Ribose-5-Phosphate Biosynthesis in *Methanocaldococcus jannaschii*

Occurs in the Absence of a Pentose-Phosphate Pathway

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Recent work has raised a question as to the involvement of erythrose-4-phosphate, a product of the pentose phosphate pathway, in the metabolism of the methanogenic archaea (*R. H. White, Biochemistry 43:7618–7627, 2004*). To address the possible absence of erythrose-4-phosphate in *Methanocaldococcus jannaschii*, we have assayed cell extracts of this methanogen for the presence of this and other intermediates in the pentose phosphate pathway and have determined and compared the labeling patterns of sugar phosphates derived metabolically from [6,6-2H2]- and [U-13C]-labeled glucose-6-phosphate incubated with cell extracts. The results of this work have established the absence of pentose phosphate pathway intermediates erythrose-4-phosphate, xylose-5-phosphate, and sedoheptulose-7-phosphate in these cells and the presence of D-arabinose-3-hexulose-6-phosphate, an intermediate in the ribulose monophosphate pathway. The labeling of the D-arabinose-3-hexulose-6-phosphate, as well as the other sugar-Ps, indicates that this hexose-6-phosphate was the precursor to ribulose-5-phosphate that in turn was converted into ribose-5-phosphate by ribose-5-phosphate isomerase. Additional work has demonstrated that ribulose-5-phosphate is derived by the loss of formaldehyde from D-arabinose-3-hexulose-6-phosphate, catalyzed by the protein product of the MJ1447 gene.

Ribose-5-phosphate is generally assumed to arise via the oxidative and nonoxidative pentose phosphate pathways (16). Early labeling studies with the *Archea* have indicated that the oxidative part of the pathway is functioning in a number of methanogenic archaea tested (4, 7–9), whereas other data have indicated that the nonoxidative branch of the pathway may be responsible for the biosynthesis of pentoses in *Methanococcus voltae* (4). Support for the nonoxidative part of the pathway comes from direct enzymatic analysis of the required enzymes in *Methanococcus maripaludis* (35) and from analyses of methanogen genomes (5, 21, 23, 28). This information indicates that the pentose phosphate pathway is operational in these cells; however, other research suggests that the all or part of the pentose phosphate pathway is nonfunctional in some archaea. *Methanothermobacter thermoautotrophicus* (4) have putative genes for both a complete nonoxidative pentose phosphate pathway and an RuMP pathway. The putative *M. jannaschii* RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes include MJ1247 (3-hexulose-6-phosphate synthase, RuMP pathway genes 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pathway. We propose that the function of the Fae domain of Hps in the methanogenic archaea is to catalyze the condensation formaldehyde with tetrahydrodiaminopentanoic (H₂MPT) to form methylene H₂MPT and that the function of ribulose monophosphate synthase domain is to catalyze the loss of the formaldehyde from 3-hexulose-6-phosphate to form ribulose monophosphate, as Verhees et al. and Soderberg and Alver proposed (25, 28).

**MATERIALS AND METHODS**

**Chemicals.** All chemicals were obtained from Aldrich/Sigma unless otherwise indicated. Labeled glucose-6-phosphates were prepared and purified as previously reported (31). Ribulose-5-phosphate was prepared in a final volume of 1 ml containing 100 mM ribose-5-phosphate, 140 U of phosphoribosylisomerase type I from spinach (Sigma), and 25 mM TES [N-tris(hydroxymethyl)methyl-2-aminooethanesulfonic acid] buffer (pH 7.5) incubated for 2 h at 30°C.

**Preparation of M. jannaschii cells extracts.** A cell extract of M. jannaschii was prepared by sonication of 4.67 g of frozen cells suspended in 10 ml of TES buffer (10 mM Tricine, 10 mM MgCl₂, pH 7.5) under argon for 5 min at 4°C. M. jannaschii cells were grown as previously described (18). The resulting mixture was centrifuged under argon (27,000 × g; 10 min) and stored frozen at −20°C until used. The M. jannaschii extract protein concentration was 30 mg/ml. Protein concentration was measured using the BCA Total Protein assay (Pierce) with bovine serum albumin as a standard.

