Purification and Characterization of Two Extremely Thermostable Enzymes, Phosphate Acetyltransferase and Acetate Kinase, from the Hyperthermophilic Eubacterium Thermotoga maritima

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Phosphate acetyltransferase (PTA) and acetate kinase (AK) of the hyperthermophilic eubacterium Thermotoga maritima have been purified 1,500- and 250-fold, respectively, to apparent homogeneity. PTA had an apparent molecular mass of 170 kDa and was composed of one subunit with a molecular mass of 34 kDa, suggesting a homotetramer (α4) structure. The N-terminal amino acid sequence showed significant identity to that of phosphate butyryltransferases from Clostridium acetobutylicum rather than to those of known phosphate acetyltransferases. The kinetic constants of the reversible enzyme reaction (acetyl-CoA + Pₐ → acetyl phosphate + CoA) were determined at the pH optimum of pH 6.5. The apparent Kₘ values for acetyl-CoA, Pₐ, acetyl phosphate, and coenzyme A (CoA) were 23, 110, 24, and 30 μM, respectively; the apparent Vₘₐₓ values (at 55°C) were 260 U/mg (acetate phosphate formation) and 570 U/mg (acetyl-CoA formation). In addition to acetyl-CoA (100%), the enzyme accepted propionyl-CoA (60%) and butyryl-CoA (30%). The enzyme had a temperature optimum at 90°C and was not inactivated by heat upon incubation at 80°C for more than 2 h. AK had an apparent molecular mass of 90 kDa and consisted of one 44-kDa subunit, indicating a homodimer (α₂) structure. The N-terminal amino acid sequence showed significant similarity to those of all known acetate kinases from eubacteria as well that of the archaeon Methanosarcina thermophila. The kinetic constants of the reversible enzyme reaction (acetyl phosphate + ADP ⇌ acetate + ATP) were determined at the pH optimum of pH 7.0. The apparent Kₘ values for acetyl phosphate, ADP, acetate, and ATP were 0.44, 3.4, 40, and 0.7 mM, respectively; the apparent Vₘₐₓ values (at 50°C) were 2,600 U/mg (acetyl phosphate formation) and 1,800 U/mg (acetyl phosphate formation). AK phosphorylated propionate (54%) in addition to acetate (100%) and used GTP (100%), ITP (163%), UTP (56%), and CTP (21%) as phosphoryl donors in addition to ATP (100%). Divalent cations were required for activity, with Mn²⁺ and Mg²⁺ being most effective. The enzyme had a temperature optimum at 90°C and was stabilized against heat inactivation by salts. In the presence of (NH₄)₂SO₄ (1 M), which was most effective, the enzyme did not lose activity upon incubation at 100°C for 3 h. The temperature optimum at 90°C and the high thermostability of both PTA and AK are in accordance with their physiological function under hyperthermophilic conditions.

Acetate is an important end product of energy-yielding fermentation processes of many anaerobic and facultative prokaryotes. Generally acetate is formed from acetyl coenzyme A (acetyl-CoA), a central intermediate of metabolism. The mechanism of conversion of acetyl-CoA to acetate in prokaryotes, which is coupled with ATP formation, has recently been shown to be dependent on the phylogenetic domain to which the organisms belong (33, 34). (i) In all eubacteria analyzed, acetyl-CoA is converted to acetate by the “classical” mechanism involving two enzymes, phosphate acetyltransferase (PTA) (EC 2.3.1.8) and acetate kinase (AK) (EC 2.7.2.1). ATP is formed in the acetate kinase reaction by the mechanism of substrate-level phosphorylation.

Acetyl-CoA + Pₐ ⇌ acetyl phosphate + CoA (PTA)

Acetyl phosphate + ADP ⇌ acetate + ATP (AK)

(ii) In all acetate forming archaea studied so far, including anaerobic hyperthermophiles and aerobic mesophilic halophiles, the conversion of acetyl-CoA to acetate and the formation of ATP from ADP and phosphate is catalyzed by only one enzyme, an acetyl-CoA synthetase (ADP forming) (33, 34).

