Patterns of Urinary Excretion of Modified Nucleosides

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ABSTRACT

A recently developed high-performance liquid chromatography method permits quantitative measurement of low levels of modified nucleosides in urine. We report on the patterns of excretion of seven modified nucleosides by normal subjects and cancer patients. It was found that the excretion of these nucleosides expressed as a function of creatinine concentration was constant, not episodic nor related to diet. Thus, randomly collected samples of urine are satisfactory sources for measurement of nucleosides, and the level of nucleosides is of significance when related to creatinine excretion. The constancy of the excretion of the modified nucleosides in normal subjects is quite remarkable. It implies strict metabolic control of transfer RNA turnover. The values for the individual nucleoside/creatinine ratios were found to be significantly elevated in the urine of colon cancer patients.

INTRODUCTION

tRNA has the most complex structure of all the biopolymers. Of its approximately 85 bases, as many as 20% may be modified. The modifications, which number over 50, may be as simple as a methyl group or may be extremely complex. All of these modifications from the simplest to the most complex are achieved after the synthesis of the primary sequence by extraordinarily specific enzymes: they are species specific, base specific, site specific, and sequence specific. Some of the modifications such as methylation are achieved by one enzyme, but the more complex modifications such as the "Q base" require the sequential intervention of several enzymes. Thus, while the synthesis of the primary sequence of tRNA probably requires but one enzyme, RNA polymerase, the completion of its structure for its many functional potencies requires scores of enzymes.

The discovery of the nature of the modifying enzymes of nucleic acids resolved a long-standing paradox of renal physiology. The presence of modified bases and nucleosides in urine had been known for a long time, but their origin remained obscure. Since the modified bases must be in specific positions, to prevent the insertion of a modified nucleoside (resulting from the turnover of tRNA) into a forbidden position, the kinases to convert modified nucleosides into nucleotide triphosphates are lacking from mammalian cells; consequently, the nucleosides are excreted (2). An example of this fail-safe system is the level of excretion of uridine and pseudouridine; the former, which is a major component of RNA, is excreted in traces only since it can be recycled into the macromolecule, but the latter, which is a minor component of RNA, is voided in quantities as large as 60 mg in a 24-hr period by a normal subject (for a detailed review, see Ref. 2).

It has been observed that cancer patients excrete elevated levels of methylated purines (7). It is obvious that some of them must stem from the breakdown of tRNA since they are present only in that nucleic acid. Whether the elevated levels originate from massive cell death or specifically increased metabolism was obscure until recently. We have shown by differential labeling of $\beta$-aminoisobutyric acid, which is a degradation product of the thymine of both DNA and tRNA, that there is a very high turnover of tRNA in the tumor tissue of an animal model (1).

The possible molecular mechanism of the origin of the elevated levels of the excretion products has been partially elucidated. The enzymes that methy1ate tRNA are aberrantly hyperactive in every malignant tumor, and every malignant tumor contains a small number of isoa11cepting tRNA's unique to it (2). One of these tumor-specific tRNA's has been purified and analyzed recently; tumor-specific tRNA$^{Phe}$ contains 2 supernumerary methylated bases (5). The increased excretion of tRNA breakdown products by cancer patients probably stems from the high turnover of tRNA, but the molecular mechanism of the high turnover remains obscure.

We have been engaged in an extensive study to determine whether the elevated levels of excretion products, nucleosides in particular, by cancer patients could be used for diagnosis or for monitoring the effectiveness of cancer therapy (9). For these studies, we have developed a rapid, reliable high-performance liquid chromatographic method of assay which has been published elsewhere (4).

MATERIALS AND METHODS

The Determination of Nucleosides. The determination of nucleosides was described in detail in Ref. 4.

Determination of Creatinine. The traditional Jaffe alkaline picrate method tends to yield somewhat variable results because of the presence in the urine of nonspecific chromogens (3). A rapid kinetic-rate measurement in the first 10 to 30 sec of the reaction eliminates such nonspecific interference. An instrument designed by Beckman Instruments for such measurements can be used routinely. It is sold under the designation Creatinine Analyzer No. 2.

Urine Collections. Urine was collected and kept at -80° until analysis without any preservative. No dietary restric-

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tions were imposed; the patients were not receiving any antitumor therapy. The samples were coded so the analysts were unaware of their source.

RESULTS

The Excretion of Modified Nucleosides Is Not Episodic.