**Cloning and expression of the MJ1447-derivative protein in Escherichia coli.** The M. jannaschii gene MJ1447 (Swiss-Prot accession number Q58642) was amplified by PCR from genomic DNA using oligonucleotide primers synthesized by Invitrogen: Fwd (5′-GGTCTATGATAAATTTTGG-3′) and Rev (5′-GCTG GATCCTCATAATTCCTC-3′). PCR was performed as described previously using a 50°C annealing temperature (12). The primers introduced an NdeI site at the 5′ end and a BamHI site at the 3′ end. The amplified PCR product was purified with a QiAquick spin column (Invitrogen), digested with restriction enzymes NdeI and BamHI and then ligated into the competent plasmid pT7-7 (USB). T4 DNA ligase (Invitrogen) was used to make the recombinant plasmid pMJ1447. DNA sequences were verified by dye-terminator sequencing at the Virginia Bioinformatics Institute’s DNA facility. The resulting plasmid, pMJ1447, was transformed into E. coli BL21-CodonPlus (DE3)-RIL (Stratagene) cells. The transformed cells were grown in Luria-Bertani medium (200 ml; Difco) supplemented with 100-μg/ml ampicillin at 37°C with shaking until they reached an absorbance of 1.0 at 600 nm. Recombinant protein production was induced by adding 1 ml of 2 MN H₄HCO₃. Evaporation of the sample to dryness with a stream of nitrogen gas, the addition of lactose to a final concentration of 28 mM. After an additional 3-h incubation, the clear extract was passed through a Dowex 50W-X8-pyridinium column (2 by 5 mm), which was washed with 1 ml of water and adjusted to pH 8 to 9 by the addition of 1 M NaOH. The sample was applied to a small column of DEAE-Sephadex (2 by 5 mm), which was washed with 1 ml of water; the sugar phosphates were eluted with 1 ml of 2 M NH₄HCO₃. Evaporation of the sample to dryness with a stream of nitrogen gas while the sample was held at 100°C removed any NH₄HCO₃. The phosphate was removed from the isolated polyol phosphates as described above. The sample was then passed through a Dowex 50W-X8-pyridinium column (2 by 10 mm) followed by a Dowex 1-X8-200 OH− column (2 by 10 mm) prior to evaporation of water and formation of the TMS derivatives.

**RESULTS AND DISCUSSION**

Levels and identity of sugars present in M. jannaschii cell extracts and incubation mixtures. Analysis of the TMS derivatives of the polyols generated by borohydride reduction and

Analysis of hexose-6-phosphate metabolism in M. jannaschii cell extracts. The incubation mixture was treated with either borohydride or borodeuteride, and the resulting polyols were isolated and were used for GC-MS analysis. With this procedure, erythrose-4-phosphate produced erythritol, ribose-5-phosphate produced ribitol, xylose-5-phosphate produced xylitol, arabinose-5-phosphate produced arabitol, ribulose-5-phosphate produced an equal mixture of arabinitol and ribitol, glucose 6-phosphate produced glucitol, fructose phosphates produced an equal mixture of mannitol and glucitol, arabino-3-hexulose phosphate produced an equal mixture of mannotetol and D-altritol (D-talitol), and sedoheptulose-7-phosphate gave rise to sedoheptitol. GC-MS analysis of the TMS derivatives was used to determine the amount of the polyols present and to estimate the extent of labeling in each polyol produced. Quantitation was based on comparing the peak heights of the polyols to the peak height of the inositol that was added as a known amount to each sample to serve as an internal standard. With the column used for the GC-MS analyses, the TMS derivatives of arabinol, xylitol, ribitol, mannotetol, glucitol, altritol, and sedoheptitol were all separated in the order listed.

