ACETYULATION OF AMINO ACIDS BY ENZYMES OF CLOSTRIDIUM KLUYVERI*

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Stadtman et al. (1) recently described a cyanide-induced acetylation of amino acids by enzymes derived from Clostridium kluyveri. The present paper gives a more detailed account of the properties and specificity of this enzyme system.

Materials and Methods

The experiments were done with dried cells of C. kluyveri, Lots Q and R, prepared by the method of Stadtman and Barker (2). Lot R contained 1.2 μM of volatile acids, consisting mostly of caproic acid with a little acetic and butyric acids, and 3.2 μM of non-protein amino nitrogen per 25 mg. of dried cells. The enzymes responsible for acetylation of amino acids are not readily inactivated by exposure to oxygen and therefore the suspensions were generally made by grinding the dried cells in a mortar with sufficient water or buffer to give a 10 per cent suspension. When cyanide was present, no other reducing agent was required.

Acetyl phosphate was used as the dilithium salt prepared by the method of Bentley (3) as modified by Lipmann and Tuttle (4), or by the method of Stadtman and Lipmann (5).

Acetylglycine was prepared by the method of Herbst and Shemin (6). The same method, appropriately modified, was used to prepare radioactive acetylglycine, labeled in the carboxyl group of the glycine moiety with C¹⁴. The specific activity of the product was approximately 0.5 μc. per mg. Synthetic ε-monoacetyl-L-lysine was prepared by the method of Neuberger and Sanger (7), and diacetyl-L-lysine by acetylation of lysine with excess acetic anhydride in the presence of barium hydroxide.

Separation and identification of acetylated amino acids were generally carried out by a modification of the one-dimensional chromatographic method of Lugg and Overell (8), with No. 1 Whatman filter paper. Samples were deproteinized and desalted by admixture with 9 volumes of 95
per cent ethanol. The supernatant was evaporated to dryness and the acetylated amino acids were extracted from the residue with a small volume of 50 per cent ethanol. A 0.01 to 0.02 ml. aliquot containing 0.2 to 1.5 μM of each N-acetyl derivative was applied to the paper and the chromatogram was developed with butanol-acetic acid-water or isoamyl alcohol-acetic acid-water.

Most of the acetylated amino acids are acidic substances that can be detected by spraying the chromatogram with a weakly alkaline 0.05 per cent solution of brom cresol green. The minimal amount of acetylated amino acid that can be detected is 0.1 μM. With more than 1.5 μM, the spot becomes diffuse and the \( R_F \) value is less reproducible. \( R_F \) values for several acetylated amino acids are given in Table I. With the pure compounds the values are reproducible to ±0.02. In the presence of salts the \( R_F \) values are more variable and streaking of the spots occurs. Alcohol-soluble buffers such as tris(hydroxymethyl)aminomethane (Tris) should be avoided for this reason.

The two monoacetyl derivatives of lysine, which are neutral compounds, were detected by spraying with a solution of ninhydrin.

Two-dimensional paper chromatograms of mixtures containing free and acetylated amino acids were made by the method of Benson et al. (9). The solvents were butanol-propionic acid-water and phenol-water. For making radioautographs of paper chromatograms, the specific activity of the substrate was chosen to give 0.001 to 0.1 μc. per spot.

Amino nitrogen was determined by the method of Moore and Stein (10) with the following modifications. The sample, usually 0.1 to 0.2 ml., was diluted to 5 ml. with 70 per cent ethanol and the precipitated protein and salt were removed by centrifugation. To remove cyanide, which
interferes even in high dilution, a suitable aliquot (0.1 to 0.2 ml.) of the neutral sample (pH 7.5 to 8.3) was placed in a test-tube and evaporated to dryness on a steam bath. The residue was dissolved in 0.1 ml. of water and used for the determination. In a few experiments, glycine was determined by the specific colorimetric method of Alexander et al. (11).

Acetyl phosphate was estimated by the method of Lipmann and Tuttle (12). In the presence of cyanide a high blank reading is obtained as a result of the formation of Prussian blue by reaction with iron salts in the system. Suitable blank corrections were made to compensate for this effect. Inorganic phosphate was estimated by the Fiske-Subbarow method.

Volatile acids were estimated by titration following steam distillation. Samples were deproteinized by admixture with 2 volumes of 0.15 \( \text{N} \) \( \text{H}_2\text{SO}_4 \) and centrifugation. Cyanide, which interferes with the titration (phenol red end-point), was removed by adding an excess of a 5 per cent suspension of silver sulfate at the beginning of the distillation.