Our initial studies were on 24-hr total urine collections (9). To avoid the inconvenience of such collections, we undertook studies on randomly collected samples with a view toward developing more convenient methods. It was found that, if the nucleoside levels are related to the creatinine level of the random sample, such determinations are as valid as those on urine voided in 24 hr. In Tables 1 to 3, the data for the excretion levels of 3 of the nucleosides in random samples related to 24-hr collections are presented. Since the creatinine output is directly related to muscle mass, the production of modified nucleosides must also be related to total muscle mass. Such estimations of nucleoside levels will obviously be invalid in subjects with syn-

Table 1
Excretion of pseudouridine relative to creatinine by normal males

Random samples and urine voided during 24 hr were collected and frozen. On aliquots of each, pseudouridine and creatinine were determined. Methods are described in Ref. 4. In these and all subsequent experiments, samples were coded so that the analysts were not aware of the origin of the samples.

<table>
<thead>
<tr>
<th>Collection and time</th>
<th>Pseudouridine (nmol/μmol creatinine)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td>σ</td>
<td>RSD%</td>
</tr>
<tr>
<td>Pseudouridine, 8 a.m.</td>
<td>10</td>
<td>22.8</td>
<td>2.60</td>
<td>11.4</td>
</tr>
<tr>
<td>Pseudouridine, 10 a.m.</td>
<td>10</td>
<td>22.9</td>
<td>1.65</td>
<td>7.2</td>
</tr>
<tr>
<td>Pseudouridine, 3 p.m.</td>
<td>10</td>
<td>22.5</td>
<td>2.98</td>
<td>13.2</td>
</tr>
<tr>
<td>Pseudouridine, 24 hr</td>
<td>10</td>
<td>22.3</td>
<td>1.30</td>
<td>5.83</td>
</tr>
<tr>
<td>Pseudouridine, total</td>
<td>40</td>
<td>22.6</td>
<td>2.16</td>
<td>9.6</td>
</tr>
</tbody>
</table>

* x/x₂₄, a ratio for the average value of each (random or total) collection to the average value for a 24-hr total collection; RSD%, relative S.D.

b Total, the combination of all data for random and 24-hr samples.

Table 2
Excretion of 1-methylinosine relative to creatinine by normal males

Method of procedure as described in Table 1.

<table>
<thead>
<tr>
<th>Collection and time</th>
<th>m₁(μmol/μmol creatinine)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td>σ</td>
<td>RSD%</td>
</tr>
<tr>
<td>m₁, 8 a.m.</td>
<td>10</td>
<td>1.05</td>
<td>0.34</td>
<td>32</td>
</tr>
<tr>
<td>m₁, 10 a.m.</td>
<td>10</td>
<td>1.18</td>
<td>0.20</td>
<td>17</td>
</tr>
<tr>
<td>m₁, 3 p.m.</td>
<td>10</td>
<td>1.13</td>
<td>0.32</td>
<td>28</td>
</tr>
<tr>
<td>m₁, 24 hr</td>
<td>10</td>
<td>1.26</td>
<td>0.16</td>
<td>13</td>
</tr>
<tr>
<td>m₁, total</td>
<td>40</td>
<td>1.15</td>
<td>0.27</td>
<td>23</td>
</tr>
</tbody>
</table>

* m₁, 1-methylinosine; x/x₂₄, a ratio for the average value of each (random or total) collection to the average value for a 24-hr total collection; RSD%, relative S.D.

b Total, the combination of all data for random and 24-hr samples.

The Range of Excretion Levels of Some Nucleosides Is Very Narrow in Normal Subjects and Widely Elevated in Cancer Patients. In Charts 1, 2, and 3, the scattergrams of the excretion levels of 5 modified nucleosides in the urine of normal subjects and in 10 patients with advanced cancer of the colon are presented. The narrowness of the range of excretion levels related to creatinine in normal subjects is

Table 3
Excretion of N²,N²-dimethylguanosine relative to creatinine by normal males

Method of procedure as described in Table 1.

<table>
<thead>
<tr>
<th>Collection and time</th>
<th>m₂G(μmol/μmol creatinine)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>x</td>
<td>σ</td>
<td>RSD%</td>
</tr>
<tr>
<td>m₂G, 8 a.m.</td>
<td>10</td>
<td>1.10</td>
<td>0.09</td>
<td>8.2</td>
</tr>
<tr>
<td>m₂G, 10 a.m.</td>
<td>10</td>
<td>1.21</td>
<td>0.12</td>
<td>9.9</td>
</tr>
<tr>
<td>m₂G, 3 p.m.</td>
<td>10</td>
<td>1.22</td>
<td>0.21</td>
<td>1.6</td>
</tr>
<tr>
<td>m₂G, 24 hr</td>
<td>10</td>
<td>1.26</td>
<td>0.11</td>
<td>17</td>
</tr>
<tr>
<td>m₂G, total</td>
<td>40</td>
<td>1.20</td>
<td>0.15</td>
<td>0.95</td>
</tr>
</tbody>
</table>

* m₂G, N²,N²-dimethylguanosine; x/x₂₄, a ratio for the average value of each (random or total) collection to the average value for a 24-hr total collection; RSD%, relative S.D.

b Total, the combination of all data for random and 24-hr samples.

