NUCLEOTIDE UTILIZATION BY ESCHERICHIA COLI*

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The utilization by the rat of yeast nucleotides labeled distinctively in the purine, sugar, and phosphate has been studied by Roll et al. (1, 2). They found that the purines of the 2' and 3' isomers of the purine nucleotides were both used to the same extent, but incorporation of the intact nucleotides was not observed. The ability of certain microorganisms to utilize efficiently the purine moieties of the 3' isomers of guanylic and adenylic acids has been demonstrated (3, 4). The inability of the 2' isomers to serve in the same manner has also been noted. We have investigated the utilization of the purine moiety and the phosphorus by microorganisms. Strains of Escherichia coli were used, and it was found that incorporation of the purine ring into the nucleic acid is accompanied or preceded by liberation of the phosphate.

EXPERIMENTAL

The glycine-2-C14 was obtained from Tracerlab, Inc. The isomeric adenylic acids and guanylic acids labeled with C14 in position 8 or with P32 were obtained from Dr. Herbert Weinfeld of this laboratory, who prepared them biosynthetically from yeast (1). The bacteria were grown in the glucose-salts medium of Gray and Tatum (5), modified by the omission of asparagine. The seed cultures were prepared by first growing the bacteria overnight on agar slants and then twice in the liquid medium used in the actual experiment. Washed saline suspensions of the bacteria were then added to the medium contained in Erlenmeyer flasks. The flasks were aerated vigorously.

E. coli W74 is a mutant of E. coli B, requiring either histidine or a purine. This organism was obtained from Dr. E. Witkin. Its nutritional responses have been described (6).

The bacteria were grown for 16 hours, washed with cold trichloroacetic acid, defatted with alcohol and ether, and hydrolyzed with 1 ml. of 1 N NaOH per 180 mg. of bacterial residue.

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For the isolation of the purines, the alkali-degraded PNA was separated from the DNA by acidification of the alkaline solution with HCl, followed by the addition of 1.5 volumes of ethyl alcohol. The DNA was removed by centrifugation and the supernatant solution containing the PNA fragments made 1 N with respect to sulfuric acid. The solution was heated at about 100° for 1 hour to hydrolyze the purine nucleotides. The purines were precipitated as silver salts, the free purines regenerated and then separated by paper chromatography, and the radioactivities determined as previously described (7, 8).

In the instances in which the PNA nucleotides were assayed, the sodium hydroxide hydrolysate was neutralized by the addition of a cation exchange (Dowex 50, H⁺ form) resin (9). A small amount of hydrochloric acid was then added to bring the pH to about 3. 1.5 volumes of alcohol were added, and, after the mixture was chilled, the DNA and protein were removed by centrifugation. The volume of the supernatant solution was reduced by evaporation in a stream of nitrogen. The volume was reduced so that an amount convenient for paper ionophoresis (about 5 to 10 µl.) contained about 8 to 10 optical density units (optical density at 260 µ × the volume in ml.). The solution was placed on a strip of Whatman No. 3 MM filter paper, 1.5 inches wide and 45 inches long. The paper was previously wet with 0.05 M ammonium formate adjusted to pH 3.6, and the excess buffer was removed by blotting (10). The paper was suspended in carbon tetrachloride as described by Markham and Smith (10), and a potential of 1000 volts was applied across the paper for 5 hours. The nucleotides were eluted from the paper with water, their concentrations and purity determined by ultraviolet absorption, and then plated on aluminum planchets.

All materials were assayed for radioactivity in films thin enough so that self-absorption could be ignored. Activities were determined with an internal Geiger-Müller flow counter (Radiation Counter Laboratories, mark 12, model 1, helium-isobutane gas). The activities of the carbon-labeled materials were determined to within standard errors of less than 5 per cent (11). The activity of the P³²-containing nucleotides was determined with a standard error of 10 per cent or less (11). No coincidence corrections were necessary. Activities are reported as relative specific activities (R. S. A.).

\[
R. \ S. \ A. = \frac{\text{c.p.m. per mole isolated compound}}{\text{c.p.m. per mole starting compound}} \times 100
\]

1 The following abbreviations have been used: PNA = pentose nucleic acid; DNA = deoxypentose nucleic acid, AMP-2' = adenosine-2'-phosphate; AMP-3' = adenosine-3'-phosphate; GMP-2' = guanosine-2'-phosphate; GMP-3' = guanosine-3'-phosphate.
RESULTS AND DISCUSSION

The incorporation of 2'- and 3'-adenylic acids was determined indirectly by measuring their ability to decrease the incorporation of glycine-2-C^{14}. The radioactivities of the nucleic acid purines isolated from bacteria grown in the presence of the substrate under study plus glycine-2-C^{14} were compared with the activities obtained when the organism was grown with glycine alone (7, 12). In the experiments with *E. coli* W74 no purine was required, since enough histidine was present to permit maximal growth. This histidine was necessary, since AMP-2' alone will not support growth. It was included in the medium with each isomer to give a suitable basis for comparison. The results (Table I) show that the purine moiety of the AMP-3' can be well utilized for the synthesis of PNA purines.