Acetylglycine was determined by a modification of the acetyl phosphate method of Lipmann and Tuttle (12). These investigators found that acetamide reacts quantitatively with hydroxylamine when the reaction mixture is boiled for 15 minutes. We have applied this reaction for the estimation of acetylglycine after the removal of acetyl phosphate and cyanide. To remove cyanide, a 0.1 to 0.2 ml. sample containing 2 to 10 \( \mu \text{M} \) of acetylglycine is added to a small tube containing twice the volume of 10 per cent (0.6 M) silver nitrate. The mixture is made up to 1.5 ml., shaken, and centrifuged. 1 ml. of the supernatant solution is heated in a boiling water bath for 5 minutes to decompose acetyl phosphate. 1 ml. of 3.5 \( \text{N} \) \( \text{NaOH} \) and 1.5 ml. of 40 per cent \( \text{NH}_2\text{OH} \cdot \text{HCl} \) solution are added to the cooled mixture and the tube is again placed in a boiling water bath for 20 minutes. To the cooled solution is added 1 ml. of 3 \( \text{N} \) \( \text{HCl} \), 1 ml. of 12 per cent trichloroacetic acid, and 1 ml. of 5 per cent \( \text{FeCl}_3 \cdot 6\text{H}_2\text{O} \) in 0.1 \( \text{N} \) \( \text{HCl} \). After 5 minutes at room temperature the sample is centrifuged and read in the Evelyn colorimeter with a 540 m\( \mu \) filter and a 18.2 mm. (outside diameter) tube. For a standard, 0.2 ml. of a 0.01 M acetylglycine solution is used. A sample containing acetyl phosphate and cyanide but no acetylglycine and one containing only cyanide should be included in the assay. These samples, which should give the same reading in the colorimeter, are used to determine the zero reading. An almost straight line is obtained when optical density \((2 - \log G)\) is plotted against the amount of acetylglycine up to 5 \( \mu \text{M} \), which gives a density reading of 0.89.

Acetylglycine was hydrolyzed to glycine and acetate by treatment with 1 \( \text{N} \) \( \text{NaOH} \) for 1.5 hours in a boiling water bath. The compound is not appreciably hydrolyzed by acid under the conditions (pH 1 to 2, 100°, 10 minutes) used for the estimation of volatile acids.
EXPERIMENTAL

Non-Enzymatic Acetylation—When acetyl phosphate and glycine are incubated in the absence of enzyme and cyanide, acetylglycine is formed. This non-enzymatic acetylation of amino acids has recently been reported independently by Koshland (13). The rate of non-enzymatic acetylation is relatively low. When 50 μM of acetyl phosphate and 50 μM of glycine are incubated in 1 ml. of 0.1 M phosphate buffer, pH 8, from 1.0 to 1.5 μM of acetylglycine are formed per hour at 26°. Propionyl phosphate reacts similarly to give propionylglycine. Since the rates of enzymatic acetylation observed in our experiments were generally 10 to 20 times more rapid than the non-enzymatic reaction, the latter generally could be disregarded. However, when samples containing acetyl phosphate and glycine are not analyzed immediately, precautions must be taken to avoid further non-enzymatic acetylation. This can be done by cooling the samples to -18°, or by heating for 10 minutes at 95° after acidification to pH 2 to destroy residual acetyl phosphate. At room temperature acetyl phosphate is relatively stable (14) and persists for several hours in a sample adjusted to pH 2.5.

Stoichiometry of Enzymatic Glycine Acetylation—Acetylglycine is the main product of the reaction between acetyl phosphate and glycine in the presence of cyanide (1). Consequently, the reaction was formulated according to Equation 1.

\[
CH_3COOPO_4^- + NH_2CH_2COOH \rightarrow CH_3CONHCH_2COOH + HPO_4^{2-}
\]

In order to establish the stoichiometry of the reaction experimentally, changes in acetyl phosphate, volatile acid, acetylglycine, amino acids, and inorganic phosphate were determined after several time intervals. The data given in Fig. 1 fully support the equation formulated above. Time curves of acetylation obtained with the two dried cell preparations are presented. With Lot Q, there is excellent agreement between the disappearance of acetyl phosphate and volatile acid and the formation of acetylglycine and inorganic phosphate. In other experiments a good agreement between the decrease in volatile acid and amino acid was noted. Thus the decrease in both substrates for ½, 1, and 4 hours was 5, 9, and 23.5 μM per ml. of volatile acid, and 4.5, 10, and 25.5 μM per ml. of glycine.