Chart 1. Pseudouridine (•) excretion relative to creatinine content in 40 normal subjects and in 10 patients with cancer of the colon. The normal control urines were collected from laboratory personnel with an age span of 20 to 60 years. The cancer patients selected had advanced colon cancer, and at the time of the urine collection the patients were not receiving any antineoplastic drugs or other antitumor therapy. The urine samples from cancer patients were obtained through the courtesy of the National Cancer Institute, Solid Tumor Service. No dietary restrictions were imposed on the subjects.
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Chart 2. Excretion patterns of 4-acetylcytidine (ac*C) and 1-methyladenosine (m'A). Conditions as in Chart 1.

Chart 3. Excretion patterns of 1-methylinosine (m'I) and N2,N2-dimethylguanosine (m'2G). Conditions as in Chart 1.

quite remarkable. It implies an extraordinarily rigid control over the turnover of tRNA, the mechanism of which is totally obscure.

The elevation of some of the nucleosides in some patients is extraordinarily high. The total daily excretion of N2,N2-dimethylguanosine in a normal subject is 2 mg. In some patients, it may be 10-fold higher, which can result only from a very high level of tRNA turnover, which has been demonstrated by the tracer methodology cited earlier (1). The excretion of elevated levels of modified nucleosides is an apparent attribute of every type of cancer. In a recent study done in collaboration with Dr. John Speer of Penrose Hospital, Colorado Springs, Colo., it was found that 26 of 27 patients with cancers of different organs had levels 2 S.D. above normal of one or more of the following 7 products: pseudouridine, β-aminoisobutyric acid, 1-methyladenosine, 1-methylinosine, N2-methylguanosine, N2,N2-dimethylguanosine, and 2-pyridone-5-carboxamide-N'-ribofuranoside, which is a metabolite of NAD+ (8). The level of elevation correlates approximately with the stage of the cancer, but the relative levels of the various nucleosides appear to be an attribute of the tissue site of the tumor (9). The reason for the variation in the elevation of the different modified nucleosides in cancer patients is obscure at present. It may stem from the selective breakdown of some tRNA's, or some of the modified nucleosides may be metabolized by the bacterial flora of the genitourinary tract. These studies will have to be extended with a view to the possible diagnosis of the sites and the severity of cancers which are inaccessible by other diagnostic means.

Thirty UV-absorbing Nucleosides in Normal Urine Are Detected and Quantitated by High-Pressure Liquid Chromatography. We have successfully separated 30 UV-absorbing nucleosides in normal urine. However, not all of them stem from tRNA turnover. Since the nucleosides are trapped from urine on an affinity gel containing an immobilized phenylboronic acid, any product which contains a ribose moiety and is UV absorbing would be isolated. In Chart 4, the level of excretion of 2 unknown cis-hydroxyl-containing, UV-absorbing products identified by their retention time is presented. These 2 and 10 other such products remain to be identified. The discovery of 12 heretofore unobserved putative nucleosides is not surprising. This has been the pattern in this area of research; as methodology became more sophisticated and discerning, increasing numbers of such products became evident. In the initial study of the origin of methylated purines in the urine of experimental animals, only 7 such products were identified (6).

The Level of Excretion of the Modified Nucleosides Returns Very Close to Normal Levels Very Soon after Effective Chemotherapy. The level of excretion of the nucleosides was followed before, during, and after therapy in 2 cancers which respond well to chemotherapy. Within 5 days of the commencement of therapy in 6 patients with
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Burkitt’s lymphoma, the excretion levels returned to normal and remained normal as long as the subjects were in remission. In one subject who experienced relapse, the excretion levels rose again (10).

After 10 days of therapy in 3 children with T-cell acute lymphocytic leukemia, a similar return to normal excretion levels was observed.³

DISCUSSION

The data presented here offer an insight into the metabolism of tRNA. Since the excretion of some of the degradation products is constant throughout the day, the turnover of tRNA must also be constant. Indeed, the constancy of excretion of these breakdown products of tRNA may provide parameters in addition to creatinine for the assessment of total tissue metabolism.

The mechanism of restoration to normal levels of excretion after effective chemotherapy is obscure. It implies an intrusion by the chemotherapeutic agents into the aberrant metabolism of the tumor tissue since all the tumor cells are not eradicated in so short a time.

Should the response to therapy of other cancers be similar, the determination of these markers may be a valuable mode of monitoring the effectiveness of therapy, especially in conditions where no other objective assessment of remission or relapse is available.

REFERENCES


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