Acetyl-CoA + ADP + Pₐ ⇌ acetate + ATP + CoA

This unusual synthetase, which was first discovered in the anaerobic eukaryote Entamoeba histolytica (23, 30), is part of a novel mechanism of acetate formation and energy conservation in prokaryotes. Acetate also serves as substrate of catabolism and anabolism in several aerobic and anaerobic prokaryotes. The activation of acetate to acetyl-CoA, which is the first step prior to its utilization in metabolism, is catalyzed either by a single enzyme, an AMP-forming acetyl-CoA synthetase (EC 6.2.1.1) (acetyl + CoA + ATP ⇌ acetyl-CoA + AMP + Pₐ) or by the AK-PTA couple operating in the reverse direction as described above (12, 33, 36, 40). Besides their function in acetate metabolism, PTA and AK play a role, via acetyl phosphate, in various other processes. For example, in Escherichia coli, acetate phosphate functions as the phosphoryl donor of response regulator proteins of two-component systems, and a function as a global regulatory signal has therefore been proposed (22, 44).

To date, acetate kinases and phosphate acetyltransferases have been purified from various bacteria and from the ar-
MgCl₂. The 100,000 g for 60 min. The resulting supernatant contained supplemented with 2 mM MgCl₂. The 100,000 g supernatant, containing starch (5 g/liter) and yeast extract (5 g/liter) as T. maritima eubacterium (34, 35), which is the mechanism of acetate formation typical of bacteria (see above).

In this communication we report on the purification and characterization of AK and PTA from the hyperthermophilic eubacterium Thermotoga maritima.

MATERIALS AND METHODS
Source of materials. All fast protein liquid chromatography materials and columns were from Pharmacia (Freiburg, Germany). CoA and ATP were from Biomol (Hamburg, Germany). All other enzymes and coenzymes were from Boehringer (Mannheim, Germany). Unless otherwise stated, other chemicals were reagent grade and were obtained from Merck (Darmstadt, Germany). T. maritima Stamm MSB 8 (DSM 3109) was grown in a 100-liter Biostat fermenter on a medium containing starch (5 g/liter) and yeast extract (5 g/liter) as carbon and energy sources.

Purification of PTA. Since the enzyme was not sensitive to oxygen, it was purified under aerobic conditions (at 15°C). Wet cells (30 g) were suspended in 190 ml of 50 mM Tris-HCl (pH 8.0)–5 mM MgCl₂. DNase I was added, and the cells were stirred for 10 min at room temperature. The cells were disrupted by sonication for 2 min with a Branson Sonifier in pulse mode (50% pulsing) with a microtip and output control of 3. Cell debris and uncropped cells were removed by centrifugation for 10 min at 48,000 × g and 4°C. The supernatant (184 ml, 4.3 g protein/ml) was centrifuged at 16,000 × g and 4°C, the resulting supernatant contained 90% of the PTA activity. The buffer used for all chromatographic steps was 20 mM Tris-HCl (pH 8.0)–2 mM MgCl₂–0.15 M NaCl. Protein was eluted at a flow rate of 2.5 ml/min with a linear gradient from 0 to 0.5 M NaCl in buffer (400 ml). The fractions containing the highest PTA activity (43 ml, 0.17 to 0.22 M NaCl) were pooled, diluted fivefold with buffer, and applied to a Q-Sepharose HiLoad 16/10 column. Protein was eluted at a flow rate of 2.5 ml/min with a linear gradient from 0 to 0.5 M NaCl in buffer (400 ml). The fractions containing the highest AK activity (20 ml, 0.21 to 0.25 M NaCl) were pooled and adjusted to a final concentration of 1 M (NH₄)₂SO₄ by adding 20 ml of buffer containing 2 M (NH₄)₂SO₄ and were subsequently applied to a phenyl-Sepharose HiLoad 26/10 column equilibrated with buffer containing 1 M (NH₄)₂SO₄. Protein was desorbed at a flow rate of 8 ml/min with a decreasing gradient from 1 to 0 M (NH₄)₂SO₄ in buffer (300 ml). The highest AK activity eluted at 0.53 to 0.47 M (NH₄)₂SO₄ (38 ml). The eluate was concentrated to 0.7 ml by ultrafiltration with Centricon 30 microconcentrator (Amicon) (cutoff of 30 kDa) and then applied to a Diaflo UM2000 HiLoad 26/60 column equilibrated with buffer containing 0.15 M NaCl. Protein was eluted at a flow rate of 1 ml/min, and the AK activity was recovered in the fractions between 180 and 195 ml. The fractions were pooled (15 ml), diluted fourfold with buffer, and applied to a Mono Q column (1 by 10 cm). Protein was eluted at a flow rate of 2 ml/min with a linear gradient from 0 to 0.25 M NaCl in buffer (160 ml). The highest AK activity eluted at 0.2 to 0.22 M NaCl (8.8 ml).

