The Isolation of Guanosine Tetrphosphate from Commercially Available Preparations of Guanosine Triphosphate*

J. A. A. GARDNER† AND MAHLON B. HOAGLAND

From the Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts 02115

(Received for publication, September 18, 1964)

The isolation of adenosine tetraphosphate and pentaphosphate from samples of adenosine triphosphate has been reported by Marrian (1) and Sacks (2). The available evidence is consistent with a structure in which the phosphate residues are linked by phosphoanhydroxide bonds to one another and are carried by the 5'-hydroxyl group of the ribose moiety (1-4). During attempts to estimate guanosine triphosphate by ion exchange thin layer chromatography (5), it was found that the samples of commercial guanosine triphosphate investigated contained a component that migrated more slowly than guanosine triphosphate, and constituted between 5 and 10% of the absorbance at 252 nm. This paper is concerned with the purification and analysis of this component, which has proved to be guanosine tetraphosphate.

METHODS AND RESULTS

Preparation

The GTP used was obtained from the Sigma Chemical Company (Batch Nos. 34B-7510 and 34B-7540) and from Pabst Laboratories (Batch No. 2017). The chromatography of GTP on Dowex 1-formate can be seen in Fig. 1. G-tetraP was precipitated from selected, pooled fractions at pH 7 by addition of barium acetate and 1 volume of ethanol. The precipitate was washed with ethanol and redissolved in 0.05 M HCl. The barium ions were precipitated with Na₂SO₄, and the preparation was rechromatographed, as indicated in Fig. 1, when it gave a single peak. Selected fractions from this peak were lyophilized, and formate was removed by vacuum sublimation. The purity of the preparations was checked by thin layer chromatography (5), and gave a yield of 102.5% when assayed by thin layer chromatography. No other nucleotide could be detected in this material.

Identification

The results of chemical analyses performed on two G-tetraP preparations are summarized in Table I.

* This work was supported by a Grant GM-07928-04 from the United States Public Health Service.
† This work was done during the tenure of a Sir Walter Mulholland Scholarship from The New Zealand Meat Board.

The Sigma Chemical Company has confirmed our findings concerning the presence of guanosine tetraphosphate in these preparations (private communication). They have assured us that GTP batches containing guanosine tetraphosphate have been withheld from the market.

To correct for a small amount of ultraviolet-absorbing material that is eluted from the Dowex 1, an equivalent number of fractions containing no nucleotide material were collected from the column adjacent to the G-tetraP peak. These fractions were lyophilized and treated in the same way as the G-tetraP and subjected to all subsequent analyses.

Guanine—Guanine was identified as a component of the nucleotide in two ways. In Table I the ultraviolet absorbance ratios of G-tetraP are compared with published values for guanine. The spectra of the two compounds were indistinguishable at pH 2, 7, and 12.

After hydrolysis in 1 M HCl at 100° for 1 hour, the picrate complex of the base residue was prepared and recrystallized from hot water. The infrared spectrum of this complex was compared with the spectrum of an authentic sample of guanine picrate and with a similarly prepared complex from GTP. These spectra clearly showed that the base residue was guanine. The spectra were, in fact, identical apart from one small peak at 1700 cm⁻¹, which had a different magnitude in all three preparations. The reason for this variability is not yet known.

Guanine was estimated by its ultraviolet absorption with the value of 13.7 × 10⁴ for the molar absorbance of guanine nucleotide at 252 nm and pH 7. (7)

Phosphorus—Determined by the method of Lohman and Jendrassik (8), phosphorus samples were digested according to Fiske and SubbaRow (9) except that 30% H₂O₂ was used as the oxidant rather than HNO₃.

Acid-labile Phosphate—This is here defined as inorganic phosphate released from organic combination with n H₂SO₄ at 100° for 12 minutes.

Enzyme Analysis—With GMP as a standard compound to avoid differences in the amount of color given by free as compared to bound ribose, pentose analysis was carried out by the method of Albaum and Umbreit (10). The GMP used was obtained from the Nutritional Biochemicals Corporation (Ohio), and gave a yield of 102.5% when assayed by thin layer chromatography. No other nucleotide could be detected in this material.

The pentose moiety was identified by paper chromatography after hydrolysis at 100° in n HCl for 1 hour (11). The solvents used were 1-butanol-ethanol-water (4:1:5, Ṽ/Ṽ/Ṽ) and phenol saturated with water at 20° (12). The pentose spots were developed with aniline hydrogen phthalate spray reagent (13). The hydrolysate gave spots corresponding to ribose and ribose 5-phosphate. No other pentose spots could be seen.

