that of GTP cyclohydrolase I in the genome of the archaeon *Methanococcus jannaschii* (3), make one suspect that the routes for the formation of neopterin may be different in the archaea. Since the conversion of GTP to H_2neopterin-P_3 requires at least four chemical steps, then it is possible that more than one enzyme is required for the formation of H_2neopterin 2′:3′-cyclic phosphate in the archaea. To test this idea, the proteins present in an extract of *M. thermophila* were separated on an FPLC Mono Q column into 12 fractions, and each fraction was assayed for its ability to produce pterin-containing compound(s) when incubated with GTP. This was established by iodine oxidation of the reaction product followed by its separation on a Sephadex G-25 column and identification of the pterins by fluorescence and TLC as described above. We found no single Mono Q fraction that produced a pterin-containing compound upon incubation with GTP, whereas the combined Mono Q fractions 5 to 10, eluting from 0.4 to 1 M KCl, readily produce H_2neopterin 2′:3′-cyclic phosphate, indicating that more than one enzyme or an enzyme and a coenzyme were required for the transformation. Since the product of this incubation was H_2neopterin 2′:3′-cyclic phosphate, this finding indicates that the enzyme(s) re-

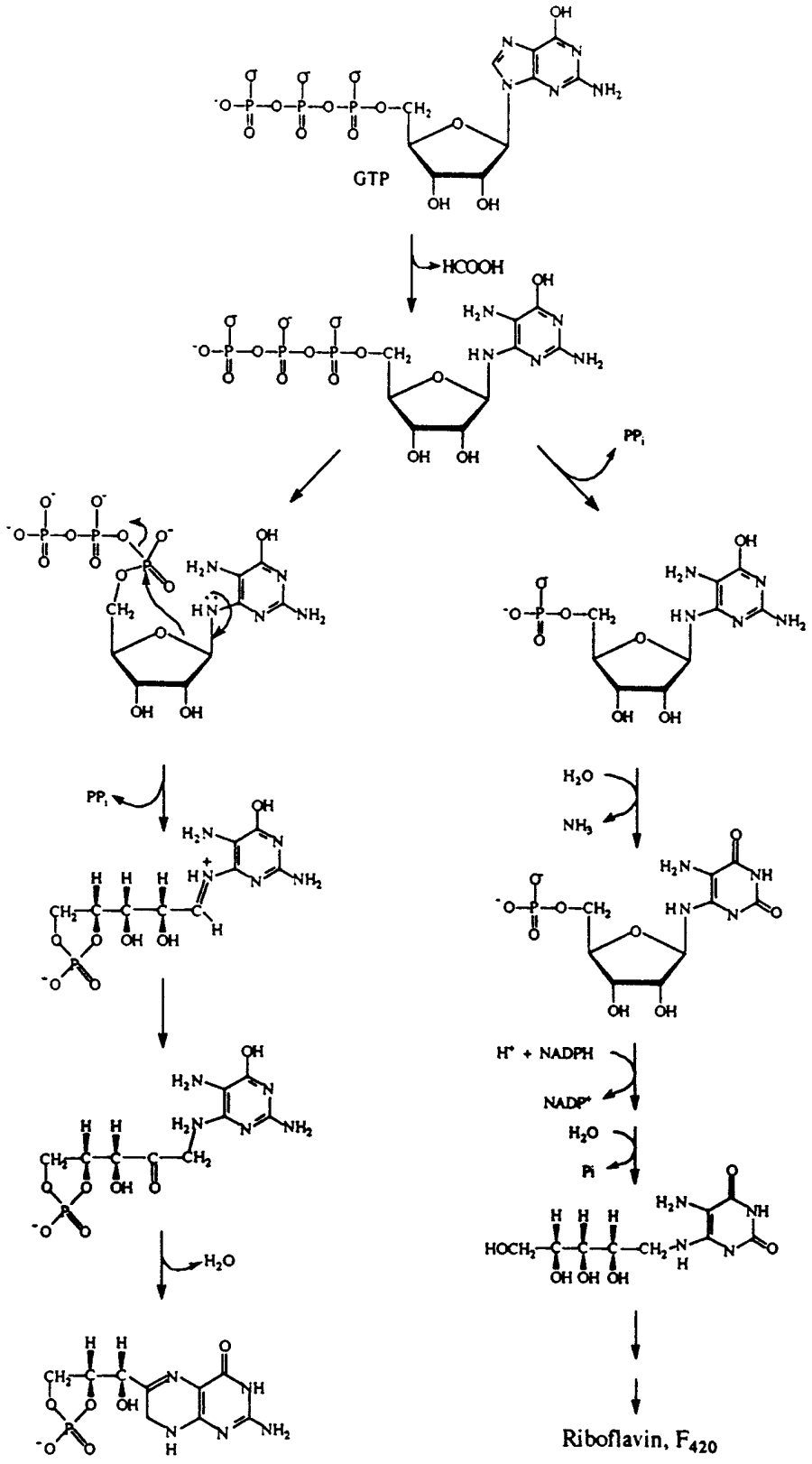

FIG. 2. Proposed pathways for the formation of D-H_2neopterin 2′:3′-cyclic phosphate, riboflavin, and F_{420} in *M. thermophila*. It is proposed that the first step in the reaction is catalyzed by new enzyme termed GTP cyclohydrolase III.

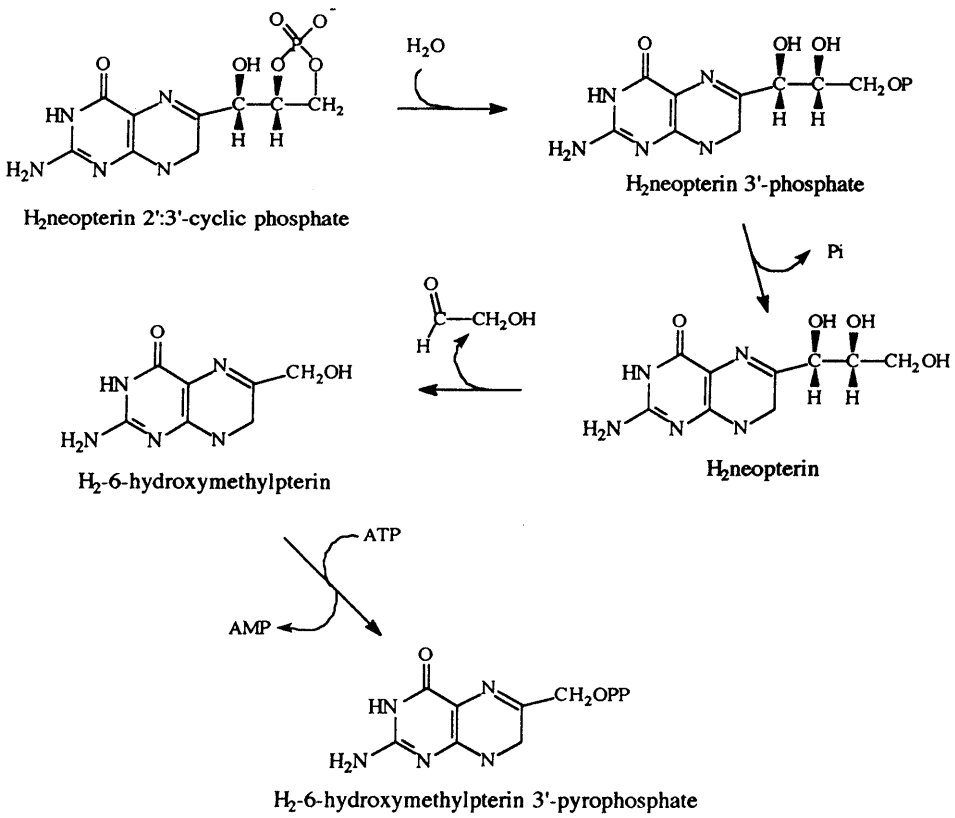

FIG. 3. Steps in the conversion of D-H_2dihydroneopterin 2′:3′-cyclic phosphate into H_2-6-hydroxymethylpterin pyrophosphate.

quired for the further metabolism of H_2neopterin 2′:3′-cyclic phosphate were not contained in Mono Q fractions 5 to 10.

