ENZYMATIC SYNTHESIS OF NUCLEOSIDE DIPHOSPHATES
AND TRIPHOSPHATES*

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It has been recognized from recent studies that nucleoside triphosphates,
other than ATP,\(^1\) function in biologic systems as phosphorylating agents
(1) and in coenzyme synthesis (2) in a manner analogous to that of ATP. The
discovery of the natural occurrence of a variety of purine and pyrimidine
nucleoside di- and triphosphates (3-5) and the likelihood that new
biologic functions for these nucleotides will be found increased our interest
in the mechanism of their biosynthesis. Several pathways are known for
the formation of UTP, including its origin from UDP, by the action of
pyruvate phosphokinase (6) or of nucleoside diphosphate kinase (7), but
no mechanism has been elucidated for the synthesis of UDP or UTP from
U5P. In a preliminary communication (8), we have reported that extracts
of yeast and liver which convert orotic acid to U5P also bring about the
synthesis of UDP and UTP. A subsequent report (9) described the action
of a partially purified enzyme preparation from yeast which effects a
transphosphorylation between adenosine, uridine, and guanosine nucleo-
tides (equations (1) to (5)) and differs from muscle myokinase (10, 11)
which acts on adenosine nucleotides only.

\begin{align*}
(1) & \quad \text{U5P} + \text{ATP} \rightleftharpoons \text{UDP} + \text{ADP} \\
(2) & \quad \text{A5P} + \text{UTP} \rightleftharpoons \text{ADP} + \text{UDP} \\
(3) & \quad \text{U5P} + \text{UTP} \rightleftharpoons 2\text{UDP} \\
(4) & \quad \text{G5P} + \text{ATP} \rightleftharpoons \text{GDP} + \text{ADP} \\
(5) & \quad 2\text{GDP} \rightleftharpoons \text{GTP} + \text{G5P}
\end{align*}

* This investigation was supported by research grants from the National Institutes of Health, Public Health Service, and the National Science Foundation.

\(^1\) The abbreviations used are adenosine triphosphate, ATP; adenosine diphosphate, ADP; adenosine-5'-phosphate, A5P; guanosine triphosphate, GTP; guanosine diphosphate, GDP; guanosine-5'-phosphate, G5P; inosine triphosphate, ITP; inosine diphosphate, IDP; uridine triphosphate, UTP; uridine diphosphate, UDP; uridine-5'-phosphate, U5P; diprophosphopyridine nucleotide, DPN; reduced DPN, DPNH; triphosphopyridine nucleotide, TPN; tris(hydroxymethyl)aminomethane, Tris.

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These reactions can be summarized by a generalized equation for the action of a nucleoside monophosphate kinase (equation (6)).

\[
\text{Nucleoside-P + nucleoside-P-P-P} \rightleftharpoons \text{nucleoside-P-P + nucleoside-P-P}
\]

This report provides the details of the partial purification and properties of the enzyme preparation from yeast which catalyzes the formation of the nucleoside di- and triphosphates. Preceding reports in this series (12-14) have described a pathway of synthesis of pyrimidine and purine nucleotides from their constituent bases.

**Materials**

2-C\textsubscript{14}-U\textsubscript{5}P was synthesized from 2-C\textsubscript{14}-sodium orotate as previously described (13) and concentrated by adsorption on and elution from Norit. 2-C\textsubscript{14}-A\textsubscript{5}P and 2-C\textsubscript{14}-G\textsubscript{5}P were prepared enzymatically from the corresponding 2-C\textsubscript{14}-labeled purine bases (14) and purified by anion exchange chromatography. U\textsubscript{5}P, UTP, and ATP were purified by anion exchange chromatography. ATP\textsuperscript{32} was prepared as previously described (12). Deoxynucleotides were products of the California Foundation for Biochemical Research. Nicotinamide ribose-5'-phosphate was prepared according to Kornberg and Pricer (15).

IDP was prepared from ITP in the presence of glucose and a catalytic amount of ADP by the combined action of nucleoside diphosphokinase (7) and hexokinase (16). The reaction was measured spectrophotometrically by the reduction of TPN in the presence of glucose-6-phosphate dehydrogenase (17). After complete conversion of the ITP (0.16 pmole per ml.), the reaction mixture was heated in a boiling water bath for 3 minutes; the supernatant solution, after centrifugation, was used as IDP.

