OCCURRENCE OF METHYLATED PURINE BASES IN YEAST RIBONUCLEIC ACID*

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(Received for publication, June 27, 1957)

A number of methylated purine bases have recently been reported to occur in small quantities in the urine of normal human subjects. These include 1-methylguanine, N²-methylguanine (6-hydroxy-2-methylaminopurine), 1-methylhypoxanthine, and 8-hydroxy-7-methylguanine (1) in addition to 7-methylguanine, previously known as a urinary constituent (2). These compounds were found to be metabolites of endogenous origin (3). Their metabolic role has not been elucidated.

The present study was undertaken to determine whether some of these methylated purines may occur as minor components of nucleic acids. Because of their ready availability, preparations of yeast ribonucleic acid and deoxyribonucleic acids from calf thymus and herring sperm were first examined.

EXPERIMENTAL

Materials and Methods—The yeast nucleic acid was purchased from the Schwarz Laboratories, Inc., the sperm deoxyribonucleic acid from the Nutritional Biochemicals Corporation, and the thymus deoxyribonucleic acid ("highly polymerized") from the Worthington Biochemical Corporation. We wish to thank Dr. G. B. Elion and Dr. G. H. Hitchings for the synthetic specimens of N²-methyladenine (6-methylaminopurine), 1-methylguanine, and N²-methylguanine used. The conditions employed throughout this study for paper chromatography and ion exchange chromatography on analytical columns of Dowex 50 were those described in a previous report (1).

Ion Exchange Chromatography of Yeast Ribonucleic Acid Hydrolysates—A solution of 250 mg. of the yeast nucleic acid preparation in 50 ml. of N sulfuric acid was heated for 1 hour at 100°. These conditions of hydroly-
sis have been reported to liberate the purines as free bases, leaving the pyrimidines largely in the form of nucleotides (4). The filtered solution, diluted with water, was chromatoographed on the Dowex 50 column. The chromatogram showed several peaks preceding the guanine peak (Fig. 1, A). These were believed to represent pyrimidine nucleosides and were not investigated further. The region where the peaks for methylated guanines would normally occur, if present (tube numbers 97 to 139, Fig. 1, A), contained too little material for investigation. The material in the peak at tube number 177, immediately after adenine, was investigated by paper chromatography and ultraviolet spectrophotometry. It contained a substance, the properties of which were similar to those of N⁶-methyladenine, which was estimated to constitute 0.1 per cent of the purine content of the nucleic acid preparation.

To secure sufficient material for further investigation, 2.5 gm. of yeast nucleic acid were hydrolyzed as before. Sulfuric acid was removed with barium hydroxide (added to pH 1) and barium chloride. The solution was concentrated and chromatographed on the Dowex 50 column. The chromatogram at this 10-fold heavier loading showed a considerable widening of the guanine and adenine bands and a shift of their peaks toward lower tube numbers (Fig. 1, B). N⁶-Methyladenine was incompletely resolved from adenine in this chromatogram and appeared in a terminal shoulder.

Fig. 1. Fractionation of a yeast ribonucleic acid hydrolysate at various loadings on identical Dowex 50 columns (Peak GU is guanine, Peak AD is adenine, Peak M is N²-methylguanine, Peak N is 1-methylguanine, Peak P is N⁶-methyladenine; the numerical values indicate the peak tube number and peak optical density, respectively, where these occur off the scale of the plot). A represents chromatography of 250 mg. of hydrolysate; B represents chromatography of 2.5 gm. of hydrolysate; C represents rechromatography of the material taken from the areas indicated in B.
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(tube numbers 150 to 180, Fig. 1, B). A minor peak also appeared between the large peaks for adenine and guanine, suggesting the possible presence of methylated guanines.

To isolate these minor constituents from the contaminating adenine and guanine, the contents of the tubes numbered 82 to 102 and 140 to 175 were pooled, concentrated in vacuo, and chromatographed on a new Dowex 50 column. In addition to the remaining guanine and adenine, this new column (Fig. 1, C) resolved three distinct small peaks.

\(N^6\)-Methyladenine—The material in Peak P of the last chromatogram (tube numbers 168 to 182, Fig. 1, C) was purified by band chromatography on Whatman No. 3MM paper developed with butanol-ammonia. The material in the principal band was eluted and again chromatographed as a band, with butanol-formic acid as the developer. The ultraviolet absorption at 260 m\(\mu\) of the substance eluted from the principal band was approximately 80 per cent that of Peak P. Part of the eluate was used for the determination of the absorption spectrum at several pH values. The remaining material served for determination of \(R_F\) values and treatment with nitrous acid.