**Incubation of cell extracts with precursors.** In a 0.5-ml plastic centrifuge tube, 100-μl portions of cell extracts were mixed with 10 μl of 0.1 M solution of labeled glucose-6-phosphates and 10 μl of the indicated cofactors, and the samples were incubated for 15 or 20 min at 70°C. After incubation, the extracts were cooled to room temperature, and 1 μl of 10 mM inositol and 2 to 3 μg of sodium borohydride or sodium borodeuteride were added with mixing. After 30 min at room temperature, 240 μl of methanol was added, and the precipitated proteins were removed by centrifugation (12,000 × g; 1 min). The clear extract was separated, acidified to pH 3 by the addition of HCl, and evaporated to dryness with a stream of nitrogen gas. To remove any borate, the samples were repeatedly suspended in methanol (three suspensions, each 0.5 ml) and the methanol was evaporated to dryness with a stream of nitrogen gas. The resulting sample was dissolved in 200 μl of water and adjusted to pH 8 to 9 by the addition of 1 M NaOH. The sample was applied to a small column of DEAE-Sephadex (2 by 5 mm), which was washed with 1 ml of water; the sugar phosphates were eluted with 1 ml of 2 M NH₄HCO₃. Evaporation of the sample to dryness with a stream of nitrogen gas while the sample was held at 100°C removed any NH₄HCO₃. The phosphate was removed from the isolated polyol phosphates as described above. The sample was then passed through a Dowex 50W-X8-pyridinium column (2 by 10 mm) followed by a Dowex 1-X8-200 OH− column (2 by 10 mm) prior to evaporation of water and formation of the TMS derivatives.

**Formation of TMS derivatives.** Samples to be assayed as TMS derivatives were dried by evaporation with a stream of nitrogen gas while being held at 100°C in a water bath and were then reacted with 20 μl of a mixture of pyridine, hexamethyldisilazane, and chlorotrimethylsilane (9:3:1 [vol/vol/vol]) for 2 min at 100°C.

**GC-MS analysis.** GC-MS analysis of the sugars in the cell extracts was obtained using a VG-70-70HEF gas chromatography-mass spectrometer operating at 70 eV and equipped with an HP-5 column (0.32 μm by 30 m) programmed from 95°C to 280°C at 10°C per min. Reduction of the sugar phosphates present in the sample with NaBH₄ was done so that each sugar phosphate would produce only one GC peak. Under the GC-MS conditions used, the TMS derivatives of the following compounds had the following retention times (in minutes) and mass spectral data (molecular weight, base peak, the most abundant ions with masses of >150 m/z are listed in order of decreasing intensities): erythritol (TMS) derivative (9.05) (410, 73, 205, 217, 189, 191, 307), xylitol (TMS) derivative (13.65) (512, 73, 217, 205, 319, 307, 422), arabitol (TMS) derivative (13.98) (512, 73, 217, 205, 319, 307, 422), ribitol (TMS) derivative (14.09) (512, 73, 217, 205, 319, 307, 427), xylitol (TMS) derivative (13.65) (512, 73, 217, 205, 319, 307, 422), mannitol (TMS) derivative (18.49) (614, 319, 205, 217, 307), glucitol (TMS) derivative (18.65) (614, 319, 205, 217, 307), galactitol (TMS) derivative (18.73) (614, 319, 205, 217, 307), altritol (TMS) derivative (18.73) (614, 319, 205, 217, 307), and pectitol (an isomer of sedoheptulitol) (TMS) derivative (24.29) (716, 319, 307, 421, 409 [range, 250 to 400]). The distribution of label incorporated into the polyols was calculated by adjusting the observed normalized isotopic cluster intensities of the labeled with the nonlabeled samples as previously described (2).

