THE ACETYLATION OF D-GLUCOSAMINE BY PIGEON LIVER EXTRACTS*

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One of the few naturally occurring acetyl derivatives in the animal body is that of glucosamine. It is well known that glucosamine is present in a large number of polysaccharides in many organisms and almost always is present there in acetylated form. During studies in this laboratory on the acetylation reaction, we, therefore, became interested in the possibility of carrying out an enzymatic acetylation of glucosamine in vitro. Pigeon liver extract had previously been used extensively in experiments on enzymatic acetylation and had been found to contain acetylating systems for several amines (1, 2). It will be reported here that this extract likewise contains the system for acetylation of glucosamine. As with previously studied acetylation reactions, glucosamine acetylation is also dependent on coenzyme A (CoA) or, more precisely, acetyl~CoA is the immediate acetyl donor in this reaction.

Materials and Methods

Enzyme Preparations—Pigeon liver acetone powder and aged extracts therefrom were prepared as described by Kaplan and Lipmann (3). The acetone fractionation of these extracts was carried out according to Chou and Lipmann (4). The transacetylase used was a partially purified preparation from Clostridium kluyveri (Stadtman et al. (5)).

Chemical Methods—Acetylglucosamine was determined by the following modification of the Morgan and Elson method (6). At the termination of the enzymatic reaction, 0.5 ml. of the reaction mixture was pipetted into a clean test-tube and 0.5 ml. of distilled water was added. 2 ml. of 5 per cent trichloroacetic acid were then added for deproteinization. After centrifuging, the supernatant fluid is poured off with draining into a 25 ml. test-tube. 1 ml. of molar sodium carbonate is added and the tubes are heated for 20 minutes in a boiling water bath and cooled in cold water. 5 ml. of 95 per cent ethanol are added to each tube with shak-

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ing, followed by 1 ml. of Ehrlich’s reagent (1.6 gm. of dimethylamino-
benzaldehyde in a mixture of 30 ml. of concentrated HCl and 30 ml. of
95 per cent ethanol). After shaking, the tubes are kept at 37° for 30
minutes for development of color, and then read on a Klett colorimeter
with a 540 mμ filter. Known amounts of acetylglucosamine, incubated
under the same conditions as the experimental tubes, serve as internal
standards.

When paper chromatograms were run, the remainder of the incubation
mixture was treated with 3 ml. of 95 per cent ethanol and the tubes were
allowed to stand half an hour for protein precipitation. After spinning,
the supernatants were evaporated to dryness in a bath at 40° or below by
means of a stream of air. The residue was extracted with 2 ml. of 95 per
cent ethanol and the extracts brought to dryness again. The final residue
dissolved in 0.1 to 0.2 ml. of water and appropriate aliquots applied
to Whatman No. 1 sheets. Ascending chromatograms were run for 16
hours with 80 per cent propanol containing 0.8 per cent ammonium acetate
as the solvent. The spots were developed by the methods described by
Partridge (7). The Rf for glucosamine and chondrosamine is 0.32. The
Rf for acetyl glucosamine is 0.46.

Results

Acetylation in Crude Pigeon Liver Extracts—With enzyme solutions simi-
lar to those used for CoA assay, a relatively weak acetylation of glucos-
amine was found. Table I shows the results of such an experiment in
which acetate-ATP-CoA served as the acetyl source. With 10 μM of
D-glucosamine and 0.3 ml. of the crude extract, generally a synthesis of
0.1 to 0.25 μM of acetylglucosamine was obtained.

Concentration of Glucosamine Acceptor Enzyme by Acetone Fractionation—
On acetone fractionation of crude liver extracts, the glucosamine-acetyl-
ing enzyme was found in the fraction precipitating between 50 and 60 per
cent acetone concentration (Fraction A-60). This fraction contains the
arylamine-acetylating system, but is free of the acetate-ATP donor system
as well as of the acetoacetate and citrate acceptor systems. In all experi-
ments with liver Fraction A-60, acetyl phosphate plus transacytase
served as the donor system.

With this concentrate a much more pronounced acetylation was obtained.
Under conditions otherwise similar to those described with crude extract
in Table I, 1.7 μM of acetylglucosamine were formed, as shown in Table
II. The activity is about 7 times greater than that with crude pigeon
liver extracts. Acetylglucosamine was furthermore identified by paper
chromatography as outlined above.

In an experiment presented in Table III, the time curves of the reaction
were studied. It was found that, up to the end of the 1st hour, synthesis proceeds almost proportionately with time. During the 2nd hour, the

**Table I**

*Acetylation of Glucosamine in Crude Pigeon Liver Extracts*

<table>
<thead>
<tr>
<th></th>
<th>Complete system</th>
<th>No CoA</th>
<th>No ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylglucosamine</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Reaction mixture, 0.3 ml. of aged pigeon liver extract (corresponding to 30 mg. of acetone powder), 50 μM of acetate, 20 μM of citrate, 5 μM of ATP, 15 units of CoA, 10 μM of cysteine hydrochloride, 10 μM of glucosamine hydrochloride, 0.2 M tris(hydroxymethyl)aminomethane buffer, pH 8.1. Total volume, 1 ml.; 120 minutes incubation at 37°.