Periodate Consumption—An estimation of periodate consumption was made according to Dixon and Lipkin (14). It may be...
seen that the values obtained are appreciably less than the theoretical value of 1 mole per mole of nucleotide. Similar low values were obtained with GTP both before and after purification on an ion exchange column, but in control experiments values close to 1 mole per mole were obtained with ATP.

The low periodate consumption seems to be due to the presence of 10 to 15% of another component with a modified 2'- or 3'-hydroxyl group. Partial separation of both the tri and tetra phosphorylated analogues of these components occurs on thin layer chromatography, where minor spots can be seen migrating immediately behind the respective nucleotides. The small peak appearing after the GTP peak in Fig. 1 is reproducible and is suspected to be due to this compound. Fractions taken from the trailing portion of the GTP peak are enriched in this component, but neither of these two contaminating nucleotides has yet been obtained pure in amounts sufficient for structural studies. They are not the deoxyribose analogues, however, since the GTP samples used contained no deoxyribose by the diphenylamine reaction (15).

**Effect on Protein Synthesis**

It has been known for some time (16) that GTP is required by certain cell-free amino acid incorporation systems. Since the present evidence shows that GTP preparations contain sub-

### Table I

**Analysis of G-tetraP compared with GTP**

<table>
<thead>
<tr>
<th></th>
<th>Guanosine</th>
<th>Phosphate</th>
<th>Adenosine phosphate</th>
<th>Guanosine 5'-phosphate</th>
<th>Pentose</th>
<th>Deoxyribose consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>moles/mole</td>
<td>moles/mole</td>
<td>moles/mole</td>
<td>moles/mole</td>
<td>moles/mole</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-tetraP</td>
<td>1.00</td>
<td>3.98</td>
<td>2.84</td>
<td>0.98</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>1.00</td>
<td>2.80</td>
<td>1.50</td>
<td>0.93</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-tetraP</td>
<td>1.00</td>
<td>3.89</td>
<td>2.81</td>
<td>0.94</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>GTP</td>
<td>1.00</td>
<td>2.92</td>
<td>1.84</td>
<td>0.97</td>
<td>0.87</td>
<td></td>
</tr>
</tbody>
</table>

### Table II

**Comparison of absorbance ratios of G-tetraP with published values for guanylic acid (6)**

The values were obtained with a Zeiss PMQ II spectrophotometer. The absorbance scale of the instrument was checked by reference to several other spectrophotometers, and we believe it to be correct within ±2% within the region of maximal precision (about 0.4 absorbance unit). The wave length scale was checked by reference to known compounds and was found to be correct within ±0.5 mμ.

<table>
<thead>
<tr>
<th>pH</th>
<th>G-tetraP</th>
<th>Published for guanylic acid*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abs. Area</td>
<td>Abs. Area</td>
</tr>
<tr>
<td>1</td>
<td>0.50</td>
<td>0.68</td>
</tr>
<tr>
<td>7</td>
<td>0.274</td>
<td>0.65</td>
</tr>
<tr>
<td>12</td>
<td>0.10</td>
<td>0.59</td>
</tr>
</tbody>
</table>

* Taken from Beaven, Holiday, and Johnson (6). The values at pH 7 and pH 12 are the same for the 2', 3', and 5' isomers. At pH 1 the values given are for the 5' isomer. The 2' isomer has A₂₉₀, A₆₅₀, A₁₉₀, and A₂₆₀/Å₆₅₀ ratios of 0.48, 0.68, and 0.90, respectively. Similarly, the 3' derivative has figures of 0.49, 0.69, and 0.93.

---

**Fig. 1.** The purification of guanosine tetraphosphate (G-tetraP). One gram of GTP (Sigma Chemical Company) was absorbed to a column (20 X 1 cm) of Dowex 1-formate (200 to 400 mesh, 8% cross-linked, acid- and alkali-washed). Elution was carried out with a linear gradient of 0.5 M to 2.0 M ammonium formate, pH 4.8 (2 liters, total eluant). The flow rate was adjusted to 0.5 ml per minute, and 5-ml fractions were collected.