**Analysis of hexose-6-phosphate metabolism in M. jannaschii cell extracts. The incubation mixture was treated with either borohydride or borodeuteride, and the resulting polyols were isolated and were used for DEAE column. The sample was then treated with phosphatase to remove the phosphohexose isomerase type I from spinach (Sigma), and 25 mM TES [N-tris(hydroxymethyl)methyl-2-aminooethanesulfonic acid] buffer (pH 7.5) incubated for 2 h at 30°C.
TABLE 1. Levels of the sugar-derived polyols and distribution of their incorporated label generated in *M. jannaschii* cell extracts incubated with [6,6-2H2]glucose-6-phosphate and [U-13C]glucose-6-phosphate

<table>
<thead>
<tr>
<th>Amt of products detected and measured distribution of label with:</th>
<th>Controlb (µM)d</th>
<th>[6,6-2H2]glucose-6-phosphatec</th>
<th>[U-13C]glucose-6-phosphatec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µM</td>
<td>0°</td>
<td>1°</td>
</tr>
<tr>
<td>Glycerol</td>
<td>28</td>
<td>135</td>
<td>40.8</td>
</tr>
<tr>
<td>D-Arabitolb</td>
<td>2</td>
<td>44</td>
<td>73.5</td>
</tr>
<tr>
<td>Ribitolb</td>
<td>6</td>
<td>120</td>
<td>82.2</td>
</tr>
<tr>
<td>Mannitold (0.3)</td>
<td>49</td>
<td>55.6</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>55.5</td>
<td>10.4</td>
<td>34.6</td>
</tr>
<tr>
<td>D-Glucitold</td>
<td>0.3</td>
<td>180</td>
<td>55.6</td>
</tr>
<tr>
<td>D-Altritol</td>
<td>ND</td>
<td>7</td>
<td>48.3</td>
</tr>
</tbody>
</table>

| a| The control sample is the analysis of a 100-µl *M. jannaschii* cell extract that was not incubated. All sugar assayed, except glycerol, contained one deuterium as a result of the sodium borodeuteride reduction. |
| b| In this experiment, 100 µl of *M. jannaschii* cell extract was incubated for 20 min at 70°C in the presence of 12 mM [6,6-2H2]glucose-6-phosphate and 4 mM (each) NADH, NADPH, ATP, GTP, UTP, TTP, and CTP prior to the analysis of sugars. |
| c| In this experiment, *M. jannaschii* cell extract (100 µl) was incubated for 30 min at 70°C in the presence of 7.7 mM [U-13C]glucose-6-phosphate, 3.8 mM NADH, and 3.8 mM NADPH. |
| d| Calculated from peak heights using inositol as an internal standard. ND, not detected. |

The distribution of label incorporated into the polyols was calculated by adjusting the observed normalized isotopic cluster intensities of the labeled with the nonlabeled samples as previously described (2). The numbers refer to the percentage of the molecules containing the indicated number of H and/or 13C.

1. Glycerol (TMS), M+ -15 ion at m/z 293 had 2H0 = 59%, 2H1 = 12%, and 2H2 = 30%; M+ -90 ion at m/z 218 had 2H0 = 70.5%, 2H1 = 18%, and 2H2 = 4.8%; and M+ -203 ion at m/z 205 had 2H0 = 80%, 2H1 = 7.2%, and 2H2 = 13%. 
2. The m/z 422 ion of the arabitol (TMS), derivative was too weak for accurate measurement of the ion intensities. The reported values were calculated from the m/z 307 and the m/z 320 ions that showed the same labeling pattern as seen in the ribitol but with a higher abundance of 13C.
3. Ribitol is derived both from D-ribose-5-phosphate and D-ribulose-5-phosphate present in the cell extracts.
4. Mannitol is considered to arise from the ribonucleotide reduction of the fructose-6-phosphate, fructose-1-phosphate, fructose-1,6-phosphate, and/or fructose-1,6-bisP present or generated in the cell extracts.
5. The m/z 319 ion from the (TMS) 7 derivative of mannitol, and D-altritol.
6. D-Glucitol is not produced by *M. jannaschii* cell extracts.
7. The m/z 307 ion from the (TMS) 7 derivative of mannitol and glucitol.
8. The m/z 319 ion from the (TMS) 7 derivative of mannitol and glucitol.