PTA activity. PTA activity (which catalyzes the reaction acetyl-CoA + Pₐ = acetate + ATP) was measured at 55°C under aerobic conditions by using two different assays. In the first assay, the phosphate-dependent release of CoA from acetyl-CoA was monitored with Ellman’s thiol reagent, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) (16), by measuring the formation of the thiophosphate anion at 412 nm (ε₂₀ = 13.5 mM⁻¹ cm⁻¹). The assay mixture (1 ml) contained 100 mM Tris-HCl (pH 7.2), 5 mM MgCl₂, 5 mM KH₂PO₄, 0.1 mM DTNB, and 0.1 mM acetyl-CoA. This assay was used (i) to routinely monitor AK activity during the purification procedure, (ii) to determine the temperature values for acetyl-CoA and phosphate as well as the temperature and pH optima of the enzyme, (iii) to test the thermostability of the enzyme between 80 and 100°C, and (iv) to determine the specificity of the enzyme for other CoA esters of known and unknown structure. In the second assay, acetyl phosphate and CoA was monitored at 233 nm (ε₂₀ = 4.44 mM⁻¹ cm⁻¹). The assay mixture (1 ml) contained 100 mM Tris-HCl (pH 7.2), 2 mM acetyl phosphate, and 0.15 mM CoA. The assay was used to determine the apparent Km values for acetyl phosphate and ATP.

AK activity. AK activity (which catalyzes the reaction acetyl-P + ADP = acetate + ATP) was measured under aerobic conditions by using three different assay systems. In the first assay, the acetate-dependent ADP formation from ATP was monitored at 55°C by coupling the reaction with the oxidation of NADH via pyruvate kinase and lactate dehydrogenase (33). This assay was used (i) to routinely monitor acetate kinase activity during the purification procedure, (ii) to determine apparent Km values for acetate and ATP, and (iii) to test the thermostability of the enzyme between 80 and 100°C. In the second assay, the formation of ATP-dependent acetyl phosphate formation from acetate was assayed at 40 to 110°C by monitoring the formation of acetyl hydroxamate from acetyl phosphate and hydroxylamine at 540 nm (ε₂₀ = 0.46 mM⁻¹ cm⁻¹) (11). This assay was used to determine (i) the specificity of the enzyme for organic acids, nucleotides, and divalent cations, (ii) the apparent Km value for Mg²⁺, and (iii) the temperature and pH optima of the enzyme. In the third assay, the formation of ATP from acetyl phosphate and ADP was monitored at 50°C by coupling the reaction with the reduction of NADPH via hexokinase and glucose-6-phosphate dehydrogenase (33). This assay was used to determine apparent Km values of acetyl phosphate and ADP.

