Carbamyl and Acetyl Phosphokinase Activities of Streptococcus faecalis and Escherichia coli*

Kareen J. I. Thorne† and Mary Ellen Jones‡

From the Graduate Department of Biochemistry, Brandeis University, Waltham 54, Massachusetts

(Received for publication, February 5, 1963)

Acetyl phosphate has been found to replace carbamyl phosphate as substrate for brain carbamyl phosphatase (1); for ornithine transcarbamylase from rat liver, frog liver, and bacteria (2, 3); for production of adenosine triphosphate with carbamyl phosphate synthetase from frog liver (3, 4); and for carbamyl phosphokinase from strain D10 group D streptococci and Escherichia coli (3, 5). The ratio of the activities of brain carbamyl phosphatase with the two substrates remained constant during three purification steps, but the $K_m$ for acetyl phosphate was $1.1 \times 10^{-2}$ M and for carbamyl phosphate was $6.4 \times 10^{-8}$ M and there was some difference between the pH optima of the two reactions (1). The activity of ornithine transcarbamylase from rat liver with the two substrates showed the same temperature stability (2). Carbamyl phosphokinase gave ATP with both substrates, the activities with the two substrates showing the same temperature sensitivity and the two substrates exhibiting competitive inhibition (4). These results suggest a close relation and possibly an identity between the enzymes using acetyl phosphate and those using carbamyl phosphate.

The present paper describes a comparative study of the carbamyl phosphokinase (ATP : carbamate phosphotransferase E.C. 2.7.2.2) and acetyl phosphokinase (ATP : acetate phosphotransferase E.C. 2.7.2.1) from Streptococcus faecalis R and from Escherichia coli W which attempted to discover whether the two enzymes are identical. A comparison is made between these enzymes from the two sources. In addition, studies on the carbamyl phosphokinase of an E. coli mutant, R 185-482, which is one of a number of E. coli mutants behaving nutritionally and genetically as though they were unable to synthesize carbamyl phosphate (6-10), are reported.

**Experimental Procedure**

**Bacterial Strains**

*Escherichia coli* W (ATCC 9637) and *Streptococcus faecalis* R (ATCC 8043) were obtained from the American Type Culture Collection. *E. coli* mutant R 185-482, requiring arginine and uracil for growth, was isolated by Dr. R. R. Roepke (6) and was a gift from Dr. A. B. Pardee.

*Publication No. 226 of the Graduate Department of Biochemistry, Brandeis University. This investigation was supported in part by research grants from the National Institutes of Health (C-3658) and the National Science Foundation (G-14717). †Present address, Nutritional Laboratory, Milton Road, Cambridge, England. ‡Scholar of the American Cancer Society.

**Growth Media and Conditions**

*E. coli*—Stock cultures were maintained on glucose-agar slopes enriched with 0.2% Difco yeast extract and 0.2% Sheffield N-Z Case. Two different liquid media were used for experimental work: "synthetic medium" containing 7 g of KH$_2$PO$_4$, 3 g of KH$_2$PO$_4$, 0.5 g of sodium citrate, 0.1 g of MgSO$_4$.7H$_2$O, 1 g of (NH$_4$)$_2$SO$_4$, and 5 g of glucose per liter (pH 7) (11) with the addition of all the amino acids except arginine and with guanosine and adenine and B vitamins as described by Novick and Maas (12); "rich medium" in which the amino acids, guanosine, adenine, and B vitamins were replaced by 0.2% Difco yeast extract and 0.2% Sheffield N-Z Case. Synthetic medium was used for repression studies in which a defined arginine- and uracil-free medium was required; rich medium was used for the preparation of bacterial extracts for enzyme studies. All liquid growth media were incubated at 37° with shaking. Bacteria were prepared by the following procedure: a loopful was transferred from the stock slope into 10 ml of rich medium and incubated for 7 hours; one loopful was transferred from this into 4 ml of the experimental medium and incubated overnight; these 4 ml were then added to 400 ml of the experimental medium which were incubated for 4 hours.

*S. faecalis*—The maintenance and growth of *S. faecalis* have been described previously (13). Arginine was present in all growth media unless otherwise stated. The final incubation of cells was for 7 hours.