By dephosphorylation of the sugar phosphates present in the incubation mixtures was used to determine the sugars present in the different samples. By using an internal standard of inositol, which is not produced by the cells, the levels of each of the compounds produced could also be determined. The specific compounds assayed by GC-MS were glycerol arising from dihydroxyacetone, hydroxyprovaldehyde, glyceraldehyde, and/or their phosphates; erythritol arising from erythrose-3-phosphate; ribitol arising from ribose-5-phosphate; xylitol arising from xylose-5-phosphate; arabitol arising from arabinose-5-phosphate; arabitol and ribitol arising from ribulose-5-phosphate; glucitol arising from glucose-6-phosphate; mannitol and glucitol arising from the fructose phosphates; D-altritol and mannitol arising from arabino-3-hexulose-6-phosphate; and sedoheptulose-7-phosphate.

By reduction of the sugars with either borohydride or borodeuteride, the number of carbonyl groups in each sugar can be determined by the increase in the nominal mass of the derivative. Changes in the masses of the fragment ions allowed for the determination of the positions where the reduction occurred. By isolating the sugar phosphates after the incubation using DEAE-Sephadex chromatography, it was possible to establish that each sugar was present as the phosphate esters. Analysis of the label distribution of the incorporated stable isotopes allowed the pattern of the label incorporation to be determined. Analysis of cell extracts incubated either in the presence or absence of glucose-6-phosphate by the methods described showed the presence of glyceral, D-arabitol, ribitol, mannitol, D-glucitol, and D-altritol (Table 1). Erythritol, xylitol, and sedoheptulose-7-phosphate were never detected; if present, the amounts would have to be 50 times smaller than the glucose-6-phosphate present in the cell extracts. This limit is based on the single ion plots of the m/z 205, 217, 189, 191, 307 ions from erythritol (TMS) derivative; the m/z 217, 205, 319, 307, 422 for the xylitol (TMS) derivative, and the m/z 319, 307, 421, 409 for the peracetol (TMS) derivative. Table 1 shows that incubation of the cell extracts with each of the labeled glucose-6-phosphates increased the amount of each polyol over the control and that in each incubation the polyls contained label derived from the labeled glucose-6-phosphates.

**Sugar metabolism as revealed by labeling studies.** Since *M. jannaschii* does not rely on the pentose phosphate pathway to produce ribose-5-phosphate, we needed to establish the route for the conversion of glucose-6-phosphate to ribose-5-phosphate. The incorporation of labeled glucose-6-phosphates into dihydroxyacetone-phosphate, glyceraldehyde-3-phosphate, ribose-5-phosphate, ribulose-5-phosphate, D-arabino-3-hexulose-6-phosphate, fructose-6-phosphate, and glucose-6-phosphate was measured. This was accomplished by measuring the incorporation of labeled glucose-6-phosphate into each of the specific polyls. Cell extracts incubated with [6,6-2H2]glucose-6-phosphate produced a fourfold increase in the amount of glyceral isolated, indicating that the amount of triose phosphate and/or glyceral phosphate had increased (Table 1). Since this sample...
was not reduced with borodeuteride, these different trioses could not be distinguished. Also, since the samples were isolated on DEAE, each sugar would have been present as phosphates. The important measurement is that around 46% of the isolated glycerol contained two deuteriums. This confirmed cleavage of fructose-6-phosphate, derived from glucose-6-phosphate, into triose phosphates labeled with two deuteriums (Fig. 1). This cleavage occurred in the absence of added ATP, which further indicates that fructose-6-phosphate and not fructose-1,6-bisphosphate was the molecule cleaved. This is consistent with the presence of dihydroxyacetone in the cell extract (31). Such cleavage is not consistent with fructose-1,6-diphosphate aldolase, which, despite relaxed acceptor aldehyde specificity, requires a conserved “dihydroxyacetone phosphate” unit in the sugar substrate (1, 24). This same pattern of labeling was also seen in the isolated arabinol and ribitol, where arabinol reflected the labeling present in the ribulose-5-phosphate and ribitol reflected the ribose-5-phosphate in the cells. The higher levels of labeling seen in arabinol over ribitol indicates that these sugars were not completely in equilibrium and that the ribulose was biosynthetically closer to the glucose-6-phosphate than the ribose-5-phosphate. Considering that mannitol, which measures the label in both fructose-6-phosphate and arabino-3-hexulose-6-phosphate, had a label distribution similar to that seen in the glucitol, then all these sugars have been labeled from glucose-6-phosphate. The operation of both glucose-6-phosphate isomerase (MJ1605) and fructose-6-phosphate isomerase (MJ1247) is consistent with the detection of altritol in the cell extracts incubated with glucose-6-phosphate.