RESULTS
Purification and properties of PTA. PTA activity in cell extracts of T. maritima was about 0.13 U/mg (55°C). Almost all activity was retained in the 100,000 × g supernatant, indicating that the enzyme is not an integral membrane protein. The subsequent purification steps involved chromatography on DEAE-Sepharose, Q-Sepharose, phenyl-Sepharose, Superdex 200, and Mono Q. During gel filtration on Superdex 200, significant amounts (>70%) of the enzyme were lost for unknown reasons. After chromatography on Mono Q, the enzyme was apparently homogeneous, since only one band was detected both on denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1) and on native PAGE (results not shown). At this stage, the enzyme was purified about 1,500-fold (225 U/mg at 55°C) with a yield of about 2%. Thus, PTA represents about 0.07% of the cellular protein.
of Thermotoga. The purified enzyme (10 μg/ml) could be stored without significant loss of activity for several weeks at 220°C in buffer (20 mM Tris-HCl [pH 8.0], 2 mM MgCl₂, 0.32 M NaCl) supplemented with glycerol (10%, vol/vol).

(i) Molecular and catalytic properties. The apparent molecular mass of native PTA was determined to be about 170 kDa by gel filtration on Superdex 200. SDS-PAGE revealed only one subunit, with an apparent molecular mass of 34 kDa, suggesting that the native enzyme has a homotetrameric (α₄) structure. PTA was colorless and exhibited a UV-visible spectrum similar to that of bovine serum albumin, indicating the absence of a chromophoric prosthetic group. The N-terminal amino acid sequence of the 34-kDa subunit (MFLEKLVEMAYKGKKLAVAAANDHIEAVYRAWRERV) showed high homology (39% identity and 49% similarity) to phosphate butyryltransferases from Clostridium acetobutylicum ATCC 824 (43) and NCIMB 8052 (27) rather than to PTAs from other bacteria and the archaeon M. thermophila. The kinetic constants of purified PTA were determined for both directions of the reaction (acetyl-CoA + P → acetyl phosphate + CoA). The apparent (Kₘ) values for acetyl-CoA, P, acetyl phosphate, and CoA, obtained from linear Lineweaver-Burk plots, were 23, 110, 235, and 30 μM, respectively; the apparent Vₘ values (at 55°C) were 260 U/mg (acetyl phosphate formation) and 570 U/mg (acetyl-CoA formation). The pH optimum for enzyme activity was at pH 6.5; about 80 and 60% of the activity were found at pH 6.0 and 8.0, respectively. In addition to acetyl-CoA (100%), PTA accepted propionyl-CoA (60%) and butyryl-CoA (33%) as substrates. KCl (up to 400 mM) and NaCl (up to 100 mM) did not affect PTA activity, and (NH₄)₂SO₄ (300 mM) inhibited PTA activity by 65%. Both NH₄Cl (200 mM), and MgCl₂ (50 mM) slightly increased PTA activity to about 140%. KH₂PO₄ at 20 mM, which inhibits M. thermophila PTA, did not affect T. maritima PTA.

(ii) Temperature optimum and stability. The temperature dependence of PTA is shown in Fig. 2. The enzyme showed little activity at 40°C, and its activity increased rapidly above 55°C. The temperature optimum was at 90°C. From the linear part of the Arrhenius plot between 40 and 100°C, an activation energy of 70.3 kJ/mol was calculated. The temperature stability of the purified enzyme was tested between 80 and 100°C in 20 mM Tris-HCl (pH 8.0)–320 mM NaCl. At 80°C the enzyme did not lose activity after incubation for 2 h; 30% and 60% of the activity were lost after incubation for 2 h at 90 and 100°C, respectively. Various salts [NaH₂PO₄, KCl, NaCl, NH₄Cl, (NH₄)₂SO₄, KH₂PO₄] at 1 M did not significantly stabilize PTA against heat inactivation.

Purification and properties of AK. AK activity in cell extracts (5.7 U/mg at 55°C), which was not sensitive to oxygen, was purified under aerobic conditions. The purification steps used were the same as described above for the purification of PTA. After the last chromatographic step on Mono Q, the enzyme appeared homogeneous; only one protein band was de-
In this communication we reported the purification and properties of PTA and AK from the hyperthermophilic eubacterium *T. maritima*. This is the first report on the characterization of these “classical” acetate-forming enzymes from a hyperthermophilic ancestral organism.