With Lot Q, the more active of the two preparations, acetylation continued until the acetyl phosphate was virtually exhausted, and the hydrolysis of acetyl phosphate, which was appreciable in the absence of glycine, was completely suppressed. The course of the reaction was somewhat different with dry cell preparation Lot R. The rate was slower, and, with glycine levels of 50 μM per ml., the reaction did not go to completion. Acetyl phosphate disappearance and acetylation were at first identical, but,
after some time, the rate of acetylation decreased sharply and hydrolysis of acetyl phosphate became apparent.

The two curves are typical for the kinetics of the reaction at optimal conditions of pH and cyanide. Under other conditions hydrolysis of acetyl phosphate proceeds from the start, and therefore the disappearance of acetyl phosphate is due to simultaneous acetylation and hydrolysis.

![Graph showing the stoichiometry of glycine acetylation.](http://www.jbc.org/) Fig. 1. The stoichiometry of glycine acetylation. The reaction mixture contained per ml. the following: 200 μM of tris(hydroxymethyl)aminomethane-H₂SO₄ buffer, 50 μM of glycine, 50 μM of dilithium acetyl phosphate, 400 μM of potassium cyanide-H₂SO₄, and 25 mg. of dry cells, Lots R and Q, respectively. Initial pH with Lot R, 8.2; Lot Q, 8.5. 27°.

**Factors Influencing Rate of Acetylation**

*Cyanide Concentration*—Preliminary experiments showed that cyanide does not induce appreciable acetylation of glycine, measured by volatile acid disappearance, unless the concentration is 0.01 M or above. By the use of a more precise analytical method, slow acetylation could undoubtedly be detected at somewhat lower cyanide concentrations.

Table II gives the results of an experiment in which cyanide concentration was varied from 0.1 to 0.8 M. In this experiment cyanide was added as the sodium salt. Later it was found that sodium ion, in contrast to potassium ion, markedly inhibits the acetylation reaction. Therefore, a correction was made for sodium inhibition, based upon the results given in
Table IV. From the corrected results, given in the last column of Table II, it appears that the rate of acetylation is almost directly proportional to cyanide concentration from 0.1 to 0.4 M. At higher levels the rate increases more slowly. In most subsequent experiments, 0.4 M cyanide was used.

In addition to stimulating acetylation, cyanide suppresses acetyl phosphate hydrolysis. This is illustrated by an experiment in which cyanide was added at 0, 0.1, and 0.2 M levels. The quantities of acetylglycine formed enzymatically were 0, 20.8, and 33.4 $\mu$M per ml., respectively, whereas the corresponding quantities of acetyl phosphate hydrolyzed (decomposition not accounted for by acetylglycine formation) were 9.2, 3.4, and 1.9 $\mu$M per ml.

**Table II**

Influence of Cyanide Concentration on Rate of Acetylation

The reaction mixture contained per ml. the following: 50 $\mu$M of glycine, 50 $\mu$M of lithium acetyl phosphate, 100 $\mu$M of potassium phosphate buffer, pH 8, 25 mg. of dried cells of Lot R, and the indicated amounts of sodium cyanide adjusted to pH 8 with sulfuric acid. Incubated anaerobically for 60 minutes at 26°. All values are corrected for non-enzymatic acetylation.

<table>
<thead>
<tr>
<th>Cyanide added $\mu$M per ml.</th>
<th>Volatile acid disappearance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corrected</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td>100</td>
<td>6.0</td>
</tr>
<tr>
<td>200</td>
<td>11.5</td>
</tr>
<tr>
<td>400</td>
<td>19.5</td>
</tr>
<tr>
<td>800</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Acetyl Phosphate Concentration—The influence of acetyl phosphate concentration on acetylation is illustrated in Fig. 2. From the data it can be estimated that a concentration of approximately 0.15 M is required to saturate the enzyme, the maximal velocity of acetylation at this concentration being 0.88 $\mu$M per ml. per minute. The Michaelis constant for acetyl phosphate is about 0.013 M. In most experiments 0.05 M acetyl phosphate was used.