**Fig. 2.** Effect of purified preparations of guanosine triphosphate and guanosine tetraphosphate on a cell-free amino acid incorporation system from rat liver. "Heavy" microsomes were prepared from the livers of normal adult rats, and pH 5 fraction from regenerating livers, as described in a recent paper (17). The microsomes were washed once by resuspension at a concentration of 0.5 mg of RNA per ml and by recentrifugation at 60,000 X g for 15 minutes. Incubations were carried out at 37° for 30 minutes with the following final concentrations of components: microsomes (about 0.6 mg of RNA per ml), pH 5 fraction from regenerating livers, as described in a recent paper (17). The microsomes were washed once by resuspension at a concentration of 0.5 mg of RNA per ml and by recentrifugation at 60,000 X g for 15 minutes. Incubations were carried out at 37° for 30 minutes with the following final concentrations of components: microsomes (about 0.6 mg of RNA per ml), pH 5 fraction, 2.6 mg of protein per ml; phosphoenol pyruvate, 0.05 mg per ml; phosphoenol pyruvate kinase, 0.05 mg per ml; pyruvate dehydrogenase, 0.01 mg; ATP, 0.3 mg; MgCl₂, 0.0025 M; sucrose, 0.075 M; L-leucine, 0.02 mM (0.1 μC). Reactions were stopped with trichloroacetic acid and proteins were washed with hot trichloroacetic acid and lipid solvents (18); dissolved in 1 N NH₄OH, plated on metal planchets, dried, and counted in a Nuclear-Chicago gas flow counter. Activity with G-tetraP, ⬤.—⬤. Activity with GTP, ●.—●.
stantial amounts of G-tetraP, we assayed purified preparations of each nucleotide for activity in protein synthesis.

Microsome preparations from normal adult rat liver afford a useful assay system as they are highly responsive to GTP. This property is apparently due to their high content of a natural inhibitor of protein synthesis, the action of which is antagonized by GTP (17). Fig. 2 shows the effects of GTP and G-tetraP on such an incorporation system. The GTP used in this experiment contained no detectable G-tetraP or GDP on assay by thin layer chromatography, but the G-tetraP preparation contained 8% GTP. It may be seen that pure GTP saturated the system at 0.3 to 0.4 mM as expected from earlier work with unresolved preparations. On the other hand, G-tetraP consistently stimulated at concentrations below 0.1 mM but inhibited at higher concentrations. Addition of a maximally stimulating concentration of G-tetraP (0.03 mM) together with 0.3 mM GTP gave no greater incorporation than that given by the latter alone.

**DISCUSSION**

The analytical data presented in Table I are consistent with a structure for the new nucleotide analogous to that proposed (1, 3, 4) for adenosine tetraphosphate. The periodate reaction shows that the 2' and 3' positions of the ribose moiety are unsubstituted, at least for about 90% of the molecules in the preparations. The spectral data would render unlikely a structure in which one or more phosphate residues are carried on the purine ring system. Finally, the acid-labile phosphate estimations are in agreement with the idea that the phosphate residues are linked to one another by phosphoanhydride bonds.

G-tetraP seems to stimulate the rat liver cell-free amino acid incorporation system somewhat, but it is clearly not as effective as pure GTP. Its activity may well be due to conversion to GTP. If this were so, it is possible that the inhibition seen at higher concentrations of G-tetraP may be due to competition between GTP and G-tetraP.

We have as yet no evidence for the natural occurrence of G-tetraP. GTP is prepared commercially from equine muscle, but whether the presence of G-tetraP in these preparations is because of its natural occurrence in the tissue or whether it is an artifact must be the subject of further study.

**SUMMARY**

The isolation of guanosine tetraphosphate from commercial preparations of guanosine triphosphate is reported. Chemical analysis indicates that the structure of this compound is guanosine 5'-tetraphosphate.

This nucleotide apparently has some activity in stimulating amino acid incorporation by rat liver microsomes but is not as active as guanosine triphosphate at low (0.05 mM) concentrations, and it inhibits the system at higher concentrations (0.4 mM).

There is as yet no evidence concerning the natural occurrence of this nucleotide.

**Acknowledgment—**The authors are indebted to Mr. David Coleman for running the infrared spectra.

**REFERENCES**

The Isolation of Guanosine Tetrrophosphate from Commercially Available Preparations of Guanosine Triphosphate
J. A. A. Gardner and Mahlon B. Hoagland


Access the most updated version of this article at http://www.jbc.org/content/240/3/1244.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/240/3/1244.citation.full.html#ref-list-1