TPN was obtained by anion exchange chromatography of a crude liver fraction (18). DPNH was prepared by Ohlmeyer's method (19) and phosphopyruvic acid by Ohlmeyer's modification of Kiessling's procedure (20).

Pyruvate phosphokinase was purified from rabbit muscle by a modification of the procedure described by Kubowitz and Ott (21). Lactic dehydrogenase was prepared by ammonium sulfate fractionation of rabbit muscle extract (22), hexokinase according to Berger *et al.* (16), and glucose-6-phosphate dehydrogenase by the method of Kornberg (17). Adenylic acid deaminase was prepared from rabbit muscle by the procedure used for Preparation A by Kalekar (23).

**Determinations**

*Enzyme Assay*—This was based on the conversion of C\textsubscript{14}-labeled U\textsubscript{5}P to UDP and UTP. The reaction mixture (1.0 ml.) contained 0.1 ml. of glycylglycine buffer (0.5 M, pH 7.5), 0.05 ml. of MgCl\textsubscript{2} (0.1 M), 0.1 ml. of
ATP (0.04 M), 0.01 ml. of potassium phosphopyruvate (0.05 M), 4 units of pyruvate phosphokinase, 0.05 ml. of 2-C14-U5P (6.8 × 10^{-4} M, 240,000 c.p.m. per pmole), and 0.6 to 2.6 units of enzyme. After 15 minutes at 36°, 1 ml. of water was added and the diluted mixture was heated in a boiling water bath for 3 minutes. The supernatant fluid obtained by centrifugation was adsorbed on a column of Dowex 1, chloride form (200 to 400 mesh, 2 per cent cross-linked; height 1.5 cm., diameter 1 cm.), contained in a columnar glass funnel with a coarse sintered glass disk. After the fluid was drawn through the column with gentle suction, the resin was washed first with 2.5 ml. of water and then with 25 ml. of HCl (0.05 N) (at a rate of about 1.5 ml. per minute) to elute the U5P. Up to six such columns were readily handled at the same time by setting up a glass manifold to connect the HCl reservoir to the columns. The uridine polyphosphates were then eluted with 4 N HCl (2.5 ml.) with the aid of suction. An aliquot (0.2 ml.) of this eluate was plated on a circular glass cover-slip (diameter 2.2 cm.), dried, and counted. A unit of enzyme was defined as the amount producing 1000 c.p.m. in the strong HCl eluate under the conditions of the assay. Specific activity was defined as units of activity per mg. of protein.

Under the assay conditions, the radioactivity of the strong HCl eluate was proportional to the amount of enzyme. Thus, assays of 0.010, 0.015, and 0.020 ml. of enzyme solution all yielded values of 80 units per ml. No counts were detected in the absence of enzyme.

Chromatographic analysis of the amounts of UDP and UTP in the strong HCl eluate under standard assay conditions with a purified enzyme fraction indicated a ratio of 1:4. Under similar conditions but with one-twentieth as much ATP (0.2 μmole per ml.), roughly equal amounts of UDP and UTP were produced.

Adenylate kinase was measured with the coupled pyruvate phosphokinase-lactic dehydrogenase system as an indicator (22). The reaction mixture (1.0 ml.) contained 0.1 ml. of glycylglycine buffer (0.5 M, pH 7.5), 0.05 ml. of MgCl₂ (0.1 M), 0.01 ml. of potassium phosphopyruvate (0.05 M), 0.05 ml. of DPNH (0.001 M), 0.01 ml. of lactic dehydrogenase (diluted 1:5000), 5 units of pyruvate phosphokinase, 0.1 ml. of ATP (3.5 × 10^{-4} M), 0.1 or 0.2 ml. of enzyme solution, and 0.02 ml. of A5P (0.025 M). The formation of ADP was calculated as a function of pyruvate appearance and measured by the decrease in optical density at 340 mμ resulting from the oxidation of DPNH (equations (7) to (9)).

\[
\begin{align*}
\text{(7)} & \quad \text{ATP} + \text{A5P} \rightleftharpoons 2\text{ADP} \\
\text{(8)} & \quad \text{Phosphopyruvate} + \text{ADP} \rightarrow \text{pyruvate} + \text{ATP} \\
\text{(9)} & \quad \text{Pyruvate} + \text{DPNH} + \text{H}^+ \rightarrow \text{lactate} + \text{DPN}
\end{align*}
\]

Nucleoside diphosphokinase was assayed with ATP and IDP in the
presence of yeast adenylate kinase and adenylic acid deaminase as described by Berg and Joklik (7).