**Preparation of Bacterial Extracts**

The cells were harvested by centrifuging at 10,000 $\times g$ for 8 minutes at 0°. They were washed once with 0.02 M sodium phosphate, pH 6.6, and resuspended in about 20 ml of buffer. They were ultrasonically disrupted in a 10-kc Raytheon sonic oscillator at maximal power for 10 minutes for *E. coli* and for 40 minutes for *S. faecalis*. The cell debris was removed by centrifugation in a Servall centrifuge at 15,000 $\times g$ for 15 minutes. The clear supernatant was analyzed directly or was stored frozen.

**Enzyme Assays**

Carbamyl Phosphokinase Activity—Carbamyl phosphokinase was assayed by incubation with ammonium carbonate, ATP, ornithine, and an excess of ornithine transcarbamylase. The carbamyl phosphate produced was measured as citrulline. The assay mixture contained 10 $\mu$moles of ATP, 140 $\mu$moles of ammonium carbonate, 10 $\mu$moles of MgCl$_2$, 100 $\mu$moles of Tris (pH...
8.5), 5 μmoles of L-ornithine, and at least 20 units of ornithine transcarbamylase purified from *S. faecalis* (13), in a total volume of 0.35 ml. To this were added bacterial extract and water to a volume of 0.5 ml and the mixture was incubated for 15 minutes at 37°. The reaction was terminated by the addition of 1 ml of 20% trichloroacetic acid. The precipitated protein was removed by centrifugation and 0.5 ml of the supernatant was assayed for citrulline by a modification of the Archibald method (14). One enzyme unit is defined as the amount of enzyme needed to synthesize 1 μmole of product in 1 minute.

**Assay of Acetyl Phosphokinase**—Acetyl phosphokinase was assayed by incubating with acetate and ATP in the presence of hydroxylamine and measuring the acetylhydroxamic acid formed with ferric chloride (15). The concentration of acetate in the assay medium was sufficient to give maximal acetyl phosphokinase activity (0.8 mM for *E. coli* and 0.12 mM for *S. faecalis*). One enzyme unit is the amount of enzyme needed to synthesize 1 μmole of product in 1 minute.

**Aspartate Transcarbamylase Activity**—Aspartate transcarbamylase was assayed by measuring the carbamyl aspartate produced from carbamyl phosphate and aspartic acid using the colorimetric method of Gerhart and Pardee (16). The extracts were incubated with 50 μmoles of Tris (pH 8.5), 5 μmoles of carbamyl phosphate, and 20 μmoles of L-aspartic acid in a total volume of 0.5 ml for 10 minutes at 37°. To avoid high blanks, the carbamyl phosphate used was precipitated from solution with an equal volume of cold 95% ethanol and redissolved in fresh solution just before use. One enzyme unit is defined as the amount of enzyme needed to synthesize 1 μmole of product in 1 minute.

**Protein Determination**

Protein concentration was measured by the Lowry modification of the Folin-Ciocalteu procedure (17).

**Column Chromatography**

**Cellulose Phosphate**—Cellulose phosphate (1 meq per g) from the Brown Company was washed repeatedly with 0.1 N NaOH until colorless and degassed. The NaOH was removed by washing with water. Before use the cellulose phosphate was stirred overnight with 0.05 M maleate, pH 5.4. It was then poured into a column (12 cm long, 1 cm in diameter) and equilibrated with 0.005 M maleate, pH 5.4. The bacterial preparations were dialyzed for 3 hours against 0.005 M maleate, pH 5.4, and a sample of about 0.5 ml was put on the column. It was eluted with 0.005 M maleate, pH 5.4, then with 0.05 M maleate, pH 5.8. Fractions of 2 ml of the weaker buffer and 6 ml of the stronger buffer were collected and analyzed for acetyl phosphokinase, carbamyl phosphokinase, and protein. All buffers contained 0.02% mercaptoethanol.

**DEAE-cellulose**—DEAE-cellulose was obtained from the Brown Company, washed with 0.1 N NaOH, and degassed. Columns (of 5-cm length) were prepared and equilibrated with 0.2 M Tris, pH 8. The bacterial preparations were dialyzed against 0.2 M Tris, pH 8, for 3 hours, put on the column, and eluted with a gradient of increasing molarity. Fractions of 5 ml were collected and analyzed for acetyl phosphokinase, carbamyl phosphokinase, and protein. All buffers contained 0.02% mercaptoethanol.