As seen in Fig. 2 and in Table I, the correspondence between the ultraviolet absorption spectrum of the substance so separated from the yeast nucleic acid hydrolysate and an authentic specimen of \(N^6\)-methyladenine is excellent at four pH values. The mobilities on the Dowex 50 column used and on paper chromatograms developed with two solvents also correspond for the yeast \(N^6\)-methyladenine and the synthetic specimen (Table

![Fig. 2. Ultraviolet absorption of a synthetic specimen of \(N^6\)-methyladenine (curves) and of the specimen of this substance from yeast nucleic acid (points) at several pH values (pH 2.0, \(\bullet\); pH 5.7, O; pH 14, \(\bigcirc\)).](http://www.jbc.org/)

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I). The identification was further confirmed by treatment of each specimen with nitrous acid (5) under the conditions previously described (1). The nitrous acid product, presumed to be \(N^6\)-nitroso-\(N^6\)-methyladenine

<table>
<thead>
<tr>
<th>Substance and source*</th>
<th>Tube No., in Dowex 50 column†</th>
<th>RF value in solvent‡</th>
<th>Ultraviolet absorption at pH 2.0</th>
<th>pH 9.0</th>
<th>pH 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Butanol-ammonia</td>
<td>Butanol-formic acid</td>
<td>(\lambda_{\text{max}})</td>
<td>(\lambda_{\text{min}})</td>
<td>(\lambda_{\text{max}})</td>
</tr>
<tr>
<td>(N^6)-Methyladenine</td>
<td>Y 172</td>
<td>0.56 0.45</td>
<td>267.5 232.5 266.5 232 273 240</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>A 169</td>
<td>0.57 0.45</td>
<td>267.5 232.5 266.5 231 273 239</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Nitroso-(N^6)- methyadenine&quot;</td>
<td>Y 172</td>
<td>0.46 0.65</td>
<td>263§ 243 266§ 247.5 273.5 242</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;Nitroso-(N^6)- methyadenine&quot; A 169</td>
<td>0.46 0.65</td>
<td>203§ 242 207§ 247.5 274 242</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N^2)-Methylguanine</td>
<td>Y 100</td>
<td>0.22 0.31</td>
<td>261.5 230 247 229 257§ 245</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>A 97</td>
<td>0.22 0.30</td>
<td>250.5 228 246.5 229 256§ 244</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-Methylguanine</td>
<td>Y 106</td>
<td>0.17 0.19</td>
<td>251 227 249.5 227.5 275 243.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;</td>
<td>A 105</td>
<td>0.18 0.21</td>
<td>250 227.5 249.5 227 276.5 241.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Y denotes the substance derived from a yeast nucleic acid hydrolysate, and A denotes the substance derived from a synthetic specimen.
† For the substances from yeast, this represents the peak tube number in the Dowex 50 column (Fig. 1, C) used for isolation of the specimens; the tube numbers for the peaks given by synthetic specimens are those published previously (1).
‡ These RF values were determined at the same time for the present purpose, and they are slightly different from those reported earlier (1).
§ These values represent shoulders, points of inflections, or poorly defined peaks and are approximate.

\(N^6\)-Methyladenine—One principal band was obtained in similar band

(5), obtained from the natural specimen and that obtained from synthetic \(N^6\)-methyladenine showed similar characteristics (Table I). The data given here for \(N^6\)-methyladenine correspond with those in the literature (5-7). The amount of \(N^6\)-methyladenine in the yeast nucleic acid specimen was again estimated to be 0.1 per cent of the purine content.

\(N^2\)-Methylguanine—One principal band was obtained in similar band
paper chromatography of the material in Peak M (tube numbers 98 to 103, Fig. 1, C). The spectral characteristics of the substance eluted from this band, as well as its mobilities on paper and on the Dowex 50 column, were similar to those of synthetic N2-methylguanine (Table I). It was estimated to constitute 0.01 per cent of the purine content of the yeast ribonucleic acid specimen.

1-Methylguanine—Similar purification of the principal component of Peak N (Fig. 1, C, tube numbers 104 to 114) gave material whose properties indicated its identity with synthetic 1-methylguanine (Table I). The yield of this substance was approximately the same as that of N2-methylguanine.

