THE SPECIFICITY OF ADENOSINE DEAMINASE AND PURINE NUCLEOSIDASE

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In the course of studies on the metabolism of purine nucleosides (1), we had the opportunity to examine two rare compounds of this class, adenine thiomethylpentoside and isoguanosine (crotonoside, 2-hydroxy-6-aminopurine-9-riboside). These substances afforded a chance to gain more insight into the rôle which substituents of the purine nucleus and of the carbohydrate part play in determining the substrate specificity. The following is a report of our findings with adenosine deaminase and purine nucleosidase.

EXPERIMENTAL

Substrates—Adenine thiomethylpentoside (formula IV) was obtained from yeast\(^1\) by a modification\(^2\) of the method described by Levene (2). The well crystallized product (m.p. 211\(^\circ\)) had the following elementary composition.

\[
\begin{align*}
\text{C}_{17}\text{H}_{15}\text{O}_{3}\text{N}_{6}\text{S} & \quad \text{Calculated. } C \, 44.44, \, H \, 5.08, \, N \, 23.55, \, S \, 10.78 \\
297.1 & \quad \text{Found.}^3 \quad C \, 44.22, \, H \, 5.12, \, N \, 23.46, \, S \, 10.98
\end{align*}
\]

The structure proposed by Wendt (3) agrees with that suggested by Suzuki, Odake, and Mori (4). Falconer and Gulland have found that the sugar is attached at position 9 of the adenine molecule (5). Hypoxanthine thiomethylpentoside was prepared from adenine thiomethylpentoside by nitrous acid treatment according to the directions of Kuhn and Henkel (6).

Isoguanosine (crotonoside) was discovered by Cherbuliez and Bernhard (7) in croton beans (Croton tiglium L.) and the structure as suggested by these authors was established beyond doubt by Spies and Drake (8). The attachment of the carbohydrate group in position 9 of the purine nucleus, as given in formula III, has been suggested by Falconer, Gulland, and Story (9). The source material for the preparation of crotonoside is almost unobtainable at present. The examination of this compound was

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\(^1\) Generously supplied by Anheuser-Busch, Inc., St. Louis.
\(^2\) Schlenk, F., to be published.
\(^3\) Dr. H. R. Morris, Galveston.
made possible by Dr. J. R. Spies who provided us with a generous sample of it.

Adenosine, xanthosine, and guanosine were prepared by the usual procedures (10).

*Enzymes*—Adenosine deaminase was prepared from small intestine by the directions of Brady (11) with additional purification steps. While this work was in progress, a new procedure was published by Kalckar (12) which is based on Schmidt and Thannhauser's earlier outline (13). Both methods yield preparations which, under suitable conditions, deaminate several times their own weight of adenosine per minute. A detailed account of our purification experiments will appear later. Nucleosidase was prepared from acetone-dehydrated rat or rabbit liver. The extract of this was purified according to Klein (14) and to Kalckar (12).

**Table I**

*Action of Adenosine Deaminase on Various Substrates*

Experimental conditions as given in the text. Concentration of deaminase, 35.4 γ per sample in Experiment A, 3540 γ per sample in Experiment B.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>NH₃ liberated</th>
<th>Experiment A</th>
<th>Experiment B</th>
</tr>
</thead>
<tbody>
<tr>
<td>No substrate</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Adenosine</td>
<td></td>
<td>37.05</td>
<td>35.90</td>
</tr>
<tr>
<td>Guanosine</td>
<td></td>
<td>&lt;0.05</td>
<td>0.23</td>
</tr>
<tr>
<td>Isoguanosine</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Adenine thiomethylpentoside</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

*Experiments with Adenosine Deaminase*—To study the rate of deamination both the conventional ammonia determination and the spectrophotometric procedures of Kalckar (12) were used.

In the former experiments the conditions were as follows: To 10 mg. of nucleoside in 1.0 ml. of 0.06 M phosphate buffer, pH 6.5, 1.0 ml. of enzyme solution was added and the mixture incubated at 37° for 2 hours. The reaction was stopped with 1.0 ml. of 1 N hydrochloric acid and the ammonia liberated was determined by distillation with an excess of alkali (15) and collection in a measured amount of 0.01 N hydrochloric acid. Control experiments showed that no significant amount of ammonia was liberated from the substrate or enzyme alone during incubation and distillation. In Table I the results of deamination experiments are shown. As can be seen, adenosine deaminase is strictly specific toward adenosine. This has been confirmed by spectrophotometric measurements. The use of spectrophotometry to follow enzymatic changes of purine compounds
was first described by Kalckar (12). We have applied his technique to our studies. The changes which would result from deamination of isoguanosine to xanthosine and of adenine thiomethylpentoside to hypoxanthine thiomethylpentoside are illustrated in Figs. 1 and 2. We found the absorption spectrum of isoguanosine rather different from that reported for this compound by Falconer, Gulland, and Story (9). There is close agreement between our data and those of these English authors with respect to adenine thiomethylpentose (5) and xanthosine (9). No data have been reported earlier for hypoxanthine thiomethylpentoside. Its absorption spectrum resembles that of inosine (16). A Beckman spectrophotometer, model DU, was used for the measurements which are given in Table II. Deamination of adenosine is indicated by the decrease in the absorption intensity at 260 mμ. Isoguanosine and adenine thiomethylpentoside are not attacked. Their absorption spectra remain unchanged.