ADP and UDP were estimated spectrophotometrically with the phosphokinase-lactic dehydrogenase system (22).

Pentose, phosphate, and protein were determined by methods previously cited (12).

C14-containing samples were plated as thin layers on aluminum disks or, in the presence of strong acid, on glass cover-slips. Radioactivity was measured in a gas flow counter. When a large amount of salt was present, a self-absorption correction factor was applied (1.25 for 0.05 M KCl; 1.35 for 0.1 M KCl). P32 was measured in solution (1.0 ml.) in a dish under a Geiger-Müller tube.

Results

Purification of Enzyme—The enzyme catalyzing the synthesis of UDP and UTP from U5P and ATP was purified about 30-fold from extracts of yeast, as shown in Table I. The operations were carried out at 0° except as indicated.

Extracts were prepared from dried brewers' yeast by incubation in 0.1 M KHCO3 for 5 hours at 30° as previously described (13).

To 36 ml. of yeast autolysate, 72 ml. of sodium acetate buffer (0.2 M, pH 4.5) were added rapidly, with stirring, and the acidified extract was cooled to −2° in an alcohol-ice bath. 45 ml. of ethanol (−14°) were then added over a 7 minute interval while the temperature was allowed to fall to −4°. The mixture was cooled to −8° and a further addition of ethanol was made (29 ml.) over a 4 minute interval at −8°. Insoluble material was removed by centrifugation for 3 minutes at 10,000 × g in a Servall centrifuge kept in a room at −14°. To the supernatant fluid, 90 ml. of ethanol were added over a 4 minute interval with the temperature maintained at −10°. The precipitate was collected by centrifugation (3 minutes at 10,000 × g), dissolved in glycylglycine buffer (0.025 M, pH 7.0), adjusted to pH 7.0 with 1 N KOH, and brought to a final volume of 36 ml. (ethanol fraction).

To the ethanol fraction (36 ml.) were added 1.44 ml. of Tris buffer (0.1 M, pH 8.0) and sufficient calcium phosphate gel (24) (260 mg. of solids) to adsorb 40 to 50 per cent of the activity. After 5 minutes, the supernatant fluid was collected by centrifugation (calcium phosphate fraction).

To the calcium phosphate fraction (50 ml.) was added sufficient aged alumina gel Cγ (25) (22.5 mg. of solids) to adsorb 90 to 95 per cent of the activity. The gel was washed with 10 ml. of potassium phosphate buffer (0.01 M, pH 7.2) and the enzyme was then eluted with 10 ml. of sodium pyrophosphate buffer (0.03 M, pH 8.5) (aluminum hydroxide gel fraction).
Survey of Nucleoside Monophosphate Kinase Activities of Enzyme Preparation—ATP was tested as phosphate donor with a number of nucleoside monophosphates in the spectrophotometric assay system described for adenylate kinase (see above). A5P, U5P, G5P, and deoxyadenosine-5'-phosphate were active as acceptors; no activity was observed with inosine-5'-phosphate, deoxycytidine-5'-phosphate, deoxyguanosine-5'-phosphate, nicotinamide ribose-5'-phosphate, or adenosine-3'-phosphate. With UTP as donor, A5P and U5P proved to be acceptors. While UTP can be tested as a phosphorylating agent in this assay procedure (i.e., UDP participates in the pyruvate phosphokinase reaction but at a much slower rate than ADP (6)), the result is far less sensitive than with ATP and negative findings are less meaningful.

