Two distinctly different L-threonine deaminases are known to be formed in Escherichia coli. One of them participates in the biosynthesis of L-isoleucine from L-threonine and is susceptible to an end product inhibition by the former compound (1). The other one seems to participate in the catabolism of L-threonine under anaerobic conditions and is activated by adenosine 5'-monophosphate (1, 2). Little has been understood, however, as to the mechanism of the AMP activation.

Hayashi, Gefter, and Weissbach (3) recently described another L-threonine deaminase from Clostridium tetanomorphum which was activated by adenosine diphosphate. ADP was found to be bound by the deaminase (4), to stabilize its activity (4), to increase the affinity of the substrate for it (3), and to regulate the anaerobic energy production in this organism (5).

In the present report is described a detailed study of the nature of activation of E. coli threonine deaminase by 5'-AMP and other nucleotides. Evidence is presented which shows that the properties of the E. coli enzyme are distinctly different from those of the clostridial enzyme in several respects, including reaction kinetics, inactivation kinetics, and conformational modifications.

**EXPERIMENTAL PROCEDURE**

**Growth of Organism**—E. coli strain W was provided by Dr. T. Yura, the Institute for Virus Research, Kyoto University. The organism was grown for 17 hours at 37° without aeration in 4.5 liters of the medium in 5-liter Erlenmeyer flasks according to the method of Umbarger and Brown (1). Cells were harvested with a Sharples centrifuge at room temperature. The yield of wet packed cells was about 1.2 g per liter of the medium.

**Threonine Deaminase Assay**—The activity of threonine deaminase was routinely determined spectrophotometrically by measuring the formation of α-ketobutyrate as its 2,4-dinitrophenylhydrazone. The standard assay system contained 100 μmoles of L-threonine, 10 μmoles of 5'-AMP, 300 μmoles of potassium phosphate buffer, pH 7.4, and the enzyme in a total volume of 1.0 ml. The reaction mixture was incubated at 37° for 10 minutes and the reaction was then terminated by the addition of 1.0 ml of 1 N HCl. The keto acid formed was determined as the 2,4-dinitrophenylhydrazone at 415 μm by a modification of the method of Katsuki et al. (6). One unit of enzyme was defined as the amount producing 1 μmole of α-ketobutyrate per minute under the standard conditions. Specific activity was expressed as units per mg of protein. Protein was determined by the method of Lowry et al. (7). All spectrophotometric determinations were carried out with a Shimadzu DU spectrophotometer.

**Sucrose Density Gradient Centrifugations**—The method of Martin and Ames (8) was used to prepare 10 to 20% sucrose density gradients. When necessary, a nucleotide was included in the gradient solutions at a designated concentration. After layering the enzyme preparation on the top of the gradient, the sample was subjected to centrifugation at 39,000 rpm for 14 hours at 3° in the SW-39 rotor of the Spinco model L centrifuge. Each tube was then punctured at the bottom and drops were collected for assay of enzyme activity.

**Paper Chromatography**—Ascending paper chromatography was carried out for the identification of the 2,4-dinitrophenylhydrazones of α-keto acids with Whatman No. 1 filter paper with the use of tert-amyl alcohol-ethyl alcohol-water (5:1:4) as solvent.

**Materials**—Sodium α-ketobutyrate was a product of the Mann Chemical Industries, Ltd. Other ribo- and deoxyribonucleotides, 5'-AMP, 5'-UMP, 5'-GMP, 5'-CMP, 5'-IMP, 2'-AMP, and 3',5'(cyclic) AMP were gifts of the Takeda Research Laboratories, the Takeda Chemical Industries, Ltd. Other ribo- and deoxyribonucleotides, laetic dehydrogenase, and DPNH were the products of the Sigma Chemical Company. All nucleotides were purified by column chromatography on Dowex 1-formate resin with the use of ammonium formate as the eluting system (10). Lithium pyruvate was a gift of Dr. H. Katsuki, Department of Chemistry, Faculty of Science, Kyoto University. All other chemicals were of analytical grade.

**RESULTS**

**Enzyme Purification**—All procedures were carried out at 0-4°.

1. Crude extracts: A portion (10 g) of the harvested cells was suspended in 20 ml of 20 mm potassium phosphate buffer, pH
The enzyme activities were determined under the standard conditions except that 0.2 mM Tris-phosphate buffer was employed in place of potassium phosphate buffer. The upper curve (●) represents the activity in the presence of 10 mM AMP (with the left side ordinate) and the lower curve (○) represents the activity in its absence (with the right side ordinate).