**RESULTS**

**Carbamyl Phosphokinase Adaptation in *S. faecalis***

When arginine is present in the growth medium of *S. faecalis*, the levels of the enzymes on the pathway of arginine breakdown are increased adaptively (18, 19). If acetyl phosphokinase and carbamyl phosphokinase were the same enzyme, the level of acetyl phosphokinase would increase to correspond with the increase in level of carbamyl phosphokinase. In Table I are shown the levels of carbamyl phosphokinase and acetyl phosphokinase after growth in the presence and absence of arginine for 5 hours and for 7 hours. The presence of arginine in the growth medium resulted in a large increase in the level of carbamyl phosphokinase over the level of acetyl phosphokinase which was evidenced by the fall in the ratio of acetyl phosphokinase to carbamyl phosphokinase. These results suggested that the two enzyme activities were associated with different proteins and attempts were made to separate them.

**Purification of Carbamyl Phosphokinase and Acetyl Phosphokinase from *S. faecalis***

**Acid Precipitation and Ammonium Sulfate Fractionation**—The extract from *S. faecalis* was dialyzed overnight against 0.02 M phosphate, pH 6.6 (containing 0.02% mercaptoethanol), and then treated with 0.20 volume of cold 5% streptomycin sulfate solution, and the precipitated nucleic acids were removed by centrifugation for 15 minutes at 15,000 × g. With 25 ml of the streptomycin-treated extract, the purification of the two enzymes by precipitation with acid and with ammonium sulfate was investigated. The solution was adjusted to pH 3.8 with 0.5 M acetic acid and the precipitate was discarded. Analysis of the material soluble at pH 3.8 showed the presence of 64% of the original carbamyl phosphokinase but only 9% of the original acetyl phosphokinase (Table II). The ratio of acetyl phosphokinase to carbamyl phosphokinase fell by a factor of 15. When one carried out an ammonium sulfate fractionation of the same crude extract as was used for acid precipitation, both acetyl phosphokinase and carbamyl phosphokinase were purified.

**TABLE I**

<table>
<thead>
<tr>
<th>Additions to growth medium</th>
<th>Carbamyl phosphokinase (units/mg protein)</th>
<th>Acetyl phosphokinase (units/mg protein)</th>
<th>Acetyl phosphokinase/Carbamyl phosphokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-hour incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.23</td>
<td>0.58</td>
<td>2.18</td>
</tr>
<tr>
<td>L-Arginine (0.05 M)</td>
<td>1.00</td>
<td>0.00</td>
<td>2.00</td>
</tr>
<tr>
<td>7-hour incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.17</td>
<td>0.97</td>
<td>2.18</td>
</tr>
<tr>
<td>L-Arginine (0.05 M)</td>
<td>1.66</td>
<td>0.61</td>
<td>2.75</td>
</tr>
</tbody>
</table>

Enzyme assay conditions are described in "Experimental Procedure."
Enzyme assay conditions are described in "Experimental Procedure"; details of fractionation are in "Results."

<table>
<thead>
<tr>
<th>Fractionation</th>
<th>Carbamyl phosphokinase</th>
<th>Acetyl phosphokinase</th>
<th>Carbamyl phosphokinase</th>
<th>Acetyl phosphokinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid precipitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>446</td>
<td>205</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>pH 3.8 soluble</td>
<td>285</td>
<td>19</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation</td>
<td>375</td>
<td>196</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>241</td>
<td>120</td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>60-80% precipitate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table II**

Purification of carbamyl phosphokinase from *S. faecalis*

Enzyme assay conditions are described in "Experimental Procedure"; details of fractionation are in "Results."

**FIG. 1.** Column chromatography of acetyl phosphokinase and carbamyl phosphokinase from *S. faecalis* on cellulose phosphate. Partially purified extract was put on the column in 0.005 M maleate, pH 5.4, and eluted with 0.005 M maleate, pH 5.4. A sample of 0.5 ml was put on the column and eluted with 0.005 M maleate, pH 5.4, followed by 0.05 M maleate, pH 5.8. As shown in Fig. 1, part of the acetyl phosphokinase (25%) was eluted with 0.005 M maleate, pH 5.4, and the remainder (68%) was eluted with 0.05 M maleate, pH 5.8. This second acetyl phosphokinase fraction had no carbamyl phosphokinase activity. Carbamyl phosphokinase was found only in the early fraction, being eluted with 0.005 M maleate, pH 5.4; its recovery was 08%. This early fraction, containing both carbamyl phosphokinase and acetyl phosphokinase, was put onto a second column in an attempt to separate the two activities. However, the acetyl phosphokinase remained associated with the carbamyl phosphokinase through the second column.