Stoichiometry in Transphosphorylation between UTP and U5P—When 2-Cl4-U5P and UTP were incubated with the purified enzyme, UDP was formed and approximately equal amounts of U5P and UTP disappeared (Table II). The UDP was isolated chromatographically and identified by its absorption spectrum (maximum at 262 mμ, λ280/λ260 = 0.37 in 0.01 N HCl) and by the molar ratios of uracil to acid-labile phosphate to total phosphate to phosphate acceptor (in the phosphopyruvate-pyruvate phosphokinase system) of 1.00:0.97:1.94:0.94. In the absence of either U5P or UTP, no UDP was formed.

The high specific radioactivity of the UTP and the low value for UDP (Table II) cannot be explained by equation (3), but they could be the result of the action of nucleoside diphosphokinase, which was observed to be

<table>
<thead>
<tr>
<th>Enzyme fraction</th>
<th>Total units</th>
<th>Over-all recovery</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autolysate</td>
<td>2860</td>
<td>100</td>
<td>5.3</td>
</tr>
<tr>
<td>Ethanol fraction</td>
<td>1410</td>
<td>49.3</td>
<td>25.1</td>
</tr>
<tr>
<td>Calcium phosphate fraction</td>
<td>707</td>
<td>24.7</td>
<td>53.2</td>
</tr>
<tr>
<td>Aluminum hydroxide gel fraction</td>
<td>591</td>
<td>20.6</td>
<td>174.0</td>
</tr>
</tbody>
</table>

The preparation and isolation of UDP in a larger amount were carried out with a 10 ml. reaction mixture, in which the amounts of U5P, UTP, and enzyme were increased 10-fold and the other components 5-fold. After incubation for 2 hours at 36°, the mixture was heated in a boiling water bath for 3 minutes and then chromatographed on a column of Dowex 1, chloride form (200 to 400 mesh, 2 per cent cross-linked, 5 cm. high, 1 cm. diameter) at 3°. 7.0 μmoles of UDP appeared between 37 and 60 resin bed volumes of 0.01 N HCl-0.05 M KCl. The eluate was concentrated by adsorption on and elution from Norit.
present in this enzyme preparation. When the experiment was repeated with an enzyme preparation that had been freed of nucleoside diphosphokinase by heating at low pH, the specific radioactivity of the UTP was reduced to less than 5 per cent of that of the UDP.

Equilibrium Constant for Uridylate Kinase Reaction—Starting with UTP and U5P on the one hand, or with UDP on the other, steady state concentrations were reached which appeared to represent an equilibrium point (Table III). Constants calculated in the direction of UDP formation in the experiment starting with UTP and U5P or with UDP were 0.92 and 0.95, respectively.

**TABLE II**

**Stoichiometry of UDP Synthesis from U5P and UTP**

<table>
<thead>
<tr>
<th></th>
<th>0 min.</th>
<th>45 min.</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles</td>
<td>c.p.m. per μmole</td>
<td>μmoles</td>
</tr>
<tr>
<td>U5P</td>
<td>2.20</td>
<td>17,000</td>
<td>1.81</td>
</tr>
<tr>
<td>UTP</td>
<td>1.64</td>
<td>0</td>
<td>1.27</td>
</tr>
<tr>
<td>UDP</td>
<td>0.00</td>
<td>0</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Transphosphorylation between UTP and A5P—A balance study of the transphosphorylation between adenosine and uridine nucleotides was carried out with 2-C14-A5P and UTP and an enzyme preparation which had been heated at acid pH (Table IV). Chromatographic analysis of the reaction mixture showed that the disappearance of 0.59 μmole of A5P was matched by the appearance of 0.61 μmole (combined) of ADP and ATP and the disappearance of 0.51 μmole of UTP by the appearance of 0.66 μmole (combined) of U5P and UDP. No reaction occurred when either A5P or UTP was omitted from the reaction mixture.

*The reaction mixture was the same as that shown in Table II, except for the specific activity of the U5P (58,000 c.p.m. per μmole) and the use of 30.1 units of enzyme which had been heated at pH 2.1 in a boiling water bath. 0.13 μmole each of U5P and UTP disappeared, and 0.27 μmole of UDP was formed. The specific activities of the nucleotides were found to be 55,300 for U5P, 1115 for UTP, and 24,450 c.p.m. per μmole for UDP.*
Synthesis of Guanosine Triphosphate On the basis of spectrophotometric evidence for the phosphorylation of G5P (see above) an attempt was made to isolate and identify the products.