7.4, containing 5 mM β-mercaptoethanol, and was disrupted in a 10-kc sonic disintegrator for 15 minutes. The cell debris was then removed by centrifugation at 20,000 × g for 20 minutes. The supernatant was designated the crude extract.

2. Streptomycin treatment: To 29 ml of the crude extract was added an equal volume of 20 mM potassium phosphate buffer, pH 6.4, containing 10 mM β-mercaptoethanol, and the mixture was treated with 23 ml of a 5% solution of streptomycin sulfate with stirring. After the mixture was kept overnight, the precipitate was removed by centrifugation at 20,000 × g for 20 minutes.

3. Ammonium sulfate fractionation: Solid ammonium sulfate (15 g) was gradually added to 81 ml of the above supernatant solution with stirring, and the mixture was allowed to stand for 3 hours. The precipitate was then removed by centrifugation at 20,000 × g for 20 minutes and dissolved in 10 ml of 20 mM potassium phosphate buffer, pH 7.4, containing 1 mM β-mercaptoethanol.

4. DEAE-cellulose column chromatography: The following procedures were carried out in the presence of 1 mM EDTA and 1 mM β-mercaptoethanol. For desalting, the enzyme solution was then passed through a Sephadex G-25 column (2 × 20 cm) which had been equilibrated with 50 mM potassium phosphate buffer, pH 7.4. The effluent fluid from the Sephadex column (200 mg of protein in 20 ml) was immediately placed on a DEAE-cellulose column (2 × 30 cm) which had been equilibrated with 50 mM potassium phosphate buffer, pH 7.4. Elution was carried out with a linear concentration gradient of potassium phosphate buffer, pH 7.4, from 0.05 to 0.40 M (mixing chamber, 500 ml of 0.05 M buffer; reservoir, 500 ml of 0.40 M buffer). The enzyme in the 620- to 640-ml effluent volume was collected and was precipitated immediately with ammonium sulfate (90% saturation), since the diluted enzyme solution was rather unstable. This fraction was used for all kinetic studies unless specified otherwise. The enzyme was purified about 30-fold with about 13% yield by the above procedures. A typical result of enzyme purification is shown in Table I.

Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg protein)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>1390</td>
<td>3320</td>
<td>2.4</td>
<td>100</td>
</tr>
<tr>
<td>Streptomycin supernatant</td>
<td>690</td>
<td>3170</td>
<td>5.1</td>
<td>100</td>
</tr>
<tr>
<td>Ammonium sulfate fraction</td>
<td>200</td>
<td>415</td>
<td>16.0</td>
<td>95</td>
</tr>
<tr>
<td>DEAE-cellulose fraction</td>
<td>6</td>
<td>415</td>
<td>69.0</td>
<td>13</td>
</tr>
</tbody>
</table>

Effect of pH, and Cofactor Requirement—The optimal pH for the deaminase activity was about 9.5 in the absence of 5'-AMP with Tris-phosphate buffer. In the presence of the nucleotide, however, a bimodal curve was obtained with peaks at pH values 8.0 and 8.5, as shown in Fig. 1. Although the deaminase is supposed to be a pyridoxal enzyme (1), the addition of pyridoxal phosphate did not cause any significant increase of activity, presumably due to a firm binding of the cofactor to the enzyme protein. Wood and Gunsalus (2) described a stimulatory effect of reduced glutathione on the crude preparation of the E. coli threonine deaminase. However, we did not observe such an effect with our purified enzyme preparations, although glutathione and β-mercaptoethanol stabilized the enzyme to some extent.

Substrate Specificity—The threonine deaminase reacted with L-threonine and L-serine. The reaction products were identified as α-ketobutyric and pyruvic acids, respectively, as the 2,4-dinitrophenylhydrazones, by paper chromatography. The $K_r$ values for the hydrazones were as follows; 0.53 (trans) and 0.84 (cis) for α-ketobutyric acid, and 0.34 (trans) and 0.55 (cis) for pyruvic acid.