**Heat Inactivation**—The sensitivities of the carbamyl phosphokinase and acetyl phosphokinase to heat were compared. The two enzymes from *S. faecalis* were separated by acid precipitation; the acetyl phosphokinase fraction was the pH 3.8 precipitate whereas the carbamyl phosphokinase was the pH 3.8 supernatant; and the preparations were adjusted to pH 7 and put into a water bath at 50°. Samples were taken at measured time intervals for determination of enzyme activity. The acetyl phosphokinase activities of the acetyl phosphokinase fraction and of the carbamyl phosphokinase fraction were measured, as was the carbamyl phosphokinase activity. The results are shown in Fig. 2a. There was an initial delay in the inactivation which could be explained by a delay in the time taken for the whole solution to come to 50°. The inactivation curves of the acetyl phosphokinase and carbamyl phosphokinase activities of the carbamyl phosphokinase fraction were similar and differed from the inactivation curve of the acetyl phosphokinase fraction, particularly during the first 2 minutes.

**COMPARISON OF ENZYMES FROM S. FAEALIS AND E. COLI**

**Mechanism of Action of E. coli Carbamyl Phosphokinase**

Utilization of Carbamate—In order to show that the carbamyl phosphokinase of *E. coli* resembles that of *S. faecalis* in using carbamate, the carbamyl phosphokinase activity of the *E. coli* extract was measured in the presence of each of the two ammonium salts. Any solution of ammonium carbonate or carbamate is converted with time to an equilibrium mixture of ammonium carbonate-carbamate. Therefore, to determine which one is a substrate, it is necessary to prepare fresh solutions. These were made by mixing equal volumes of 2 M ammonium chloride with 1 M potassium carbonate to give 0.5 M ammonium carbonate and by freshly dissolving ammonium carbamate in water to a concentration of 0.5 M. The solutions were kept at 10°, as at this temperature the rate of conversion of the salts to the equilibrium mixture is slow, equilibrium being reached only after about 3 hours. Aliquots of these two solutions were taken at various intervals and incubated at 37° with a given volume of crude extract of *E. coli* and an assay mixture of ATP, magnesium chloride, Tris (pH 8.5), ornithine, and ornithine transcarbamylase for 2 minutes; the amount of carbamyl phosphate formed was measured as citrulline (Fig. 3). This short incubation at 37°
minimized further equilibration of the salts. The results show that initially the ammonium carbamate was a more active substrate for carbamyl phosphokinase than ammonium carbonate, but with increasing time the activity of the ammonium carbamate solution fell and that of the ammonium carbonate solution increased. This is in agreement with the concept of carbamate as the true substrate, the carbamyl phosphate formed being a measure of the amount of carbamate present in each of the two solutions. With increasing time, the percentage of carbamate present tends to approach the equilibrium level. The difference between the two solutions at equilibrium can be explained by the presence of KCl in the ammonium carbonate solution. KCl both influences the composition of the final equilibrium mixture and also inhibits carbamyl phosphokinase, giving a false low value for the carbamyl phosphokinase activity.

The enzyme from E. coli resembles all the other carbamyl phosphate-synthesizing enzymes in requiring magnesium. Since on dialysis of the enzyme from E. coli the activity is retained in the absence of added acetyl glutamate, it seems probable that acetyl glutamate is not a cofactor as it is for the ureotelic vertebrate enzyme, carbamyl phosphate synthetase (20). The extract from E. coli showed no carbamyl phosphokinase activity when ammonium carbonate was replaced by glutamine and potassium carbonate, thereby eliminating the possibility that this activity is related to the fungal enzyme occurring in Agaricus bisporus (21).