The reaction mixture (50 ml.) contained 4.5 ml. of glycylglycine buffer (0.5 M, pH 7.5), 2.25 ml. of MgCl2 (0.1 M), 15.0 ml. of ATP (2.7 × 10^{-4} M),

<table>
<thead>
<tr>
<th>Products</th>
<th>UDP synthesis, μmole</th>
<th>UDP dismutation, μmole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min. 50 min. 100 min. 150 min.</td>
<td>0 min. 120 min. 180 min.</td>
</tr>
<tr>
<td>U5P</td>
<td>0.50* 0.41 0.36 0.34</td>
<td>0.24 0.26 0.27</td>
</tr>
<tr>
<td>UDP</td>
<td>0.17 0.26 0.29</td>
<td>0.76* 0.26 0.25</td>
</tr>
<tr>
<td>UTP</td>
<td>0.44* 0.31 0.28 0.27</td>
<td>0.21 0.24</td>
</tr>
<tr>
<td>K&lt;sub&gt;eq&lt;/sub&gt;†</td>
<td>0.92</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* Calculated values.
† Calculated in the direction of UDP synthesis.

15.0 ml. of 2-C<sup>14</sup>-G5P (8 × 10<sup>-5</sup> M, 15,800 c.p.m. per μmole), and 300 units of enzyme (ethanol fraction, heated for 3 minutes at pH 1.7 in a boiling water bath). In order to maintain a high level of ATP, 0.25 ml. of phosphopyruvate (0.05 M) and 5 units of pyruvate phosphokinase were also added. After incubation at 36° for 1 hour, the reaction mixture was heated in a boiling water bath for 3 minutes and then rapidly cooled. The entire reaction mixture was then chromatographed at 3° on a column of Dowex 1, chloride form (2 per cent cross-linked; height 3 cm., diameter 1 cm.). To elute any unchanged G5P, the column was first treated with 34
resin bed volumes of a solution of 0.01 N HCl-0.05 M KCl. Only negligible amounts of radioactivity were eluted and the eluent was changed to 0.01 N HCl-0.1 M KCl. The product, presumed to be GTP, was detected by radioactivity and spectrophotometric measurements and appeared as a

### Table IV

#### Transphosphorylation between Uridine and Adenosine Nucleotides

The reaction mixture (2.05 ml.) contained 0.2 ml. of glycylglycine buffer (0.5 M, pH 7.5), 0.1 ml. of MgCl₂ (0.1 M), 0.5 ml. of 2-C³⁴-A³P (0.002 M, 82,000 c.p.m. per µmole), 0.25 ml. of UTP (0.004 M), and 16 units of heated enzyme (ethanol fraction heated for 3 minutes at pH 1.5 in a boiling water bath; specific activity 28.3). After incubation at 36° for 1 hour, the reaction mixture was heated in a boiling water bath for 3 minutes. The entire reaction mixture was chromatographed on a column of Dowex 1, chloride form (2 per cent cross-linked; height 2 cm., diameter 1 cm.), at 3°. A³P was eluted with 0.01 N HCl. U³P and ADP were eluted together with 0.01 N HCl-0.05 M KCl, and UDP (separated from the other nucleotides except in a few ATP-containing fractions) was eluted with the same solvent. ATP was eluted with 0.01 N HCl-0.1 M KCl and UTP with a solution of 0.1 N HCl-0.1 M KCl. A³P was estimated spectrophotometrically at 260 µm and by radioactivity measurements and ADP by radioactive measurements and with pyruvate phosphokinase (0.29 µmole found). ATP was measured by radioactivity, by spectrophotometric determinations, and by enzymatic assay (17). U³P was estimated spectrophotometrically at 260 µm, with a correction for the ADP present. UDP was likewise measured spectrophotometrically (260 µm), with a correction for the ATP present, and, in addition, the two peak fractions were estimated with pyruvate phosphokinase (6). Spectrophotometric measurements showed the presence of 0.09 and 0.11 µmole and the enzymatic assay 0.08 and 0.10 µmole of UDP in the two fractions, respectively. UTP was estimated spectrophotometrically at 260 µm.