Glycine Concentration—The effect of glycine concentration on acetylation is illustrated in Fig. 2. In contrast with acetyl phosphate, increasing the glycine level above 0.01 M has little effect on the reaction rate. The slow acetylation in the absence of added glycine is due to acetylation of free amino acids and proteins in the enzyme preparation. In most experiments 0.05 M glycine was used.

Enzyme Concentration—In the range between 14 and 25 mg. of dried...
cells of Lot Q per ml., the rate of acetylation is directly proportional to the quantity of cells. Other levels were not tested. Under optimal conditions acetylglycine was formed at the rate of approximately 2.0 μM per hour per mg. of dried cells.

pH—The data given in Table III show that acetylation occurs in the range from about pH 6.0 to above pH 9.4. The rate is maximal in the region between pH 7.7 and 8.3. In other experiments, the rate at pH 10.0 was found to be approximately half of that at pH 8.0. Above pH 10, the increasing lability of acetyl phosphate makes it difficult to obtain reliable data. Below pH 7.5, the rate of acetylation decreases more rapidly than the rate of hydrolysis; hence acetyl phosphate disappearance is not a satisfactory measure of acetylation.

Since acid is formed during acetylation, the initial pH of the reaction mixture should be 8.3 to 8.5 to insure a maximal rate over a period of time. Tris buffer at a concentration of 0.2 M gives satisfactory buffering in this range.

Potassium and Sodium Ion Concentration—Stadtman (15) found that potassium or ammonium ions are essential for the action of the enzyme phosphotransacetylase, whereas sodium and to a lesser extent lithium ions are inhibitory. Since phosphotransacetylase is probably a component
of the amino acid-acetylating system, the influence of the potassium-sodium ratio on the rate of acetylation was investigated.

The data of Table IV show that the rate of acetylation is markedly dependent upon the relative concentrations of the two cations. The rate is most rapid when potassium is the predominant cation. However, even with a very low potassium-sodium ratio, acetylation occurs at an appreciable rate. It should be noted that several of the experiments reported in this paper were done with reaction mixtures similar to Sample 3 (Table IV) in which the rate is approximately 60 per cent of that in a high potassium system.

**Table III**

*Influence of pH on Rate of Acetylation*

The reaction mixture contained per ml. the following: 60 μM of glycine, 70 μM of dilithium acetyl phosphate, 400 μM of sodium cyanide-H₂SO₄, 100 μM of K₂HPO₄, and 25 mg. of dried cells of Lot Q. The pH was adjusted as indicated with H₂SO₄. Incubated for 60 minutes at 26°.

<table>
<thead>
<tr>
<th>pH</th>
<th>Initial</th>
<th>Final</th>
<th>Volatile acid disappearing μeq. per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.4</td>
<td>6.1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6.9</td>
<td>6.5</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>7.8</td>
<td>6.9</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>7.8</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>9.0</td>
<td>8.7</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>9.4</td>
<td>9.0</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

The response of this system to sodium and potassium ions suggests that phosphotransacetylase is involved in the acetylation reaction.

**Replacement of Cyanide by Other Compounds**—Cyanide has a variety of properties, any one of which might be responsible for catalyzing the acetylation of amino acids. For example, it combines with metalloporphyrins to form stable complexes, reacts with carbonyl groups to give cyanhydrin, and functions as a reducing agent. In an attempt to define the action of cyanide, several other compounds were tested for ability to activate glycine acetylation.

The compounds tested were sodium azide, sodium sulfide, sodium sulfite, cysteine, hydrazine, semicarbazide, and α,α'-dipyridyl. The concentration used was 0.1 M, except with dipyridyl which was tested at 0.005 and 0.01 M. Other conditions were the same as those described in Fig. 1. The results of the tests were determined by measuring acetyl phosphate and volatile acid disappearance, acetylglycine formation, and by making paper chromatograms to identify acetylglycine.
The only compound found to replace cyanide was sodium azide. With sulfide the decomposition of acetyl phosphate occurred at the same rate as in a control without cyanide or other activators. When sulfide was added alone with cyanide, both compounds being 0.1 M, the rate of acetylation was the same as with cyanide alone. Sulfite, hydrazine, and semicarbazide greatly accelerated the decomposition of acetyl phosphate but did not cause acetylation of glycine. Cysteine and dipyridyl had no significant effect.

