On the Biological Methylation of Histamine. 3

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It is well known that many biological transmethylation reactions require methionine. However, in the case of guanidinoacetate and nicotineamide it has been shown by Cantoni and co-workers that the active methylating agent is S-adenosylmethionine.

In an earlier study it was demonstrated that radioactive methylhistamine was formed, when methionine labelled with $^{14}$C in the methyl group was incubated with histamine, enzyme extract from mouse liver, and appropriate cofactors. It was thus concluded that the methyl group of methionine can be utilized for the methylation of histamine.

In this communication it is demonstrated that the histamine methylating enzyme in mouse liver utilizes with great efficiency S-adenosyl-methionine as methyl donor.

Methods. Enzyme extract. Fresh mouse liver was ground in a blender with 3 volumes of cold 0.1 M sodium acetate buffer pH 5.6. The homogenate was centrifuged for 30 min at 25,000 g in the cold. The supernatant liquid was heated to 47°C for 10 min, centrifuged, and dialyzed for 2 h against 0.05 M sodium acetate buffer pH 6.0.

Determination of histamine according to Shayer and co-workers. Histamine dihydrochloride was added to the sample as carrier. After removal of the proteins, the piperyl derivative was prepared, and recrystallized until the radioactivity count was unchanged for at least three successive crystallizations. The average value of the constant samples was used.

Determination of methylhistamine. Carrier methylhistamine was added to the sample, extracted with chloroform after protein precipitation, and converted into the picrate which was recrystallized until constant radioactivity was attained. The activity was considered constant, if three successive values coincided within experimental errors.

S-adenosylmethionine was isolated from yeast according to Schlenk and De Palma. A chromatographically pure product was obtained by extraction with HClO$_4$, chromatography on Dowex 50, and precipitation with phosphotungstic acid.

Results. Histamine-2-$^{14}$C-dihydrochloride was incubated with the enzyme extract obtained from mouse liver for 1 h at 37°C. The incubation mixture was analyzed for histamine and its N-methylated metabolite, 1-methyl-4-(β-aminoethyl) imidazole (methylhistamine), according to the isotope dilution method, developed by Shayer and co-workers (see under Methods).

It was found that nearly all histamine remained intact after 1 h incubation, and that very little methylhistamine was formed. However, when S-adenosylmethionine was added to the incubation mixture, virtually all histamine had disappeared after 1 h, and the radioactivity was recovered as methylhistamine.

The essential figures from an experiment are given in Table 1. From these results, in comparison with earlier studies, it is obvious that the soluble fraction of mouse liver contains a histamine methylating enzyme which utilizes S-adenosylmethionine as methyl donor with great efficiency.

Table 1. The complete system contained 0.5 ml of the enzyme preparation, 15.5 μg $^{14}$C histamine dihydrochloride, 1.5 μmole S-adenosylmethionine, and Tris 0.5 M, pH 7.4, to make 1 ml. The calculated maximal radioactivity count is denoted as 100%.

<table>
<thead>
<tr>
<th>% of radioactivity recovered as</th>
<th>Methyl-Histamine</th>
</tr>
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<tbody>
<tr>
<td>No enzyme</td>
<td>97</td>
</tr>
<tr>
<td>No S-adenosylmethionine</td>
<td>92</td>
</tr>
<tr>
<td>Complete system</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>100</td>
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</table>

* Methylhistamine was obtained from Dr. R. W. Schayer, Rahway, N. J. which is gratefully acknowledged.

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efficiency. The metabolism of histamine corresponds to at least 110 μg/h/g fresh liver.


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On the Applicability of the $H_0$ Acidity Function to the Case of Two Competing Mechanisms

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The $H_0$ acidity function has become the subject of a great number of studies on the mechanisms of acid catalysis $^{1,2}$ although cases have been also reported in which this function is of a limited applicability $^3$. Apart from the fundamental restrictions involved in assuming certain activity coefficient terms to remain constant in different media, there is the following particular case, in which the use of the acidity function may lead, at least partly, to fallacious conclusions.

Let us consider an acid-catalysed hydrolysis reaction in which the unimolecular and bimolecular mechanisms are in competition. The overall rate coefficient $k$, which can be measured experimentally, is then made up of the two individual rate coefficients, $k_1$ and $k_2$, which refer to the different mechanisms. If the Zucker-Hammett hypothesis is valid, $k_2$ should be proportional to the acid concentration, whereas log $k_1$ should vary similarly as the value of $-H_0$ of the solution. Fig. 1 shows some examples calculated for the dependence of log $k$ on $-H_0$ in such cases. The $H_0$ values of aqueous hydrochloric acid solutions used in the calculations are those revised by Paul and Long $^4$.

The most striking feature is that the contribution of the bimolecular mechanism becomes largely masked by the unimolecular mechanism, when higher acidities are approached. Thus the curves are experimentally indistinguishable from a straight line of a slope of nearly unity, which would correspond to a purely unimolecular reaction, except that case where the bimolecular mechanism is the prominent in dilute acid; here the curve shows a clearly notable point of inflexion. Such a behaviour is thus highly characteristic of competing mechanisms which depend on the acidity of the solution in a different way.

An example of the masking of a mixed unimolecular and bimolecular reaction by

Fig. 1. A plot of log $k$ against $-H_0$ for competing uni- and bimolecular reactions. Aqueous hydrochloric acid solutions, 25°C. In dilute acid the bimolecular reaction assumes 25% (curve 1), 50% (curve 2), and 75% (curve 3) of the overall reaction.