Experiments with Nucleosidase—For determination of nucleosidase activity the iodometric procedures of Gryenberg (17) and Dmochowski (18) as adapted by Klein (14) were used. Nucleosides do not react with hypoiodite, but, after splitting them into their components, the aldehyde
group and some of the purine bases react with iodine. Guanine reacts with 4 equivalents of iodine, while hypoxanthine reacts with about 1 equivalent. Adenine does not react under the conditions of the Willstätter-Schudel titration (19). Data for isoguanosine and isoguanine have not yet been listed in the literature. We found that isoguanosine does not reduce hypoiodite, while isoguanine, like guanine, reacts with 4 equivalents of iodine. Therefore, the splitting of isoguanosine may be followed by the iodometric technique as in the case of guanosine.

The conditions are more complicated with adenine thiomethylpentoside. Wendt (3) has found that the thiomethyl group reduces 2 equivalents of iodine and yields the corresponding sulfoxide. He used 0.1 N iodine and alkali solution. Using more dilute solutions (0.01 N iodine and 0.02 N alkali), we found that less than 2 equivalents of iodine are bound by adenine and hypoxanthine thiomethylpentoside. Regardless of this, the

### Table II

**Action of Deaminase on Adenosine and Related Purine Nucleosides.**

Spectrophotometric Technique

Concentration of substrates, 16.6 γ per ml., deaminase 2.0 γ per ml. of 0.066 M phosphate buffer, pH 7.5.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Wave-length</th>
<th>Density readings after incubation for</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>Adenosine</td>
<td>260</td>
<td>0.729</td>
</tr>
<tr>
<td>Isoguanosine</td>
<td>290</td>
<td>0.780</td>
</tr>
<tr>
<td>Adenine thiomethylpentoside</td>
<td>260</td>
<td>0.722</td>
</tr>
</tbody>
</table>

### Table III

**Action of Nucleosidase on Purine Nucleosides**

Each sample contained 17 micromoles of substrate and 8.7 mg. of nucleosidase in 3.5 ml. of 0.02 M phosphate buffer, pH 7.5. Incubation at 37°. Perchloric acid was used for deproteinizing (20).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Increase in microequivalents of iodine reduced after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
</tr>
<tr>
<td>No substrate</td>
<td>0</td>
</tr>
<tr>
<td>Guanosine</td>
<td>10.2</td>
</tr>
<tr>
<td>Isoguanosine</td>
<td>1.3</td>
</tr>
<tr>
<td>Adenine thiomethylpentoside</td>
<td>0</td>
</tr>
<tr>
<td>Hypoxanthine thiomethylpentoside</td>
<td>0</td>
</tr>
</tbody>
</table>
splitting of the compounds under consideration by nucleosidase would be indicated by increasing amounts of iodine reduced due to the liberation of the aldehyde group. Our observations with nucleosidase are listed in Table III, which gives the increment of iodine reduction after incubation.

It is evident that the nucleosidase preparation does not split adenine thiomethylpentoside and isoguanosine. The action on hypoxanthine thiomethylpentoside is very limited and perhaps insignificant. According to Kalckar (12), nucleosidase action is a phosphorolytic process, yielding the labile ribose-1-phosphate. Accordingly, we checked the iodometric

DISCUSSION

The relationship of the purine nucleosides used in our experiments may be seen from their formulas. It is known that guanosine is resistant toward adenosine deaminase (22). Nucleosides other than adenosine with the amino group in position 6 have not been examined earlier. The failure of deaminase to attack them is remarkable, because isoguanosine is different from adenosine only in that it has a hydroxy group instead of hydrogen in position 2, and adenine thiomethylpentoside differs in having a thiomethyl group in the carbohydrate moiety. The inability of deaminase to

\[
\begin{align*}
\text{I, adenosine; II, guanosine; III, isoguanosine; and IV, adenine thiomethylpentoside.}
\end{align*}
\]
split the latter compound was expected, because it resembles somewhat adenosine-5'-phosphoric acid, which is resistant to this enzyme. Not all modifications of the carbohydrate part, however, interfere; adenine deoxyriboside is deaminated by adenosine deaminase (11).

Similar considerations hold for the results with nucleosidase. Earlier investigators (12, 23) found it capable of hydrolyzing only inosine and guanosine. The inertia of adenine thiomethylpentoside is analogous to that of adenosine, in which the amino group interferes with nucleosidase action. However, the failure of nucleosidase to split hypoxanthine thiomethylpentoside emphasizes the fact that the carbohydrate part also contributes to the specificity. A perfect fit (23) of the purine nucleus alone to the enzyme is not sufficient.

Our data amplify the information on specificity of enzymes concerned with nucleic acid metabolism. We agree with Dixon and Lemberg (23) in their criticism of the term "purine nucleosidase." It should be replaced by a more specific name.

SUMMARY

1. Experiments with adenosine deaminase from small intestine showed that 2-hydroxy-6-aminopurine-n-riboside (crotonoside, isoguanosine) and adenine thiomethylpentoside are not deaminated.

2. The same compounds are not split by nucleosidase from mammalian tissue.

3. The implications of these findings with respect to enzyme specificity are discussed.

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