Another characteristic of the *E. coli* GTP cyclohydrolase I is the lack of any detectable intermediate(s) in its enzymatic conversion of GTP to H_2neopterin-P_3 (27). This is in contrast to our observations where we have tentatively identified 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-triphosphate (Fig. 2) as an intermediate in the series of reactions in which H_2neopterin 2′:3′-cyclic phosphate is the product. The identification of this intermediate is based on the detection of a molecule, produced in an incubation mixture of a cell extract of *M. thermophila*, which elutes from a DEAE-Sephadex column in the same position as ATP and GTP and produces 6,7-dimethylpterin when reacted with 2,3-butanedione. These observations are all consistent with 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-triphosphate being present in the incubation mixture. The DEAE-Sephadex column separation of the incubation mixture also identified, at about the same concentration, a compound that eluted at the position of AMP and GMP and also produced 6,7-dimethylpterin when heated with 2,3-butanedione. This compound is most likely 2,5-diamino-6-(ribosylamino)-4(3H)pyrimidinone 5′-phosphate, the first intermediate in the biosynthesis of riboflavin, and arises from the GTP cyclohydrolase II present in the extract (2).

H_2neopterin 2′:3′-cyclic phosphate was first thought to be produced by the GTP cyclohydrolase present in *Comamonas* sp. (4) but was later found to be an artifact of the chromatographic methods used in the analysis of the enzymatic products (15). Later results showed that the true reaction product was H_2neopterin 3′-triphosphate (15). The reason for the error in the identification was traced to the strongly basic solvents used in the chromatographic analytical methods employed. This cannot be the reason for the H_2neopterin 2′:3′-cyclic phosphate detection here, since none of those analytical methods were used. It should be noted that H_2neopterin 2′:3′-cyclic phosphate has been reported several times as a microbiological product (18, 26), which may indicate its involvement in neopterin biosynthesis in organisms other than archaea.

The formation of H_2neopterin 2′:3′-cyclic phosphate can be rationalized by the following route. An intramolecular reaction in the 2,5-diamino-6-ribosylamino-4(3H)-pyrimidinone 5′-triphosphate would lead to pyrophosphate and 2,5-diamino-6-(ribitylimino)-4(3H)pyrimidinone 4′:5′-cyclic phosphate as shown in Fig. 2. An aldolase-type rearrangement of the 2,5-diamino-6-(ribitylimino)-4(3H)pyrimidinone 4′:5′-cyclic phosphate followed by cyclization of the resulting product would then produce 7,8-H_2neopterin 2′:3′-cyclic phosphate. Hydrolysis of the cyclic phosphate would then produce H_2neopterin, a known precursor to the pterin portion of methanopterin, as shown in Fig. 3.

The *Methanococcus jannaschii* genome has a gene sequence encoding a protein with a sequence homologous to that of GTP cyclohydrolase II (3). GTP cyclohydrolase II of *E. coli* is responsible for the first step in the biosynthesis riboflavin and converts GTP into 2,5-diamino-6-(ribosylamino)-4(3H) pyrimidinone 5′-phosphate, pyrophosphate, and formate (5). Since this enzyme catalyzes a portion of the same chemistry required for the GTP cyclohydrolase I reaction, it is possible that this one enzyme is responsible for the first step in the biosynthesis of both riboflavin and pterin in the archaea. This can be most easily rationalized if the enzymatic product of archaeal GTP cyclohydrolase II is 2,5-diamino-6-(ribosylamino)-4(3H) pyrim-

idinone 5′-triphosphate as shown in Fig. 2. Loss of pyrophosphate from this molecule would then produce 2,5-diamino-6-(ribosylamino)-4(3H)pyrimidinone 5′-phosphate, the established intermediate in riboflavin biosynthesis (1). The presence of 2,5-diamino-6-(ribosylamino)-4(3H)-pyrimidinone 5′-phosphate in the archaea is supported by the presence of a riboflavin-specific deaminase in *Methanococcus jannaschii* (3). We thus propose that the GTP cyclohydrolase in archaea be named GTP cyclohydrolase III, since its product is different from that of GTP cyclohydrolase I or II.

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