About 3% of these hexitols, but not the pentitols, were found to contain 3H. This distribution would arise by the breaking down of [6,6-2H2]fructose-6-phosphate, derived from [6,6-2H2]glucose-6-phosphate, to dihydroxyacetone and [6,6-2H2]glyceraldehyde-3-phosphate. After [6,6-2H2]glyceraldehyde-3-phosphate equilibrates to [6,6-2H2]dihydroxyacetone phosphate, the two recombine to form [1,1,6,6-2H4]fructose-1,6-phosphate. The fact that arabinol had only two deuteriums indicates that no transketolase reaction from [6,6-2H2]fructose-6-phosphate was involved in the formation of [5,5,2H2]ribulose-5-phosphate.

The glycerol and the mannitol and glucitol together contain 46% and 35%, respectively, of the molecules with two deuteriums. The presence of unlabeled glucose-6-phosphate and fructose-6-phosphate present in the cell extract diluted the label. Based on the fragmentation of the isolated polysols, these two deuteriums were present at the C-5 position of the ribose-5-phosphate and ribulose-5-phosphate and the C-6 position of the hexose-6-phosphates. Dilution of the label, which began with the unlabeled glucose-6-phosphate in the cell extract, continued as the label moved to the other metabolites and was increased by other unlabeled compounds present in the cell extract. In each case, some of the molecules were found with 3H. These data clearly established that the ribose-5-phosphate is generated from [6,6-2H2]glucose-6-phosphate when incubated with cell extract, but does not indicate the pathway(s) involved.

Repeating the experiment with [U-13C]glucose-6-phosphate allowed for analysis of the breakdown and reassembly of the carbon chains. Extensive scrambling of the carbon skeleton was seen in all of the polysols from the [U-13C]glucose-6-phosphate experiment (Table 1). This was measured by the generation of hexose-phosphates containing from one to six 13C's. This scrambling most significantly resulted from the breakdown of labeled and unlabeled fructose-6-phosphate to labeled and unlabeled glyceraldehyde-3-phosphate and dihydroxyacetone, followed by random recombining of labeled and unlabeled components to re-form fructose-6-phosphate (Fig. 1). This produced the largest abundance of molecules with 13C0, 13C1, 13C2, 13C3, plus 13C5, and 13C6 units. This resulted, in part, from the equilibration of fructose-6-phosphate and ribulose-phosphate via arabinino-3-hexulose-6-phosphate with subsequent incorporation of labeled and unlabeled formaldehyde (Fig. 1). This would not occur with a pentose phosphate pathway, but it is consistent with the RuMP pathway. In addition, altritol, derived from arabinino-3-hexulose-6-phosphate, was found with about the same distribution of 13C as the mannitol, confirming its direct formation from the fructose-6-phosphate.