<table>
<thead>
<tr>
<th></th>
<th>0 min.</th>
<th>60 min.</th>
<th>∆</th>
<th>total c.p.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A³P</td>
<td>0.89</td>
<td>0.30</td>
<td>-0.59</td>
<td>-45,260</td>
</tr>
<tr>
<td>UTP</td>
<td>0.83</td>
<td>0.32</td>
<td>-0.51</td>
<td>+26,800</td>
</tr>
<tr>
<td>ADP</td>
<td>0.00</td>
<td>0.33</td>
<td>+0.33</td>
<td>+23,100</td>
</tr>
<tr>
<td>ATP</td>
<td>0.00</td>
<td>0.28</td>
<td>+0.28</td>
<td></td>
</tr>
<tr>
<td>U³P</td>
<td>0.00</td>
<td>0.31</td>
<td>+0.31</td>
<td></td>
</tr>
<tr>
<td>UDP</td>
<td>0.00</td>
<td>0.35</td>
<td>+0.35</td>
<td></td>
</tr>
</tbody>
</table>

Discrete, symmetrical peak between 1.9 and 14.6 resin bed volumes of the stronger eluent, with a peak at 7.4 volumes. It represented at least 70 per cent of the counts applied to the column. GTP was identified by its absorption spectrum (λ250/λ260 = 1.0, λ280/λ260 = 0.66, peak at 255 µm, in 0.01 N HCl; λ250/λ260 = 1.12, λ280/λ260 = 0.55, at pH 7) and by the

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⁴ ATP was eluted in part with the weaker eluent (first appearing at 11.9 resin bed volumes of eluent) and was completely removed in the early fractions of the stronger eluent (0 to 5.6 resin bed volumes of this eluent).
molar ratios of guanine to pentose to acid-labile phosphate to total phosphate of 1.00:0.96:2.02:3.02. The specific activity of the GTP was 15,700 c.p.m. per µmole.6

In a second experiment carried out with G5P (0.04 µmole), ATP32 (0.14 µmole, 7 X 10^6 c.p.m. per µmole), and a similar enzyme preparation, but without phosphopyruvate and pyruvate phosphokinase, chromatographic analysis of the reaction mixture revealed two peaks not present when G5P was omitted from the reaction mixture. The first peak (39,000 c.p.m.) appeared between 13 and 20 resin bed volumes of eluent (0.01 N HCl-0.05 M KCl) and was presumed to be GDP. The second peak (97,200 c.p.m.), considered to be GTP, was eluted with a stronger eluent (0.01 N HCl-0.1 M KCl) and appeared immediately after ATP with a peak at 12 resin bed volumes of this eluent.

**TABLE V**

**Differentiation of Uridylate and Adenylate Kinases by Heat Lability**

The heating was for 3 minutes in a boiling water bath.

<table>
<thead>
<tr>
<th>pH 7</th>
<th>Uridylate kinase remaining</th>
<th>Adenylate kinase remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>&quot; 7 + Mg^{++} (0.01 M)</td>
<td>&lt;1</td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>&lt;2</td>
<td>36.9</td>
</tr>
</tbody>
</table>

**Differentiation of Enzyme from Adenylate Kinase**—The activity responsible for the nucleotide transphosphorylation reactions described above was relatively stable to heating at low pH. Thus, after incubation of the ethanol fraction for 3 minutes in a boiling water bath at pH 1.3, 2.7, or 3.7, 53.2, 50.1, and 39.4 per cent, respectively, of the original mixed myokinase activities were recovered. While the adenylate kinase activity of yeast is also relatively stable to heating at acid pH (22), the two enzymes were readily distinguished by their heat stabilities at neutral pH (Table V).