The $K_m$ values for both L-threonine and L-serine decreased about 5-fold and the maximal velocities for both substrates increased almost 10-fold in the presence of 5'-AMP, as shown in Table II. N-Threonine competitively inhibited the deamination not only of L-threonine, but also of L-serine. Similarly, n-serine competitively inhibited the deamination of both L-serine and L-threonine as seen in Table III. However, the $K_i$ values were not affected significantly by the presence of 5'-AMP. L-Allothreonine was also deaminated by the enzyme preparation.

**Table II**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>With AMP</th>
<th>Without AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Threonine</td>
<td>4.2</td>
<td>20</td>
</tr>
<tr>
<td>L-Serine</td>
<td>4.0</td>
<td>18</td>
</tr>
</tbody>
</table>

* The concentration of AMP was 10 mM.
† When L-serine was employed as substrate, the reaction product, pyruvic acid, was determined by the standard system, except that lithium pyruvate was used as standard.
activation was dependent on the nucleotide concentration as shown in Fig. 2, A and B. In contrast to the kinetic behavior of the same enzyme of C. tetanomorphum (3), the enzyme prepared from E. coli showed typical Michaelis-Menten kinetics. Namely, when the rate of the reaction was plotted against substrate concentration, a hyperbolic curve was obtained even in the absence of the added nucleotide and the Lineweaver-Burk plot was linear both in the presence and absence of adenylic acid. When the reciprocal of the reaction rate was plotted against the reciprocal of the concentrations of 5'-AMP at different substrate concentrations the $K_a$ (Michaelis constant for activation) values for 5'-AMP thus obtained were found to be dependent on the concentration of l-threonine, as shown in Fig. 3. For example, at a threonine concentration of 100 mM, the $K_a$ value for 5'-AMP was 0.5 mM, whereas at a threonine concentration of 5 mM, the value was approximately 1.7 mM.

The $K_a$ values and maximal velocities with various nucleotides are shown in Table IV. 5'-AMP was most effective both in terms of $K_a$ and $V_{max}$. Other 5'-nucleoside monophosphates such as 5'-GMP, 5'-CMP, 5'-IMP, and 5'-UMP did not give significant activation at 0.5 mM and were about 60 to 80% as active as 5'-AMP at 30 mM. Other nucleotides including ATP, ADP, 5'-TMP, 5'-dAMP, 3',5'-AMP, 2'-AMP, 3'-AMP, adenosine, and adenine were ineffective throughout the concentration range examined.

**Table III**

$K_a$ values of d-isomers

The deaminase activity was determined by the standard system. The enzyme (10 μg of protein per tube) used in these experiments was a preparation of the ammonium sulfate fraction (Table I).

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>$K_a$ (mM)</th>
<th>$V_{max}$ (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'-AMP</td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>5'-GMP</td>
<td>4.0</td>
<td>33</td>
</tr>
<tr>
<td>5'-CMP</td>
<td>5.0</td>
<td>40</td>
</tr>
<tr>
<td>5'-IMP</td>
<td>10.0</td>
<td>40</td>
</tr>
<tr>
<td>5'-UMP</td>
<td>10.0</td>
<td>33</td>
</tr>
<tr>
<td>None</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**Inactivation and Protection of Enzyme**—The enzyme was readily inactivated upon dilution. As seen in Fig. 4, when the enzyme was diluted with water under the conditions described in the legend, almost 75% of the activity was lost within 10 minutes. The addition of d-threonine gave no protection. But in the presence of 5'-AMP at 10 mM, only about 10% of the activity was lost. Higher concentrations of the phosphate buffer appeared to protect against inactivation to some extent.

Although d-threonine alone did not protect the enzyme from inactivation, the protective effect of 5'-AMP appeared to be enhanced in the presence of d-threonine. For example, when the enzyme was diluted with a mixture of 100 mM d-threonine and 10 mM 5'-AMP, almost all the activity remained even after a 1-hour period. As seen in Fig. 5, the degree of the protection (as expressed by the remaining activity) was then plotted against the concentrations of 5'-AMP in the presence and absence of d-threonine and its absence. The $K_p$ (Michaelis constant for protection) values for 5'-AMP thus obtained by the Lineweaver-Burk plot of the results were 0.5 mM and 2 mM, in the presence of 100 mM d-threonine and its absence, respectively. 5'-CMP and 5'-GMP gave 31 and 15% protection, respectively, as compared with 5'-AMP, whereas 5'-UMP, 5'-AMP, 2'-AMP, 5'-AMP, 5'-dAMP, ADP, ATP, adenosine, and adenine did not give significant protection.
Nucleotide Activation of Threonine Deaminase