Another route to ribose-5-phosphate. These labeling data indicate that M. jannaschii must have another route to ribose-P that does not utilize the nonoxidative pentose phosphate pathway. Based on genomic analysis, this could proceed through the RuMP pathway (Fig. 2). The RuMP pathway is used by some methylotrophic bacteria for formaldehyde fixation, and several gene clusters have been cloned (13, 33, 34, 36). Soderberg and Alver proposed this pathway as an alternate route to ribose biosynthesis in archaea (25). This alternate route to ribose-5-phosphate would involve the protein products of the MJ1603, MJ1247, and MJ1447 genes. MJ1247 has been previously characterized, and its crystal structure has been solved (15).

We have confirmed the function of the MJ1447 gene by recombinantly expressing its protein product and determining that it does catalyze the addition of formaldehyde to ribulose-5-P. M. jannaschii arabinino-3-hexulose-6-phosphate synthase incubated with ribulose-5-phosphate and formaldehyde resulted in the production of arabinino-3-hexulose-6-phosphate that was detected as mannitol and talitol by GC-MS of the reduced, dephosphorylated sugar. The reaction was further confirmed by the incorporation of two deuteriums at C-1 when the reaction was conducted with [3H]formaldehyde. The Fae domain of the molecule is expected to transfer formaldehyde to H4MPT for subsequent metabolism via the methanogenesis pathway. While the manuscript was in preparation, the facB-lpsB gene product from Methanosarcina barkeri, which is a fusion of the Fae and Hps domains as seen in MJ1447, was shown to catalyze both of the reactions discussed above (11). Since there is no clear mechanistic connection between the release of formaldehyde from the ribulose-5-P and the combination of the formaldehyde with H4MPT, this enzyme may represent an example of intraprotein channeling.

Implications of these results. Our results show that M. jannaschii does not produce any intermediate expected in the pentose phosphate pathway and that the RuMP pathway is functioning in ribose-5-phosphate biosynthesis. An important question to be asked is why this pathway is functioning in M. jannaschii, which contains putative genes for each of the non-oxidative pentose phosphate pathway enzymes. Soderberg and Alver’s genomic analysis of archaeal genomes indicated that...
FIG. 1. Labeling of sugar phosphates in *M. jannaschii* cell extracts incubated with labeled hexose phosphate. *, $^{13}$C.
genes for the RuMP pathway enzymes are present in the majority of archaea, while a complete nonoxidative pentose phosphate pathway appears to be absent (25). Xavier et al. suggested a novel glycolytic pathway in *Thermococcus zilligii*, based on formate formation from glucose in this organism (32). Verhees et al. later reinterpreted their results to suggest that the RuMP pathway may be operating in conjunction with a formaldehyde oxidoreductase to produce the observed formate (28). The proposed importance of the RuMP pathway in archaeal metabolism has been supported by Orita et al.’s recent work, which showed that in *Pyrococcus horikoshii* the two central enzymes of the RuMP pathway, Hps and Phi, are fused into a single polypeptide that is constitutively expressed (19). Several archaea (*M. jannaschii*, *Methanococcus maripaludis*, *Thermoplasma acidophilum*, and *Thermoplasma volcanium*), however, are predicted to have the complete nonoxidative pentose phosphate pathway (14, 25). Two of these species, *T. acidophilum* and *T. volcanium*, do not have homologs of the RuMP enzymes or a homolog of 2-amino-3,7-dideoxy-D-threohex-6-ulosonic acid synthase (ADTH synthase) (MJ0400), an enzyme that is involved in the erythrose-4-phosphate-independent pathway to aromatic amino acids (31). It is likely that the nonoxidative pentose phosphate pathway, as well as the typical erythrose-4-phosphate pathway for aromatic amino acid biosynthesis, functions in these organisms. *M. jannaschii* and *M. maripaludis* are unique because they contain a complete set of putative genes for both pathways (25). It is interesting that while *M. maripaludis* has been shown to have a functioning nonoxidative pentose phosphate pathway (14, 27, 35), it does not utilize erythrose-4-phosphate in the formation of aromatic amino acids and instead utilizes the alternate pathway found in *M. jannaschii* (unpublished results).