PTA of *T. maritima* had a native molecular mass of about 170 kDa and was composed of a single subunit of about 35 kDa, suggesting a homotrameric structure. PTA had been isolated from various eubacteria and the archaeon *M. thermophila*; their molecular properties and kinetic constants are given in Table 1. All PTAs that have been analyzed for this property, consist of a single subunit with relative molecular masses ranging from 20 kDa (*C. thermoaceticum*) to 80 kDa (*E. coli*). Comparison with molecular masses of the native enzymes indicate monomeric, dimeric, and tetrameric structures. Like the PTA from *Clostridium thermoaceticum*, the *Thermotoga* enzyme is apparently homotrameric, but it has twice the molecular mass of subunits and native enzyme.

PTA from *Thermotoga* exhibits the lowest apparent $K_m$ values of all substrates (Table 1) and one of the highest $V_{max}$ values (approximately 1,000 to 2,000 U/mg at 90°C, taking into account the temperature dependence of enzyme activity). Like

FIG. 4. Effect of various salts on the thermostability of AK from *T. maritima* at 100°C. The 0.1-ml incubation mixtures contained 20 mM Tris-HCl (pH 8.0), 150 mM NaCl, and 0.36 μg of enzyme. Salts (1 M) were included as indicated. At the times indicated, the remaining enzyme activity was measured at 55°C in the direction of acetyl phosphate formation (pyruvate kinase/lactate dehydrogenase assay).
the PTA from other organisms (e.g., from Bacillus subtilis, Clostridium kluyveri, and Rhodopseudomonas palustris), the Thermotoga enzyme used propionyl-CoA (60%) and butyryl-CoA (30%) as substrates in addition to acetyl-CoA (100%). In contrast to PTAs from bacteria and from the archaeon M. thermophila, which are stimulated by KCl and inhibited by NaCl, these salts did not affect PTA from Thermotoga (for a comparison of specific activities and of metal effects of various PTAs, see reference 21).

The N-terminal amino acid sequence of Thermotoga PTA shows a higher homology to the corresponding sequences of phosphate butyryltransferases from Clostridium acetobutylicum. However, alignments of complete amino acid sequences of PTAs from Escherichia coli, Clostridium acetobutylicum, Bacillus subtilis, Paracoccus denitrificans, Methanosarcina thermophila, Mycoplasma genitalium, and Mycoplasma capricolum as deduced from available gene (pta) sequences in databases (5, 47) showed that they had a high overall homology to each other, ranging from 40 to 60% identity and 60 to 70% similarity. A phylogenetic tree of sequenced PTAs is given by Zhu et al. (47) and Rasche et al. (29). Since all amino acid sequences exhibited a rather poor N-terminal homology (5), only the comparison of the overall amino acid sequence of Thermotoga PTA will give conclusive information about homology to other PTAs. This will have to await the completion of the entire sequence of the PTA gene of thermophile. Sequencing of the complete T. maritima genome is in progress.

PTA from T. maritima showed the highest temperature optimum (about 90°C) and the highest thermostability of all PTAs analyzed. The enzyme did not lose activity upon incubation for 2 h at 80°C and lost only 30% of its activity upon incubation at 90°C (2 h). For comparison, PTA from the moderate thermophile M. thermophila had a temperature optimum at about 40°C and was completely inactivated after incubation for 5 min at 80°C (21). PTA from Clostridium thermoaceticum showed a temperature optimum of 75°C (11).

AK of T. maritima had a native molecular mass of 90 kDa and was composed of a single subunit of 44 kDa, indicating a homodimeric structure. As shown in Table 2, a homodimeric structure is typical of most AKs from eubacteria and the archaeon M. thermophila. Exceptions are the AKs of Clostridium thermoaceticum and of Bacillus stea"erothermophilus, which have been reported to be monomeric and homotetrameric enzymes, respectively. Furthermore, comparison of the N-terminal amino acid sequence of Thermotoga AK (MRYVLIN S GSSS) revealed a high degree of homology to AK from eubacteria and the archaeon M. thermophila, with the underlined amino acids being almost completely conserved (>80% identity within the first 15 N-terminal amino acids). Alignments of N-terminal and complete amino acid sequences of AKs, including those of, e.g.,