**Enzyme Fractionation**—As shown in Table II, the carbamyl phosphokinase from S. faecalis is soluble at pH 3.8 and precipitates between 60% and 80% saturated ammonium sulfate. The enzyme from E. coli was completely precipitated by pH 4.8 and when fractionated with ammonium sulfate precipitated between 55% and 65% saturation.

Column chromatography of the E. coli extract on cellulose phosphate did not result in the separation of the acetyl phosphokinase and carbamyl phosphokinase. About 30% of the two activities emerged from the column without retardation and the remaining 70% was not recovered, even when the column was eluted with M Tris, pH 8.5. Better recovery of the activity was achieved when the enzymes were chromatographed on DEAE-cellulose; however, the two activities were not resolved, both being eluted with 0.3 M Tris, pH 8.

**Heat Inactivation**—The heat inactivation curves shown in Fig. 2a indicate that S. faecalis contains two acetyl phosphokinase activities, one of which resembles carbamyl phosphokinase, whereas the other differs from it. For comparison, the heat inactivation curves of acetyl phosphokinase and carbamyl phosphokinase from E. coli are shown in Fig. 2b. Only small differences were found between the curves for the two enzymes from E. coli and both resembled the curve for S. faecalis carbamyl phosphokinase.

**Substrate Requirements**—As shown above, the carbamyl phosphokinase activities of S. faecalis and E. coli act by the same mechanism and utilize the same substrates. For a closer comparison of the two proteins' substrate requirements have been investigated in more detail. Extracts from S. faecalis were separated by acid precipitation into acetyl phosphokinase and carbamyl phosphokinase fractions and the substrate requirements of the acetyl phosphokinase fraction and also of the carbamyl phosphokinase and carbamyl phosphokinase activities of the carbamyl phosphokinase fraction were measured. In addition, the acetyl phosphokinase and carbamyl phosphokinase activities of E. coli were investigated. The Michaelis constants of these enzymes for carbamate, acetate, and ATP are shown in Table III. The carbamyl phosphokinase activity was measured at different ammonium carbonate concentrations and the amount...
Activity with ATP was defined as 100%. Incubation conditions and substrate concentrations, except as noted, were the same as given for enzyme assay in ‘Experimental Procedure.’

<table>
<thead>
<tr>
<th>Enzyme and Substrate</th>
<th>ATP</th>
<th>GTP</th>
<th>ITP</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamyl phosphokinase activity</td>
<td>100</td>
<td>143</td>
<td>43</td>
</tr>
<tr>
<td>Acetyl phosphokinase activity</td>
<td>100</td>
<td>88</td>
<td>08</td>
</tr>
<tr>
<td>S. faecalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamyl phosphokinase activity of carbamyl phosphokinase</td>
<td>100</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Acetyl phosphokinase activity of carbamyl phosphokinase</td>
<td>100</td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>Acetyl phosphokinase activity of acetyl phosphokinase</td>
<td>100</td>
<td>94</td>
<td>106</td>
</tr>
</tbody>
</table>

of carbamate present at 37° was calculated from the equation (22)

\[
\frac{[\text{NH}_2\text{HCO}_3^-]}{[\text{NH}_2\text{COO}^-]} = 0.33
\]

Both the acetyl phosphokinase and carbamyl phosphokinase fractions from S. faecalis had lower \( K_m \) values for acetate and carbamate, respectively, than the corresponding enzyme activities from E. coli, and in both species, the carbamyl phosphokinase used a lower level of substrate than the acetyl phosphokinase. The acetyl phosphokinase activity associated with carbamyl phosphokinase in S. faecalis had a very high \( K_m \) for acetate.\(^1\) There was little difference between the \( K_m \) values for ATP.

A comparison of the utilization of ATP, GTP, and ITP by the five enzyme activities is shown in Table IV. For this experiment the acetyl phosphokinase and carbamyl phosphokinase from E. coli were separated by chromatography on cellulose phosphate. The results are given as percentages of the activity with ATP. The two enzymes from E. coli had a similar response to ATP but differed in their reactions with GTP, which was a better substrate than ATP for the carbamyl phosphokinase but not for acetyl phosphokinase. It is of interest that AMP and GMP are competitive inhibitors of carbamyl phosphokinase, the \( K_i \) for GMP being 2.7 mM, whereas the \( K_i \) for AMP is 5.1 mM, a result which corresponds with GTP being a better substrate than ATP. The acetyl phosphokinase of S. faecalis used ATP, ITP, and GTP equally, but the carbamyl phosphokinase and acetyl phosphokinase activities of the carbamyl phosphokinase fraction had negligible activity with ITP and only small activity with GTP.\(^2\) The experiments with E. coli were done at 1.25 mM, or below the \( K_m \) for ATP; experiments with S. faecalis were done with 10 mM substrates or at maximal velocity. The E. coli system was therefore more sensitive than the S. faecalis system.