The results presented here indicate that the RuMP pathway may be the only pathway for ribose-5-phosphate biosynthesis in *M. jannaschii*. This raises several evolutionary questions concerning the origins of sugar and aromatic amino acid biosynthesis. In *M. jannaschii*, condensation of formaldehyde and ribulose to form hexoses, as well as the reverse reaction, may have developed from a prebiotic formaldehyde condensing reaction, the so-called formose reaction, as Decker et al. proposed (6). Alternatively, this pathway may have evolved since *M. jannaschii* no longer required erythrose-4-phosphate for aromatic amino acid biosynthesis; as a result, the cells had no use for erythrose-4-phosphate. This would, in turn, make the nonoxidative pentose phosphate pathway no longer necessary because an alternate route to ribose existed. Although *M. jannaschii* is predicted to contain a full complement of nonoxidative pentose phosphate pathway enzymes, it apparently does not utilize this pathway for either ribose-5-phosphate or aromatic amino acid biosynthesis. *M. maripaludis* may

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**FIG. 2.** The RuMP pathway and its associated genes in *M. jannaschii*. MJ1605 is the gene encoding glucose-6-phosphate isomerase, MJ1247 is the gene encoding fructose-6-phosphate isomerase, MJ1447 is the gene encoding 3-oxohexulose-6-phosphate synthase and the formaldehyde-activating enzyme, and MJ1603 is the gene encoding ribose-6-phosphate isomerase.
represent an evolutionary intermediate between organisms that utilize either the nonoxidative pentose phosphate pathway or the RuMP pathway for ribose biosynthesis. Although \textit{M. maripaludis} contains both pathways, it utilizes the nonoxidative pentose phosphate pathway for ribose biosynthesis. However, it does not use the erythrose-4-phosphate intermediate for aromatic amino acid biosynthesis but rather uses the archaeal pathway involving aspartate semialdehyde and 6-deoxy-5-keto-fructose-1-phosphate.

An important secondary question that remains to be addressed is the function of the annotated gene products that were considered to be involved in the nonoxidative pentose phosphate pathway in \textit{M. jannaschii}. Soderberg recently showed that MJ0960 encodes a transaldolase that catalyzed the reaction of fructose-6-phosphate with erythrose-4-phosphate to form sedoheptulose-7-phosphate (25). Based on sequence homology this enzyme was expected to be like the transaldolase that catalyzed the pathway involving aspartate semialdehyde and 6-deoxy-5-ketohexose (26). However, the pentose phosphate pathway for ribose biosynthesis. However, it does not use the erythrose-4-phosphate intermediate for aromatic amino acid biosynthesis but rather uses the archaeal pathway involving aspartate semialdehyde and 6-deoxy-5-keto-fructose-1-phosphate.

Based on sequence homology this enzyme was expected to be like the transaldolase that catalyzed the reaction of fructose-6-phosphate with erythrose-4-phosphate to form sedoheptulose-7-phosphate (25). The result is in conflict, since our results show no erythrose-4-phosphate in the cell extracts of \textit{M. jannaschii} and may simply represent an example of a promiscuous enzyme capable of utilizing a wide number of other sugars. We suggest that each of the proposed pentose phosphate pathway gene products carry out a function(s) not directly related to the operation of the pentose phosphate cycle. These genes may have become nonfunctional or may function in ribose-5-phosphate biosynthesis under certain physiological conditions. Another alternative is that they are used for the biosynthesis of presently unknown sugars involved in the production of S-layer glycoproteins (29) or flagellum glycons (17).

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