TABLE 1. Molecular and kinetic properties of purified PTA from eubacteria and M. thermophila

<table>
<thead>
<tr>
<th>Organism</th>
<th>Molecular massa (kDa)</th>
<th>Native enzyme</th>
<th>Subunit</th>
<th>Apparent $K_{\text{m}}^b$ (mM)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotoga maritima</td>
<td>170 ($\alpha_2$)</td>
<td>34</td>
<td>0.023</td>
<td>0.024</td>
<td>185</td>
</tr>
<tr>
<td>Methanosarcina thermophila</td>
<td>52 ($\alpha$)</td>
<td>43</td>
<td>17</td>
<td>0.12</td>
<td>21</td>
</tr>
<tr>
<td>Clostridium thermoacetum</td>
<td>88 ($\alpha_4$)</td>
<td>20</td>
<td>0.096</td>
<td>0.09</td>
<td>31</td>
</tr>
<tr>
<td>Clostridium acetici</td>
<td>63–75 (a)</td>
<td>—</td>
<td>0.12</td>
<td>0.12</td>
<td>11</td>
</tr>
<tr>
<td>Clostridium kluyveri</td>
<td>38–41 (a)</td>
<td>—</td>
<td>0.12</td>
<td>0.12</td>
<td>31</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>160–250 ($\alpha_2$)</td>
<td>81</td>
<td>0.12</td>
<td>0.12</td>
<td>28</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>68</td>
<td>—</td>
<td>0.12</td>
<td>0.12</td>
<td>37, 46</td>
</tr>
<tr>
<td>Lactobacillus fermenti</td>
<td>55 (a)</td>
<td>52.5</td>
<td>0.06</td>
<td>0.06</td>
<td>42</td>
</tr>
<tr>
<td>Rhodopseudomonas palustris</td>
<td>75–85 ($\alpha_2$)</td>
<td>32–40</td>
<td>0.06</td>
<td>0.06</td>
<td>45</td>
</tr>
</tbody>
</table>

a Molecular masses of native enzymes were determined by gel filtration, and those of subunits were determined by SDS-PAGE. The proposed subunit composition of the native enzyme is given in parentheses.

b Ac, acetyl; P, phosphate; —, not determined.

d Acetyl; P, phosphate; —, not determined.

Values given in Table 2 are ref. 80% identity within the first 15 N-terminal amino acids. Alignments of N-terminal and complete amino acid sequences of AKs, including those of, e.g.,

TABLE 2. Molecular and kinetic properties of purified AK from eubacteria and M. thermophila

<table>
<thead>
<tr>
<th>Organism</th>
<th>Molecular massa (kDa)</th>
<th>Native enzyme</th>
<th>Subunit</th>
<th>Acetate</th>
<th>ATP</th>
<th>Ac-P</th>
<th>ADP</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermotoga maritima</td>
<td>90 ($\alpha_2$)</td>
<td>44</td>
<td>40</td>
<td>0.7</td>
<td>0.44</td>
<td>3</td>
<td>This work</td>
<td></td>
</tr>
<tr>
<td>Methanosarcina thermophila</td>
<td>94 ($\alpha_2$)</td>
<td>53</td>
<td>22</td>
<td>2.8</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clostridium thermoacetum</td>
<td>60 ($\alpha$)</td>
<td>135</td>
<td>1.6</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Bacillus stea&quot;erothermophilus</td>
<td>160 ($\alpha_4$)</td>
<td>120</td>
<td>7</td>
<td>0.07</td>
<td>0.16</td>
<td>0.5</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>70 ($\alpha_2$)</td>
<td>40</td>
<td>7</td>
<td>0.07</td>
<td>0.16</td>
<td>0.5</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>70 ($\alpha_2$)</td>
<td>40</td>
<td>7</td>
<td>0.07</td>
<td>0.16</td>
<td>0.5</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Rhodopseudomonas palustris</td>
<td>47 ($\alpha_2$)</td>
<td>45</td>
<td>40</td>
<td>1.1</td>
<td>0.0026</td>
<td>0.087</td>
<td>41</td>
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</tr>
<tr>
<td>Veillonella alcalescens</td>
<td>88 ($\alpha_2$)</td>
<td>42</td>
<td>170</td>
<td>10$^d$</td>
<td>5$^c$</td>
<td>1.3$^c$</td>
<td>4, 25</td>
<td></td>
</tr>
<tr>
<td>Clostridium acetobutylicum</td>
<td>78 ($\alpha_2$)</td>
<td>42</td>
<td>160</td>
<td>2.5</td>
<td>&lt;1</td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Acholeplasma laidlawii</td>
<td>120–130 ($\alpha_2$)</td>
<td>51</td>
<td>38.5$^{d}$</td>
<td>0.3$^{d}$</td>
<td>0.1$^{d}$</td>
<td>0.24$^{d}$</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