**Table II**

*Acetylation of Glucosamine by Liver Fraction A-60*

<table>
<thead>
<tr>
<th></th>
<th>Acetylglucosamine</th>
<th>μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>No CoA</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>&quot; acetyl phosphate</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&quot; Fraction A-60</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&quot; transacetylase</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The samples were incubated in a total volume of 1 ml., containing 0.3 ml. of liver Fraction A-60 (5), 0.05 ml. of transacetylase, 10 μM of cysteine hydrochloride, 25 μM of acetyl phosphate, 10 μM of glucosamine, 2.5 μM of MgCl₂, 20 units of CoA, and 0.2 M tris(hydroxymethyl)aminomethane buffer, pH 8.1. 120 minutes incubation at 28°.

**Table III**

*Time Course of Reaction*

<table>
<thead>
<tr>
<th></th>
<th>15 min.</th>
<th>30 min.</th>
<th>60 min.</th>
<th>120 min.</th>
<th>180 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylglucosamine</td>
<td>0.39</td>
<td>0.62</td>
<td>1.18</td>
<td>1.60</td>
<td>1.60</td>
</tr>
</tbody>
</table>

Complete system, as in Table II.

rate diminishes rapidly, and there is no increase in the 3rd hour. This tapering off with time probably is due to enzyme inactivation.

**Acetylation of Galactosamine (Chondrosamine)—**A small quantity of chondrosamine hydrochloride was kindly made available to us by Dr. Karl Meyer. The formation of acetyl chondrosamine was followed by the Morgan and Elson (6) reaction. When judged by the colorimetric test,
similar amounts of chondrosamine and glucosamine were synthesized under comparable conditions (Table IV). We were, however, unsuccessful in isolating the acetyl chondrosamine chromatographically, owing probably to the relatively small amount synthesized.

Identity of Enzyme Involved in Amino Sugar Acetylation—The amino sugar-acetylating enzyme is probably not identical with that which catalyzes the acetylation of arylamines. With a combination of 2 μM p-aminohippuric acid and 10 μM of glucosamine, both substrates were acetylated to the same extent as when each was present separately. Furthermore, it was found by one of us (M. S.) that 0.005 M of benzylpenicillin will inhibit the acetylation of arylamines (8) but not the synthesis of acetylglucosamine.

**TABLE IV**

Acetylation of Glucosamine and Chondrosamine

<table>
<thead>
<tr>
<th>CoA units</th>
<th>Glucosamine μM</th>
<th>Galactosamine μM</th>
<th>Acetylated amino sugar μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>5</td>
<td>0</td>
<td>0.53</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>0</td>
<td>0.16</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>0</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Reaction mixture, 0.3 ml. of liver Fraction A-60 (5), 0.1 ml. of transacetylase, 0.1 M NH₄Cl, 2.5 μM of MgCl₂, 20 units of CoA, 10 μM of cysteine hydrochloride, and 0.2 M tris(hydroxymethyl)aminomethane buffer. Total volume, 1 ml.; 120 minutes incubation.

Comments

During the last few years, the interest in the metabolism of glucosamine has been increasing. Some time ago Lutwak-Mann published an interesting report on the metabolism, mainly the breakdown, of glucosamine in animal tissues and microorganisms (9). Of particular interest in relation to the present problem are more recent studies by Harpur and Quastel (10). They found that glucosamine is phosphorylated in brain extracts. Acetylglucosamine did not react but rather inhibited phosphorylation. Nevertheless, the relatively easy acetylation of free glucosamine described makes one wonder whether acetylglucosamine may serve as the substrate for synthesis of larger molecules or whether acetylation may occur after condensation. The results reported by Harpur and Quastel would seem to indicate the latter possibility; however, their phosphorylation experiments were done with brain extracts and seem to indicate that glucosamine is phosphorylated by brain fructokinase rather than by a special enzyme. It should be of interest to test for phosphorylation in other organs, with
free glucosamine as well as with acetylglucosamine, on the assumption that phosphogluco-
samine or phosphoacetylglucosamine represents a preliminary
substrate for further condensation reactions.

SUMMARY

Pigeon liver extracts were found to contain an enzyme which transfers
acetyl groups from acetyl-CoA to glucosamine and chondrosamine. The
enzyme may be concentrated considerably by acetone fractionation in the
cold. As acetyl donor system, (1) the ATP-acetate reaction of pigeon
liver extracts and (2) acetyl phosphate by way of transacetylase-CoA
were used. The transfer of acetyl to glucosamine is most likely due to a
special enzyme.

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