### Carbamyl Phosphokinase of E. coli Mutant R185-482

Mutant R185-482 requires both arginine and uracil for growth and arginine can be replaced by citrulline and uracil by carbamyl aspartic acid (7). However, the bacteria are not deficient in carbamyl phosphokinase activity. Several properties of the carbamyl phosphokinase activity of this mutant have been investigated in an attempt to explain its nutritional requirements.

#### Enzyme Repression

The control of enzyme levels by repression has been demonstrated in the inhibition of ornithine transcarbamylase synthesis by arginine in E. coli W (23) and in the inhibition of aspartate transcarbamylase synthesis by uracil in pyrimidine-requiring mutants of E. coli and in E. coli B under conditions of pyrimidine depletion (8, 24). It seemed possible that arginine and uracil might exert a similar repressive effect on carbamyl phosphokinase, since carbamyl phosphate is required for the biosynthesis of both these substances. E. coli W was grown for 4 hours in the synthetic medium alone and in the presence of arginine, uracil, and arginine and uracil together.

As R185-482 would not grow in the absence of arginine and uracil it was grown for 3½ hours in the presence of these two substances and then harvested, washed, and resuspended in the deficient medium for ½ hour. The levels of carbamyl phosphokinase, ornithine transcarbamylase, and aspartate transcarbamylase after growth in the presence of arginine and uracil are shown in Table V. In this experiment the carbamyl phosphokinase levels of E. coli W and R185-482 resembled one another; however, values as low as 0.06 unit per mg of protein have been obtained for the mutant. The carbamyl phosphokinase was not sensitive to repression by arginine or uracil in either species. When E. coli W was grown in the simple salt medium of Davis and Mingioli (11), the level of carbamyl phosphokinase was only 0.60 unit per mg of protein. The levels of ornithine transcarbamylase and aspartate transcarbamylase in the two species were similar and the ornithine transcarbamylase of both was repressed by arginine. However, only the aspartate transcarbamylase of the mutant was repressed by uracil, a result which agrees with the work of Yates and Pardee (8, 24), who demonstrated repression only under conditions of pyrimidine depletion.

#### End Product Inhibition of Carbamyl Phosphokinase Activity

A study of the wild-type enzyme indicated that neither arginine nor pyridine nucleotides were potent enough inhibitors to be of importance in vivo. The mononucleotide inhibition rather closely parallels the ability of the various trinucleotides to serve as substrates for carbamyl phosphokinase with purine nucleotides being more effective inhibitors than the pyrimidine nucleotides. Arginine inhibition not only becomes less pronounced as the ammonium carbonate concentration increases, but, at low carbonate concentrations (0.14 M), 50% inhibition requires between 0.15 and 0.2 mM arginine. A similar concentration of arginine yields a 50% inhibition of the acetyl phosphokinase activity of the same extracts.

\(^1\) Owing to the high levels of GTP used, this activity might be due to ATP contamination of the GTP.
TABLE V
Effect of arginine and uracil on synthesis of carbamyl phosphokinase, ornithine transcarbamylase, and aspartate transcarbamylase by E. coli W and mutant R185-482