At 0.025 M, azide is as effective as cyanide in inducing acetylation, but at higher concentrations it is less effective. In an experiment in which the effects of the two compounds were compared at levels of 0.1 and 0.2 M, azide caused the formation of 13.2 and 12.5 μM of acetylglucose per ml.

### Table IV
Influence of Potassium and Sodium Ions on Rate of Acetylation

The reaction mixture contained per ml. the following: 50 μM of lithium acetyl phosphate, 50 μM of glycine, 100 μM of phosphate buffer, pH 8.0, 450 μM of cyanide-H₂SO₄, pH 8.1, and 25 mg. of dried cells of Lot R. The cations associated with the cyanide and phosphate were varied to give the indicated potassium-sodium ratios. The total concentration of the two ions in all samples was 654 μeq. per ml. Incubated anaerobically at 26°C.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Molar ratio, K⁺ / K⁺ + Na⁺</th>
<th>Volatile acid disappearance, μeq. per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.003*</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>0.04</td>
<td>8.0</td>
</tr>
<tr>
<td>3</td>
<td>0.31</td>
<td>14.5</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>25.0</td>
</tr>
</tbody>
</table>

* This corresponds to the potassium present in the bacterial preparation; no K⁺ was added to this sample.

respectively, whereas the corresponding values with cyanide were 20.8 and 33.4 μM per ml. It will be noted that the effectiveness of azide does not increase with concentration above 0.1 M.

The above observations permit some negative conclusions concerning the mode of action of cyanide and azide. The inactivity of sulfide and cysteine indicates that the reducing properties of cyanide and azide do not account for their effects. The inactivity of dipyridyl shows that the binding of ferrous iron is not the critical factor.

**Specificity of Acetyl Acceptor**—Stadtman et al. (1) reported that several amino acids including glycine, leucine, serine, lysine, and proline can serve as acetyl acceptors in the C. kluyveri system. In the present study a number of additional amino compounds have been tested as substrates for the acetylation reaction. The tests were carried out under the conditions described in Fig. 1, except that the amino compounds were generally
The reaction was followed by the disappearance of acetyl phosphate and volatile acid. With a few compounds, the decrease of the substrate was determined directly.

The results showed that glycyl-L-leucine, DL-alanyl-L-glycine, glycine ethyl ester, and ethanolamine react at about the same rate as glycine. Several compounds react 50 to 75 per cent as fast as glycine, including glucosamine, methylamine, dimethylamine, ethylamine, and β-alanine. Aromatic amines react much more slowly. Sulfanilamide and p-aminobenzoic acid, provided at a concentration of 0.02 M, are acetylated about one-tenth as fast as glycine. This was determined by following the disappearance of these compounds by the method of Bratton and Marshall (16). Three compounds tested, choline, ammonia, and pantothenic acid, did not react to a demonstrable extent.

Acetylation of Lysine—In preliminary experiments (1) on the enzymatic acetylation of the mixed amino acids present in casein hydrolysate, it was observed by the use of paper chromatography that several amino acids, including lysine, were almost completely used up and some new compounds were formed that reacted with ninhydrin. A possible explanation of this result is the conversion of lysine to a monoacetyllysine that would still react with ninhydrin by virtue of its free amino group. An investigation of the product of lysine acetylation was undertaken to clarify this point.

The enzymatic acetylation of L-lysine was done in a reaction mixture containing radioactive acetate which was allowed to equilibrate with acetyl phosphate before the sodium cyanide (0.1 N) and lysine were added. Otherwise the conditions were essentially those given in Fig. 1. Paper chromatograms of the reaction mixture were examined for acidic substances by spraying with brom cresol green indicator, for amino compounds by spraying with ninhydrin, and for radioactive compounds by making radioautographs.

The paper chromatograms showed the presence of two radioactive compounds, one at least 10 times as abundant as the other. The more abundant compound had an RF value of 0.41 to 0.45, gave a purple color with ninhydrin, and was non-acidic. These properties indicate it to be a monoacetyllysine. Since the RF value of synthetic ε-acetyllysine was found to be 0.39, the enzymatic product was probably α-acetyllysine. The less abundant compound had an RF value of 0.78, was acidic, and did not react with ninhydrin. These properties agree with those of diacetyllysine (RF, 0.78).

