metal ion requirement in formation of threonyl-sRNA

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In addition to oxidation and phosphorylation, 3-phosphoglyceraldehyde dehydrogenase catalyzes transacylation (1, 2) and phosphatase reactions (3) with acetyl phosphate as substrate, and an esterase reaction (4) with p-nitrophenyl acetate. These reactions have different pH sensitivities and requirements for diphosphopyridine nucleotide and sulfhydryl groups on the enzyme (1–4). In an effort to relate enzyme structure to these catalytic activities, we determined the amino acid sequence of an octapeptide.

A cysteine residue in this peptide was acetylated by p-nitrophenyl acetate. These reactions to successive ionophoreses at pH 3.5 and pH 2.1. The acetyl-enzyme complexes turned over so rapidly that neither N-acetyl nor S-acetyl bands could be detected (Fig. 1, Panel 4). At pH 7.0, with DPN added to the medium, the lysine (6). At pH 7.0, with DPN added to the medium, the lysine (6).

When DPN-free enzyme was labeled with 14C-acetyl-P at pH 7.0 in the present study, there appeared three new, performic acid-stable bands (Fig. 1, Panel 5, Bands N1, N2, and N3) in which the acetyl group was attached to the e-amino group of an arginine residue. When the enzyme was acetylated with acetyl-P at pH 8.5, or when DPN-free enzyme was labeled with 14C-acetyl-P at pH 7.0 with subsequent adjustment to 8.5, N-acetyl peptides were obtained predominantly (Fig. 2). At pH 7.0 or 8.5 the acetyl to enzyme ratios were between 3:1 and 4:1. A qualitatively similar pattern was found with p-nitrophenyl acetate as substrate (Fig. 2), but it was more difficult to acetylate the lysine moiety with the given experimental conditions.

The peptides, N1, N2, and N3, were purified on a preparative scale by exposing the enzyme to acetyl phosphate at pH 8.5, passing a pepin digest of the acetyl-enzyme complex through a Sephadex G 25 column, and then submitting the collected fractions to successive ionophoresis at pH 3.5 and pH 2.1. The amino acid compositions of these three peptides were determined on an amino acid analyzer after performic acid oxidation and hydrolysis for 24 hours with 6 N HCl and are shown below.

[Table showing amino acid compositions]

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| N1: (Asp, Thr, Ser, Val, Pro, Gly, Leu, Lys) |
| HN+14COOH |
| N2: (Asp, Thr, Ser, Val, Pro, Gly, Leu, Lys) |
| HN+14COOH |
| N3: (Asp, Thr, Ser, Val, Pro, Gly, Leu, Lys) |
| HN+14COOH |

Sum: (Asp, Thr, Ser, Val, Pro, Gly, Leu, Lys)
Fig. 1. Autoradiographs of $^{14}$C-acetyl peptides produced by pepsin digestion of $^{14}$C-acetyl enzymes described in the text. The experimental procedures for the ionophoresis are detailed in previous publications (5). In the experiment with acetyl phosphate and added DPN (Fig. 4 and 5), phosphate was also present as a product of the acetylation reaction and as an impurity in the $^{14}$C-acetyl phosphate. DPN was shown to facilitate the rapid transfer of the acetyl group from the enzyme to Pi. However, DPN alone does not deacylate an acetyl enzyme. PNPA, p-nitrophenyl acetate; AcP, acetyl phosphate.

Although the sequence of the tridecapeptide has not yet been determined, certain conclusions can be reached.

1. N-Acetyllysine is the NH$_2$-terminal amino acid in Peptide N3 since it could be released by leucine aminopeptidase. Its position was also established by the overlapping peptides, N2 and N1. Leucine aminopeptidase or subtilisin digestion of N3 showed that only the NH$_2$-terminal lysine was acetylated, and that the second lysine was at least six amino acids away.

2. The three or four $^{14}$C-acetyllysine sites on the enzyme (mol. wt. 140,000) labeled at pH 8.5 are identical since virtually all of the radioactivity was associated with this tridecapeptide.