<table>
<thead>
<tr>
<th>Additions to growth medium</th>
<th>Carbamyl phosphokinase</th>
<th>Ornithine transcarbamylase</th>
<th>Aspartate transcarbamylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.15</td>
<td>5.0</td>
<td>0.27</td>
</tr>
<tr>
<td>1-Arginine (4 mM)</td>
<td>0.13</td>
<td>0.03</td>
<td>0.15</td>
</tr>
<tr>
<td>Uracil (0.5 mM)</td>
<td>0.17</td>
<td>0.03</td>
<td>0.36</td>
</tr>
<tr>
<td>Arginine and uracil</td>
<td>0.10</td>
<td>0.2</td>
<td>0.12</td>
</tr>
<tr>
<td>R185-482†</td>
<td>0.15</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
<td>1-Arginine (1.2 mM)</td>
<td>0.15</td>
<td>0.2</td>
<td>0.36</td>
</tr>
<tr>
<td>Uracil (0.2 mM)</td>
<td>0.15</td>
<td>9.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Arginine and uracil</td>
<td>0.14</td>
<td>0.5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* Prepared by incubating a loopful taken from 7-hour growth in rich medium with 4 ml of synthetic medium and the specified addition overnight and then transferring the 4 ml of bacterial suspension to 400 ml of the same medium and incubating for a further 4 hours. Final concentration of bacteria, about 200 µg (dry weight) per ml of growth medium.

† Prepared by incubating a loopful taken from 7-hour growth in rich medium with 4 ml of synthetic medium containing both arginine and uracil overnight and then transferring the 4 ml of bacterial suspension to 400 ml of the same medium and incubating for a further 3½ hours, finally harvesting the bacteria, washing them, and resuspending in synthetic medium with the specific addition and incubation for ½ hour. Final concentration of bacteria, about 100 µg (dry weight) per ml of growth medium.

DISCUSSION

The increase in carbamyl phosphokinase level of S. faecalis on adaptation with arginine without a corresponding increase in the acetyl phosphokinase level suggested that the two activities were associated with different proteins. Chromatography on cellulose phosphate resulted in the separation of acetyl phosphokinase into two fractions, about one-quarter fractionating with the carbamyl phosphokinase and the remainder being completely separable from carbamyl phosphokinase. Similarly, on acid precipitation, only one-sixth of the acetyl phosphokinase remained with the carbamyl phosphokinase. Comparison of the heat sensitivity and nucleotide requirements of the enzymes showed that the acetyl phosphokinase of the carbamyl phosphokinase fraction resembled carbamyl phosphokinase but differed from the remainder of the acetyl phosphokinase. The evidence suggests that in S. faecalis carbamyl phosphokinase and acetyl phosphokinase are two separate enzymes, but that the carbamyl phosphokinase has a small affinity for acetate, so that the acetyl phosphokinase activity of this enzyme probably has little importance in vivo.

Comparison of the carbamyl phosphokinases from E. coli and S. faecalis showed that the two proteins are quite different. They behaved differently on ammonium sulfate precipitation, acid precipitation, and chromatography. In addition, they had different affinities for their substrates and showed different specificities for nucleotides.

Attempts were made to distinguish the acetyl phosphokinase of E. coli from the carbamyl phosphokinase. No systematic purification of the enzymes, such as has been described by Rose et al. (15), was undertaken, although it is possible that this technique might result in separation of the two activities. Instead, several individual procedures have been used in an attempt to show a divergence in behavior between the two enzymes. The two activities remained together during acetone precipitation, ammonium sulfate precipitation, and chromatography on cellulose phosphate and DEAE-cellulose. Only in their sensitivity to heat and in their nucleotide specificity was there any difference between the two activities and here the differences were too small for definite conclusions to be drawn. Since the function of carbamyl phosphokinase is concerned with the biosynthesis of protein and nucleic acid whereas the function of acetyl phosphokinase, under the present growth conditions, is probably to produce acetate from glucose via acetyl-CoA and acetyl phosphate (25), it is difficult to understand why the two activities should reside in the same enzyme.

Our investigations to explore enzymatically the genetic lesion in mutant R185-482, a prototype of a number of single gene mutants with the double nutritional requirement for arginine and uracil, have revealed that this organism contains the same level of carbamyl phosphokinase activity as E. coli W. In addition, the measured activity was not repressed or subject to feedback inhibition by arginine or pyrimidine nucleotides. On the other hand, the nutritional requirement of these single gene mutants strongly supports the concept that they lack carbamyl phosphokinase. Our data support the near identity of the measurable carbamyl phosphokinase activity with acetyl phosphokinase activity in E. coli W, whereas in S. faecalis these two activities are separable.