Deoxyribonucleic Acids—The deoxyribonucleic acid present as a contaminant (8) in the yeast ribonucleic acid specimen used was obtained nearly pure by alkali treatment and alcohol precipitation (8). A 200 mg. sample of the resulting deoxyribonucleic acid was hydrolyzed with perchloric acid (9). After removal of the perchlorate as the potassium salt, the hydrolysate was chromatographed on the Dowex 50 column. No indication of the presence of methylated guanines or N6-methyladenine was found. Similar negative results were obtained with hydrolysates of commercial deoxyribonucleic acid preparations from herring sperm and from calf thymus.

**DISCUSSION**

Demonstration of the occurrence in various deoxypentose nucleic acids of nitrogenous bases other than adenine, guanine, cytosine, or thymine has been made possible by the introduction of appropriate sensitive techniques for their separation and identification. Thus, 5-methylcytosine has been discovered to occur in amounts ranging from 6 to 0.2 per cent of the base content in deoxypentose nucleic acids from sources including wheat germ, herring sperm, calf thymus, and the locust (10). 5-Hydroxymethylcytosine has been found to replace cytosine entirely in the deoxypentose nucleic acids of some *Escherichia coli* bacteriophages (11). N6-Methyladenine has been found to occur in amounts approximating 0.4 per cent of the base content in the deoxypentose nucleic acids of some strains of *E. coli* and in amounts as large as 4 per cent for thymine-requiring mutants grown in thymine-deficient media (5).

In the case of the pentose nucleic acids, the occurrence of similar so-called “satellite” (12) nitrogenous bases, as described here, apparently has escaped previous notice. The demonstration of N6-methyladenine, 1-methylguanine, and N2-methylguanine in the yeast nucleic acid specimen examined was made possible by techniques specifically designed for the detection of trace components. A closer examination than heretofore may
well reveal similar minor components in pentose nucleic acids from other sources. That the trace substances found do not originate from the deoxyribose nucleic acid present as a contaminant has been demonstrated under "Experimental." While the possibility that they might originate in substances adsorbed on the nucleic acid appears unlikely, it cannot be entirely excluded by the present experiments.

The relative proportion of 1-methylguanine or N²-methylguanine now found in the yeast nucleic acid specimen, 0.01 per cent of the purine content, is rather smaller than the proportion of these bases previously found in human urine, 0.1 per cent of the uric acid content (3). It may be pointed out that N⁶-methyladenine has not been detected in human urine, although it is recoverable when added (1). The presence in yeast nucleic acid of 7-methylguanine, the principal endogenous methylated purine base of human urine, would not be detected by the present methods because of interference by the large amount of adenine. However, the occurrence of 7-methylguanine in any polynucleotide would be unexpected, since ribose is linked to the purines in position 9 in the known naturally occurring ribonucleotides, and this position is blocked by the 7-methyl substitution.

Ribonucleic acids from various sources including yeast are known to be grossly inhomogeneous as judged by physical (13, 14), chemical (15, 16), and metabolic (cf. (16) and (17) for bibliography) criteria. The number of distinct polymeric species present in any specimen is unknown. The methylated purine bases reported here may be randomly incorporated into the various component nucleic acids in the specimens as the result of incompletely specific enzymatic action. On the other hand, it appears possible that the methylated purines represent functional constituents of particular nucleic acids. In this case, because of the low apparent molecular weight of yeast ribonucleic acid (18) and the small amounts of these bases, the nucleic acids containing them would be expected to be minor components (as little as 1 per cent) of the total ribonucleic acid.

**SUMMARY**

Investigation of the occurrence in yeast ribonucleic acid hydrolysates of trace amounts of purine bases other than adenine or guanine revealed the presence of N⁶-methyladenine (6-methylaminopurine), N²-methylguanine (6-hydroxy-2-methylaminopurine), and 1-methylguanine. These substances were identified by spectrophotometric and chromatographic comparisons with authentic specimens. The N⁶-methyladenine constituted 0.1 per cent of the purine content of the specimen examined. Each of the methylated guanines was present in about one-tenth this amount. The methylated purines found were not detected in hydrolysates of deoxyribose nucleic acid from yeast, calf thymus, or herring sperm.
BIBLIOGRAPHY

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