Vol. 240, No. 4

1714

Fig. 4. Inactivation of the enzyme activity upon dilution
Enzyme preparation, 6.5 mg per ml of protein, in 0.3 M phosphate
buffer, pH 7.4, containing 1 mM EDTA, was subjected to 100-fold
dilution with one of the following solutions at 37°: 10 mM 5'-AMP
(O------O), 1 M sodium phosphate buffer, pH 7.4 (O—O),
106 mM n-threonine (△—△), and water (△—△). The re-
main ing activity was then determined at different time intervals
by the standard system in the presence of 10 mM 5'-AMP.

Fig. 5. Synergistic effect for protection by n-threonine and
5'-AMP against inactivation by dilution. Enzyme preparation
(7.3 mg per ml of protein) was diluted 100-fold with various con-
centrations of 5'-AMP in the presence of 100 mM n-threonine
(O—O), or its absence (O—O), at 37°. After 10 minutes
the enzyme activity was determined under the standard condi-
tions.

The enzyme was inhibited by CMB1 and the inhibition was
reversed by the addition of β-mercaptoethanol. 5'-AMP pre-
vented the inhibition to some extent as shown in Table V. The
addition of n-threonine was not effective, but in the presence of
both n-threonine and 5'-AMP, the inhibition was prevented more
effectively than by 5'-AMP alone.

Hydroxylamine, an inhibitor of pyridoxal enzymes, inhibited
the deaminase activity. As seen in Table VI, in the presence of
5'-AMP at 2 mM, or n-threonine at 100 mM, hydroxylamine at
1.4 mM gave 70 and 77% inhibition, respectively, while only 2%
of the activity remained without 5'-AMP or n-threonine. How-
ever, almost 90% of the original activity remained when both
compounds were present together.

5'-AMP also protected the enzyme against heat inactivation.
When an ammonium sulfate fraction (74 μg of protein per ml of
1 m potassium phosphate buffer, pH 7.4) was heated for 10
minutes at 55°, 73% of the activity was lost, whereas the in-
activation was insignificant in the presence of 10 mM 5'-AMP
over a period of 30 minutes. As shown in Table VII the K_m
values for L-threonine, both in the presence and absence of 5'-
AMP, increased about 3-fold by heat treatment.

Sedimentation Analysis—The sucrose density gradient tech-
nique was employed to see if there was any change in the sedi-
mentation pattern of the enzyme in the presence of 5'-AMP.

Table V
Inhibition by p-chloromercuribenzoate and protection by 5'-AMP
and n-threonine

To 43 μg of the enzyme in 0.007 ml of 1 M phosphate buffer, pH
7.4, was added 0.043 ml of water, and the mixture was left for 5
minutes at 37°. The enzyme activity was then determined by the
standard system. Where indicated 1 m CMB, 2 mM AMP, and
100 mM n-threonine were added during the preincubation.

Table VI
Inhibition by hydroxylamine and protection by 5'-AMP
and n-threonine

To 35 μg of the enzyme in 0.01 ml of 1 M phosphate buffer, pH
7.4, was added 0.04 ml of water and the mixture was left for 5 min-
utes at 37°. The enzyme activity was then determined by the
standard system. Where indicated 1.4 mM hydroxylamine, 2
mM AMP, and 100 mM n-threonine were added during the prein-
cubation.

Table VII
K_m values for L-threonine before and after heat treatment

The ammonium sulfate fraction (Table I) (0.26 mg of protein
per ml) in 1.0 M sodium phosphate buffer, pH 8.0, containing 6 mM
β-mercaptoethanol was heated at 55° for 30 minutes and the de-
aminase activity was then determined by the standard system in
the presence and absence of 10 mM AMP.