The results for *E. coli* were confirmed directly by the use of adenylic acid labeled with C^{14} in position 8 of the purine ring (Table I). Glycine-2-C^{14} would be expected to label positions 2, 5, and 8 (13), and the exogenous purine is labeled only in position 8. The fact that similar values are obtained in both cases suggests, by the reasoning indicated in work with *Lactobacillus casei* (7), that the purine ring is utilized intact.

To determine whether the entire ribotide might be utilized, nucleotides labeled with P^{32} were employed. The labeled adenylic acids were diluted with the respective unlabeled isomers to obtain enough material for several experiments. Since unlabeled samples of the individual isomers of the guanylic acids were not available for diluting, only one experiment could be run with each of the guanylic isomers. The results (Table II)

### Table I

*Utilization of Adenylic Acids for PNA Synthesis*

<table>
<thead>
<tr>
<th>Strain of <em>E. coli</em></th>
<th>Substrate</th>
<th>Per cent PNA purine derived from substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenine</td>
<td>Guanine</td>
</tr>
<tr>
<td>B</td>
<td>AMP-2'</td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>AMP-3'</td>
<td></td>
</tr>
<tr>
<td>W74</td>
<td>AMP-2'</td>
<td>+ histidine</td>
</tr>
<tr>
<td>&quot;</td>
<td>AMP-3'</td>
<td>+ &quot;</td>
</tr>
<tr>
<td>&quot;</td>
<td>AMP-2't + &quot;</td>
<td>2.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>AMP-3't + &quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

*100 - (R. S. A. of isolated material)/(R. S. A. of isolated material of control) × 100, where the control differs only in having no adenylic acid. The medium contained 20 γ per ml. (2.7 × 10^{-4} m) of glycine-2-C^{14}.

† The nucleotides were labeled with C^{14} in the 8 position.
show no specific utilization of the phosphorus of the proffered nucleotides. There was negligible incorporation of the phosphate of the 2' isomers, which can be correlated with the results with the purine-labeled compounds as well as with the fact that there appears to be little or no 2'-phosphatase in *E. coli*, although there is a 3'-phosphatase (14). Perhaps the phosphate moiety in the 3' isomers permits the transport of the molecule across cellular membranes and brings it to a locus where it can be converted to a derivative which can be utilized for nucleic acid synthesis. The phosphate liberated mixes with the phosphate available from the medium and is utilized non-specifically, resulting in a nucleic acid with all

**Table II**

Utilization of $P^{32}$ of Nucleotides for Nucleic Acid Synthesis

<table>
<thead>
<tr>
<th>Strain of <em>E. coli</em></th>
<th>Substrate</th>
<th>Relative specific activity</th>
<th>DNA, total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adenyllic acid</td>
<td>Guanylic acid</td>
</tr>
<tr>
<td>W74</td>
<td>AMP-2'† + histidine</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>AMP-3'† + &quot;</td>
<td>0.4</td>
<td>0.15</td>
</tr>
<tr>
<td>B</td>
<td>AMP-2'‡</td>
<td>0.4</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>AMP-3'</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>GMP-2'§</td>
<td>0.17</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>GMP-3'§</td>
<td>0.13</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* In all cases the activities are those of the mixed 2' and 3' isomers.  
† $1.13 \times 10^{-4}$ M.  
‡ $2.25 \times 10^{-4}$ M.  
§ $1.5 \times 10^{-4}$ M.

phosphates labeled to a small extent. On the basis of this information it is difficult to determine whether or not ribosides are intermediates in this sequence of reactions. Adenosine is well utilized by *E. coli* (2), but with *L. casei* all ribosides are less extensively utilized than are the 3'-ribotides (3). The patterns of interconversion of the purine moieties are quite different; hence simple dephosphorylation is not necessarily a major step in the process of incorporation of the purine moieties of the ribotides (3), and a mechanism which does not invoke this explanation is of more general applicability.

 Similar observations with phosphorus-labeled nucleotides were made by Rose and Carter (15). These authors did not use purine-labeled compounds, thus making extensive comparison of our data difficult.
In view of the observations discussed here and the results obtained in the rat (1, 2), it appears that ribotides serve for at least the efficient transport of certain heterocyclic bases. Their bases are in equilibrium with actual intermediates in nucleic acid synthesis. If the ribotides are true intermediates in nucleic acid synthesis, as has been suggested (3), then the purine and ribose phosphate moieties of the 3'-ribotides must be in rapid equilibrium with other sources of each entity, the nucleotide intermediates in equilibrium with these pools, and the ribose phosphate also in equilibrium with inorganic phosphate. Such a hypothesis is consistent with the known facts and, though not a unique explanation, does permit an interpretation applicable to more than one species.

SUMMARY

The incorporation of the phosphate and purine moieties of yeast adenylic and guanylic acids into the nucleic acid of *Escherichia coli* has been determined. The purines of the 3' isomers but not of the 2' isomers were well utilized. The phosphorus was used non-specifically from the 3' isomers, and to a negligible degree from the 2' isomers.

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