An attempt was made to increase the yield of diacetyllysine by carrying out the acetylation reactions with an excess of acetyl phosphate. Even under these conditions the yield was much lower than that of the monoacetyl derivative, judged from the relative sizes of the spots on a paper chromatogram. This indicates that one of the amino groups of lysine,
probably that on the ε position, is much more slowly acetylated than the
other or that the acetylation of one group interferes with the acetylation of
the other.

**Acetate Activation by Adenosinetriphosphate (ATP)**—Since acetyl phos-
phate reacts with adenosinemo- and diphosphates to form ATP in the *C.
kluyveri* system, an experiment was performed to find out whether ATP
can activate acetate for the cyanide-induced acetylation of glycine. The
data of Table V show that ATP is much less effective than acetyl phos-
phate as an energy source. A possible inhibitory effect of ATP was ex-
cluded by showing that the addition of ATP to a reaction mixture con-
taining acetyl phosphate did not reduce the rate of acetylation.

**Other Possible Reactions of Acetylglycine**—A possible explanation for the
rôle of cyanide in the accumulation of acetylated amino acids is that it

**Table V**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Acetylglycine μM per ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>0.0</td>
</tr>
<tr>
<td>&quot; + ATP</td>
<td>0.5</td>
</tr>
<tr>
<td>Acetyl phosphate</td>
<td>18.0</td>
</tr>
</tbody>
</table>

inhibits an enzyme which, in the absence of cyanide, destroys the acety-
lated compounds as fast as they are formed. However, no evidence could
be obtained for the hydrolysis, phosphorolysis, or arsenolysis of acetylgly-
cine in the absence of cyanide. Also attempts to show an acetyl transfer
reaction between acetylglycine and leucine, both in the presence or absence
of cyanide, were unsuccessful.

**DISCUSSION**

The results here reported prove that dried cell preparations of *C. kluy-
veri* contain an enzyme system which, in the presence of acetyl phosphate
and a suitable concentration of cyanide or azide, can acetylate a large
number of amino acids and aliphatic amines. Compounds readily acety-
lated include the common amino acids (both D and L forms), dipeptides,
methylamine, glucosamine, and ethanolamine. The acetylation of egg
albumin in this system was previously reported (1). Aromatic amines
and choline, which are readily acetylated by specific animal enzymes (17,
18), react very slowly or not at all in the bacterial system. In general, the *C. kluyveri* system is able to acetylate a much greater variety of compounds than the animal systems. It is not yet known whether the versatility of the system is due to one or a group of enzymes, although the former seems more likely.

The present study confirms the earlier observation (1) that a rather high cyanide concentration is required for maximal activation of the acetylating system. Whereas cyanide is generally effective as an inhibitor in biological systems at concentrations of 0.005 M or less, in the acetylation reaction the activating effect of cyanide becomes just noticeable at 0.01 M and increases with concentration up to at least 0.8 M. Azide can replace cyanide as an activator but is considerably less effective, particularly at a level of 0.1 M or above. The mode of action of cyanide and azide is not apparent, although it is probable that neither the reducing nor metal-binding properties of the compounds are involved. These compounds do not act by inhibiting the decomposition of acetylated amino acids.

Active acetate can be provided in biological systems by the action of the enzyme phosphotransacetylase on acetyl phosphate or by an enzyme complex that acts on ATP and acetate. The demonstration that acetyl phosphate is a much more effective acetate donor than ATP and acetate in the *C. kluyveri* acetylation system indicates that acetyl phosphate is serving directly as an acetate donor through the mediation of phosphotransacetylase and coenzyme A rather than as a phosphate donor to the adenylic acid system. Further indirect evidence for the participation of phosphotransacetylase in acetylation is provided by the observation that sodium ion inhibits and potassium ion accelerates the process. Similar effects have been observed with purified phosphotransacetylase (14).

The authors wish to thank Dr. E. M. Gal for providing the acetylalanine, acetylvaline, and acetylleucine.

**SUMMARY**

Acetyl phosphate and glycine have been shown to be converted almost quantitatively to acetylglycine by dried cell preparations of *Clostridium kluyveri* in the presence of cyanide. The influence of substrate concentrations and other factors on the reaction rate have been studied and the optimal conditions established. Amino acids and a variety of aliphatic amines are reactive in this system, but not aromatic amines or choline. Mono-acetylleucine, probably substituted in the α position, is the main product of lysine acetylation. Acetylated amino acids do not undergo hydrolysis or participate in transacetylation reactions either in the presence or absence
of cyanide. A method for the estimation of acetylated amino acids is described.

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