<table>
<thead>
<tr>
<th>Conditions used</th>
<th>K_m values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+AMP</td>
</tr>
<tr>
<td>Before heat treatment</td>
<td>4</td>
</tr>
<tr>
<td>After heat treatment</td>
<td>13</td>
</tr>
</tbody>
</table>

1 The abbreviation used is: CMB, p-chloromercuribenzoate.
phosphate buffer, pH 7.4) containing 0.5 mg of protein was layered over 4.5 ml of 10 to 20% (w/v) linear sucrose density gradient in solution (0.1 ml of ammonium sulfate fraction in 50 mM potassium phosphate buffer, pH 7.4). Metapyrocatechase (0.2 mg of protein of crystalline enzyme) was included in the system as an internal standard, the activity of which was determined as described by Nosaki et al. (12). Centrifugation was carried out for 14 hours at 30,000 r.p.m. at 3° and the activities of threonine deaminase (O—O) (with the left side ordinate) and metapyrocatechase (O—O) (with the right side ordinate) were determined with aliquots of each fraction. A, with 1 mM; B, with 0.1 mM; and C, without 5'-AMP.

Fig. 6. Sucrose density gradient centrifugations. Enzyme solution (0.1 ml of ammonium sulfate fraction in 50 mM potassium phosphate buffer, pH 7.4) containing 0.5 mg of protein was layered over 4.5 ml of 10 to 20% (w/v) linear sucrose density gradient in 0.05 M potassium phosphate buffer, pH 7.4. Metapyrocatechase (0.2 mg of protein of crystalline enzyme) was included in the system as an internal standard, the activity of which was determined as described by Nosaki et al. (12). Centrifugation was carried out for 14 hours at 30,000 r.p.m. at 3° and the activities of threonine deaminase (O—O) (with the left side ordinate) and metapyrocatechase (O—O) (with the right side ordinate) were determined with aliquots of each fraction. A, with 1 mM; B, with 0.1 mM; and C, without 5'-AMP.

Fig. 7. Retention pattern on Sephadex G-100 column. An aliquot (0.2 ml) of the ammonium sulfate fraction containing 3.4 mg of protein was applied to a column of Sephadex G-100 (2.2 X 20.0 cm), which had been previously equilibrated with 25 mM potassium phosphate buffer, pH 7.4, or with the same buffer plus 5 mM 5'-AMP (B). Each 10 drops (0.3 ml) were collected, then protein (O—O) and the activity of threonine deaminase (O—O) were determined by the standard systems.

Crystalline metapyrocatechase (molecular weight, 140,000) (12) was used as the internal standard. The results are shown in Fig. 6. The presence of 5'-AMP gave rise to a faster sedimentation pattern than the control. Metapyrocatechase, however, was not affected at all by the presence of the nucleotide. Assuming the partial specific volume of the enzyme protein to be 0.725, the S values for the deaminase were estimated to be 4.4, 6.4, and 8.0, in the absence of 5'-AMP and in the presence of 0.1 mM and 1 mM 5'-AMP, respectively.

When 5'-IMP, 5'-GMP, 5'-UMP, and 5'-CMP were examined in the above type of experiment, similar effects on the sedimentation pattern were observed, although the effective concentrations were much higher. For example, with 5'-IMP at 1 mM, the S value was 5.0, and it was 7.1 at 10 mM. 3'-AMP, which did not activate the deaminase, caused only insignificant changes in the sedimentation pattern of the enzyme even at 10 mM.

Gel Filtration on Sephadex Column—The enzyme, after ammonium sulfate fractionation (3.4 mg in 0.2 ml), was applied to a column (1.2 X 20 cm) of Sephadex G-100 and elution was carried out with 25 mM potassium phosphate buffer, pH 7.4, at a flow rate of 0.3 ml per minute. The enzyme activity and the concentration of protein in each fraction (0.3 ml per tube) were then determined by the standard assay systems. The result is shown in Fig. 7A. When the enzyme preparation was applied to the column equilibrated with the same buffer containing 5 mM 5'-AMP, and elution was carried out in the same way, most of the enzyme activity came out in earlier fractions (Fig. 7B). When ATP at 5 mM was used in lieu of 5'-AMP, no significant change in the retention pattern was observed as compared with the control. When the enzyme was applied to a column of Sephadex G-200, the enzyme activity also appeared in earlier fractions in the presence of 5 mM 5'-AMP, than it did in the absence of the nucleotide. However, the enzyme was retained by the Sephadex G-200 column even in the presence of the nucleotide.