The enigma presented by mutants like R185-482 could be explained if (a) E. coli possessed a carbamyl phosphokinase separable from acetyl phosphokinase, so that the activity that we and other workers have measured represented mainly the acetyl phosphokinase which presumably can recognize carbamate, and (b) the "true" carbamyl phosphokinase of E. coli were so sensitive to end product repression that, like aspartate transcarbamylase (8, 24), it would be almost always repressed unless special conditions for derepression were utilized. In line with this reasoning, mutant R185-482 would lack the gene for this repressible enzyme and would merely appear to possess it because the acetyl phosphokinase could recognize carbamate. Although there are at present no data to support the existence of separable acetyl and carbamyl phosphokinases in E. coli (assumption a, above), there are data available to support the concept that carbamyl phosphokinase is repressed in wild-type and a variety of mutants of E. coli (assumption b, above). Beckwith et al. (26) have observed an increased carbamyl phosphokinase activity in E. coli under conditions which lead to derepression of the enzymes of the uridylic acid pathway. Gorini and Kalman (27) find an increase in carbamyl phosphokinase of E. coli U when aspartate transcarbamylase is derepressed. More particularly, these authors have isolated from E. coli U a mutant, U28, which requires uracil for optimal growth and has 8- to 10-fold the carbamyl phosphokinase activity of the parent when grown in the absence of uracil. This high enzyme activity can be repressed to the parental level by addition of uracil to the medium. It would seem, therefore, that the data of these
two laboratories support the idea that the carbamyl phosphokinase of E. coli is repressed by uracil and that this enzyme can be derepressed in wild type and mutants. The identification of this "true" carbamyl phosphokinase remains to be explored. If E. coli does contain separable carbamyl and acetyl phosphokinases, E. coli and S. faecalis would represent reverse examples of the overlap of these two activities. E. coli may have an acetyl phosphokinase able to handle both acetate and carbamate whereas in S. faecalis the acetyl phosphokinase cannot recognize carbamate but the carbamyl phosphokinase can recognize acetate.

SUMMARY

An investigation has been made of the carbamyl phosphokinase and acetyl phosphokinase of Escherichia coli and of Streptococcus faecalis. Two protein fractions with acetyl phosphokinase activity were found in S. faecalis, one of which was inseparable from carbamyl phosphokinase and resembled carbamyl phosphokinase from S. faecalis in catalyzing the synthesis of carbamyl phosphate from carbamate and adenosine triphosphate in the presence of magnesium. A comparison of the carbamyl phosphokinase from S. faecalis with the carbamyl phosphokinase from E. coli showed that the two proteins are quite different. The carbamyl phosphokinase of E. coli also had associated acetyl phosphokinase activity and all attempts to separate these two activities were unsuccessful. Escherichia coli W has been shown to resemble the carbamyl phosphokinase from S. faecalis in catalyzing the synthesis of carbamyl phosphate from carbamate and adenosine triphosphate. This acetyl phosphokinase activity differed from the bulk of the acetyl phosphokinase in having an unusually high K_m for acetate and it is suggested that it has little biological importance. Carbamyl phosphokinase from E. coli W has been shown to resemble the carbamyl phosphokinase from S. faecalis in catalyzing the synthesis of carbamyl phosphate from carbamate and adenosine triphosphate in the presence of magnesium. A comparison of the carbamyl phosphokinase from S. faecalis with the carbamyl phosphokinase from E. coli showed that the two proteins are quite different. The carbamyl phosphokinase of E. coli also had associated acetyl phosphokinase activity and all attempts to separate these two activities were unsuccessful. Escherichia coli W and mutant R185-482, requiring arginine and uracil, have the same level of carbamyl phosphokinase activity and all attempts to separate these two activities were unsuccessful. Escherichia coli W has been shown to resemble the "true" carbamyl phosphokinase in E. coli.

Acknowledgment—The authors are grateful to Mrs. Martha Bethall for the experiments on the inhibition by arginine of E. coli acetyl phosphokinase.

REFERENCES

Carbamyl and Acetyl Phosphokinase Activities of *Streptococcus faecalis* and *Escherichia coli*

Kareen J. I. Thorne and Mary Ellen Jones


Access the most updated version of this article at [http://www.jbc.org/content/238/9/2992.citation](http://www.jbc.org/content/238/9/2992.citation)

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 0 references, 0 of which can be accessed free at [http://www.jbc.org/content/238/9/2992.citation.full.html#ref-list-1](http://www.jbc.org/content/238/9/2992.citation.full.html#ref-list-1)