**Influence of pH, Mg^{++}, and KCl on Enzyme**—Maximal rates of UDP + UTP formation were observed (under standard assay conditions, except that Tris-maleate buffer replaced glycylglycine) around pH 7.9. At pH values of 5.0, 5.6, 6.7, and 9.5, the rates were 24, 37, 63, and 87 per cent, respectively, of the maximal value. In the absence of added Mg^{++}, the rate was 18 per cent of that observed with 0.005 M Mg^{++}. With KCl at a

5 To avoid an absorption correction in radioactivity measurements, an aliquot of one of the fractions (1.5 ml.) was desalted by passing it through a column of Dowex 50 (H^{+}) resin bed volume of 1.0 ml. An aliquot of the desalted sample was dried on a glass cover-slip and counted.
concentration of 0.5 M, there was only a 30 per cent inhibition of the rate, and at a level of 0.2 M there was no effect at all.

DISCUSSION

The results of these studies indicate that UTP can be formed from U5P and ATP and that ATP can be formed from A5P and UTP. It seems clear that the mechanism is essentially similar to that of the adenylyl kinase ("myokinase") reaction (10, 11): there is a reversible transphosphorylation of the terminal phosphate from a nucleoside triphosphate to a nucleoside monophosphate to form two nucleoside diphosphates. It has been shown that the adenylyl kinase of muscle is strictly specific for adenosine nucleotides and it is evident from our studies that there is an adenylyl kinase in yeast with a similar specificity. It is now apparent that another enzyme (or possibly several enzymes) is present in yeast which catalyzes a transphosphorylation between uridine and adenosine nucleotides, guanosine and adenosine nucleotides, and apparently between uridine nucleotides. With regard to the latter reaction (U5P + UTP ⇌ 2UDP), the possibility exists that an adenosine nucleotide, as a trace contaminant in one of the reaction components, could catalytically effect a primary transphosphorylation with a uridine nucleotide. Thus far in our studies with yeast there is no evidence for the participation of an adenosine nucleotide in this reaction, but a rigorous test of this possibility must await further purification of the enzyme preparation. Such purification may also be helpful in determining whether a single enzyme with a wide range of specificity among the nucleotides, or a family of enzymes with restricted specificities, is responsible for the observed transphosphorylations.

SUMMARY

1. A partially purified enzyme preparation from yeast has been observed to catalyze transphosphorylations between adenosine, uridine, and guanosine nucleotides. The various nucleoside di- and triphosphates appeared

6 Similar reactions appear to have been observed by others with animal tissues (26) and in yeast (27).

7 Strominger, Heppel, and Maxwell observed no reaction with purified UDP preparations in their calf liver enzyme system. We found a UDP sample, which they kindly furnished us, to be active with our yeast enzyme. When 1.3 μmoles of this UDP preparation were incubated with 47.7 units of the partially purified yeast enzyme (aluminum hydroxide gel fraction, see Table I) for 105 minutes, the disappearance of 0.54 μmole of UDP was accompanied by the appearance of 0.32 and 0.23 μmole of U5P and UTP, respectively. Under the same conditions, incubation of the U5P (1.32 μmoles) and of UTP (0.78 μmole) preparations used in previous experiments yielded 0.50 μmole of UDP.
to be formed by a mechanism summarized by the general equations

\[(a) \quad \text{nucleoside-P} + \text{nucleoside-P-P-P} \rightleftharpoons \text{nucleoside-P-P} + \text{nucleoside-P-P} \]

\[(b) \quad 2 \text{nucleoside-P-P} \rightleftharpoons \text{nucleoside-P} + \text{nucleoside-P-P-P} \]

\[(c) \quad 2 \text{nucleoside-P-P} \rightleftharpoons \text{nucleoside-P} + \text{nucleoside-P-P-P} \]

These reactions provide a mechanism, hitherto unavailable, for the biosynthesis of UTP, GTP, and probably other nucleoside triphosphates from their respective monophosphates.

2. The enzyme (or enzymes) responsible for these reactions was distinguished from yeast adenylate kinase ("myokinase") by its lability to heating at neutral pH; like adenylate kinase, it is relatively stable to heating at acid pH.

3. Equilibrium constants and balance data for some of these reactions were determined.

**BIBLIOGRAPHY**

ENZYMATIC SYNTHESIS OF NUCLEOSIDE DIPHOSPHATES AND TRIPHOSPHATES
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