3. p-Nitrophenyl acetate labels the same lysine and cysteine residues as acetyl-P, since the peptides from the proteolytic digestions of the acetyl enzymes gave identical patterns on ionophoresis at pH 2.1, 3.5, and 6.5.

4. The N-$^{14}$C-acetyllysine is not part of the octadecapeptide described earlier (5), since the neighboring amino acids differ from those of both the NH$_2$-terminal and COOH-terminal lysines of the octadecapeptide. We cannot therefore support Polgar's
FIG. 2. Autoradiographs of $^{14}$C-acetyl peptides produced by pepsin digestion of $^{14}$C-acetyl enzymes prepared at pH 7.0 and at pH 7.0 with subsequent adjustment to 8.5. The electrophoretic patterns of samples initially labeled at 8.5 are identical to those in which the pH is raised from 7.0 to 8.5. Temperature was 22° for experiments with acetyl phosphate (AcP) and 0° with p-nitrophenyl acetate (PNPA).

suggestion that p-nitrophenyl acetate acetylates the NH$_2$-terminal lysine of the octadecapeptide (7).

The lysine-bound, $^{14}$C-acetyl moiety does not participate in acetate transfer reactions, as shown in two ways. First, $^{14}$C-acetyl enzyme was prepared at pH values from 7.0 to 9.0 in order to obtain predominantly S-acetyl in the lower pH range and N-acetyl in the higher pH range. An irreversible arsenolysis of the complexes was induced by addition of DPN and arsenate. The S-acetyl enzyme was almost completely deacylated, but the N-acetyl enzyme was not affected (Table I). Autoradiographs of the acetylated peptides from these preparations confirmed these conclusions. Secondly, the inert state of the N-$^{14}$C-acetyl-lysine group was demonstrated by the fact that it could not be exchanged with $^{14}$C-acetyl phosphate whereas the S-$^{14}$C-acetyl residue was readily exchanged.

The lysine residue which bound the acetyl groups appeared to be involved in the binding of DPN to the enzyme. It could be shown that DPN binding was reduced approximately in proportion to the N-acetylation of the enzyme (Table II). DPN binding was not reduced at pH 4.5 where only S-acetylation occurred. Coenzyme binding was moderately to markedly reduced with N-acetylation of the enzyme at pH 7.0 and 8.5. Previously bound DPN, however, could not be displaced by exposure to acetyl phosphate (Table II). The N-acetylated enzyme was
**Table I**

<table>
<thead>
<tr>
<th>pH 7.0</th>
<th>pH 7.5</th>
<th>pH 8.0</th>
<th>pH 8.5</th>
<th>pH 9.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.4</td>
<td>2.2</td>
<td>2.3</td>
<td>2.5</td>
</tr>
<tr>
<td>DPN + AsO₄</td>
<td>0.2</td>
<td>0.4</td>
<td>1.4</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The charcoal-treated, DPN-free dehydrogenase (0.1 μmole) was incubated with ¹⁴C-acetyl phosphate (4.0 μmoles) in a volume of 1.0 ml at the designated pH. The reaction did not give maximal acylation of the enzyme because the acetyl phosphate concentration was less than 0.01 M in order that the acetylation at pH 7.0 be predominantly on the cysteine. Arsenolysis of the complex was induced by addition of DPN (1.0 μM) and arsenate (2.0 μM). The protein was precipitated 10 minutes later, and the specific activity of the labeled enzyme was determined (6).

**Summary**

Acetyl phosphate or p-nitrophenyl acetate can acetylate a specific cysteine or lysine residue in the active center of 3-phosphoglyceraldehyde dehydrogenase. The cysteine is involved as an acyl-thioester intermediate in the dehydrogenase, transacylase, and esterase reactions, whereas the lysine moiety may participate in the binding of diphosphopyridine nucleotide.

**REFERENCES**

N-Acetylation of 3-Phosphoglyceraldehyde Dehydrogenase by Substrates
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