a Molecular masses of native enzymes were determined by gel filtration, and those of subunits were determined by SDS-PAGE. The proposed subunit composition of the native enzyme is given in parentheses.

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Bacillus subtilis, Escherichia coli, Mycoplasma capricolum, Mycoplasma genitalium, Clostridium acetobutylicum, Haemophilus influenzae, and M. thermophila, are given in references 5, 9, and 47 (for a phylogenetic tree of sequenced AKs, see reference 47).

The kinetic properties of Thermotoga AK were very similar to the enzymes of eubacteria and the archaean M. thermophila. As shown in Table 2, the apparent $K_m$ values for substrates vary somewhat but all AKs have high values for acetate independent of whether the enzymes catalyze the activation (as, e.g., in M. thermophila) or the production of acetate in the metabolism. The Thermotoga enzyme showed a very high specific activity up to about 6,000 U/mg at 90°C (2,600 U/mg at 50°C), taking into account the temperature dependence of the enzyme.

Like all known AKs, the Thermotoga enzyme requires divalent cations for activity; Mg$^{2+}$ and Mn$^{2+}$ are the most effective. The enzyme has an optimal Mg$^{2+}$/ATP ratio of 1:1, suggesting that Mg$^{2+}$ is required only to complex ATP rather than to have additional effects on enzyme function or stability. A 1:1 ratio has also been determined for the enzymes of Escherichia coli, Salmonella typhimurium, and M. thermophila (1, 13). For AK from C. acetobutylicum, an optimal Mg$^{2+}$/ATP ratio of 2 has been reported (10). Furthermore, the substrate specificities of AK with respect to the acids other than acetate and nucleotides other than ATP were similar to those of most AKs; for example, like the AK from M. thermophila (1), the Thermotoga enzyme phosphorylated propionate (54%) in addition to acetate phosphorylation.

AK showed a temperature optimum at 90°C, which is the highest value of all AKs analyzed. Furthermore, the enzyme exhibited an extreme thermostability, which was increased by the addition of salts. In the presence 1 M (NH$_4$)$_2$SO$_4$, the enzyme did not lose activity upon incubation for 180 min at 100°C. AK from the moderate thermophile M. thermophila was almost completely inactivated after a 15-min incubation at 75°C. An increase of thermostability by salts has also been demonstrated for other enzymes from hyperthermophiles, e.g., Pyrococcus furiosus, Methanopyrus kandleri, and Archaeoglobus fulgidus (17, 14; for reviews, see references 2 and 20).

In summary, both PTA and AK of the hyperthermophile T. maritima showed similar molecular and catalytic properties to those of their mesophilic and moderately thermostable counterparts. They differ, however, in their extremely high temperature optimum and their high thermostability, which is in accordance with the hyperthermophilic nature of Thermotoga. In this respect, both PTA and AK may serve as model enzymes for analyses of the reasons for the thermostability of proteins in general. Cloning of the genes encoding both AK and PTA from Thermotoga and their expression in E. coli is in progress with the aim of crystallizing the proteins. So far, crystallization of the AK from M. thermophila and a prediction of its folding have been reported (9).

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REFERENCES