DISCUSSION

An increasing number of reports has recently been published from a number of laboratories indicating the general importance of adenine nucleotides as metabolic regulators. For example, nicotin ribonucleotidase of beef liver was shown to be converted to nicotin ribonucleotide pyrophosphorylase in the presence of ATP (13) and thiamine pyrophosphate phosphohydrolase of rat liver was activated by ATP (14). ADP and 5'-AMP activate DPN-linked isocitric dehydrogenases of bovine heart (15) and of yeast (16), respectively, regulating the ATP formation and substrate oxidation in mitochondria. The role of 5'-AMP in the activation of phosphorylase has been the subject of intensive investigation by Hehnreich and Cori (17) for a number of years. The physiological significance of the activation by AMP of E. coli threonine deaminase is at present unclear and currently under investigation in our laboratory. A plausible interpretation of a similar phenomenon was previously proposed from our laboratory for the activation of the clostridial threonine deaminase by ADP (5). In the latter case, threonine serves as the major source of energy for the growth of the microorganism, yielding propionyl phosphate by way of α-ketobutyrate; propionyl phosphate then generates ATP in the presence of ADP and an acyl kinase. Thus, the accumulation of ADP in the cell increases the rate of the first, and presumably the rate-limiting, reaction in the catabolic pathway providing more energy for the synthesis of ATP. In E. coli 5'-AMP may be acting as a metabolic regulator in a manner similar to that proposed for ADP in the case of C. tetanomorphum.

The kinetics of the reaction catalyzed by the E. coli enzyme appear to be different from those of the clostridial enzyme in terms of interactions between substrates and activators. For example, the maximal velocities and $K_m$ values were both affected by the nucleotide in the case of the E. coli enzyme, whereas the maximal velocities were not affected at all in the case of the clostridial enzyme (3). Typical Michaelis-Menten type kinetics were obtained with the E. coli enzyme even in the absence of the nucleotide, while a sigmoidal curve was observed with the clostridial enzyme in the absence of the nucleotide (18). These
Nucleotide Activation of Threonine Deaminase

Threonine deaminase was purified about 30-fold from Escherichia coli grown anaerobically. The enzyme was activated by adenosine 5'-monophosphate. Other ribonucleoside 5'-monophosphates such as UMP, CMP, GMP, and IMP were partially effective. Deoxyribonucleoside (dAMP or TMP) nucleoside polyphosphates (ADP or ATP) and nucleoside monophosphates linked at positions other than 5 (2'-AMP, 3'-AMP, or 3',5'-AMP) were not effective.

The Kₐ value for L-threonine decreased progressively, and the maximal velocity of the enzyme reaction increased also progressively, as the concentration of 5'-AMP increased. Similarly, the affinity of 5'-AMP for the enzyme protein was found to be dependent on the concentration of L-threonine. The Kₐ value and the maximal velocity for the deamination of L-serine by the same enzyme were also similarly affected by 5'-AMP. Kₐ values of L-threonine and L-serine were not, however, affected by the presence of 5'-AMP.

The nucleotides were effective in protecting the enzyme against inactivation by dilution and heat, or inhibition by p-chloromercuribenzoate and hydroxylamine. D-Threonine, a substrate analogue, was not effective in this regard. However, d-threonine acted as a protective agent if the nucleotide was also present. The degree of protection by d-threonine against inactivation by dilution was found to be dependent on the concentration of 5'-AMP. The Michaelis-Menten constant of 5'-AMP for the protection was of the same order of magnitude as that for the activation.

These results suggest that functions of the catalytic and regulatory sites of the enzyme protein are mutually dependent.

By a sedimentation study with a sucrose density gradient, the S value of the enzyme protein was calculated to be 8.0 in the presence of 1 mM 5'-AMP, and 4.4 in its absence, indicating a considerable modification of the molecular conformation. Sephadex gel filtration indicated that association of enzyme units to form a larger one seems to occur in the presence of the nucleotide.

Acknowledgment—We are indebted to Miss E. Komabayashi for her skilful and devoted assistance in this investigation. Supply of nucleotides from the Takeda Research Institute is greatly appreciated.

REFERENCES

Nucleotide Activation of Threonine Deaminase from *Escherichia coli*
Masaharu Hirata, Masanobu Tokushige, Akira Inagaki and Osamu Hayaishi


Access the most updated version of this article at [http://www.jbc.org/content/240/4/1711.citation](http://www.jbc.org/content/240/4/1711.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/240/4/1711.citation.full.html#ref-list-1](http://www.jbc.org/content/240/4/1711.citation.full.html#ref-list-1)