THE KETENE ACETYLATION OF THE PARATHYROID HORMONE*

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In addition to the numerous reactions of the parathyroid hormone which are typical of proteins, it undergoes several others which indicate a relation between its activity and the presence of free amino (or imino) groups within the molecule. It was observed by Tweedy and Torigoe (1) that its activity was completely destroyed by treatment with formaldehyde, and that the resultant product could be reactivated to the extent of about 25 to 50 per cent by boiling with very dilute hydrochloric acid. The hormone was also completely inactivated by aqueous nitrous acid, a result which might be attributed to reaction with the amino groups. In a further study of this (2) it was found that deamination to the extent of 35 per cent resulted in complete inactivation. This sensitivity to nitrous acid led to the conclusion that some reaction other than deamination was playing a role, a belief which was supported by the ease with which the hormone was inactivated by oxidation with hydrogen peroxide. The effect of nitrous acid might therefore be attributed to deamination, oxidation, or simple substitution, resulting in the formation of nitroso derivatives.

The use of ketene, which reacts with free amino groups and the phenolic hydroxyls of tyrosine, offers an admirable means of determining the dependence of the hormone on these respective groupings. Hydrolysis of the O-acetyl residues after complete saturation of the protein with ketene should provide a molecule in which the only alteration would involve acetylation of the free amino groups. The reaction with ketene has accordingly been investigated in much the same manner as that employed by other authors (3–5).

EXPERIMENTAL

The acetylation of the parathyroid hormone was carried out in the apparatus utilized by Ross and Christensen for treating proteins with carbon suboxide (6). The preparation actually employed was obtained according to the procedure described in the preceding communication (7) and had a nitrogen potency of approximately 180 units. Twice distilled ketene, in 5 times the quantity equivalent to the total reacting groups

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(i.e. the sum of the amino and phenolic hydroxyls) was added over a period of 45 minutes at 0° to a fine suspension of this product containing

<table>
<thead>
<tr>
<th>Time</th>
<th>Hydrolysis of O-acetyl groups per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>hrs.</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>38</td>
</tr>
<tr>
<td>1.75</td>
<td>71</td>
</tr>
<tr>
<td>4.5</td>
<td>100</td>
</tr>
<tr>
<td>8.5</td>
<td>97</td>
</tr>
<tr>
<td>44.5</td>
<td>101</td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Preparation</th>
<th>Fraction of total Amino groups covered per cent</th>
<th>Fraction of total phenolic hydroxyls covered per cent</th>
<th>Nitrogen potency, U.S.P. units per mg. N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Original protein</td>
<td>0</td>
<td>0</td>
<td>160 (2.2, Dog 3)</td>
</tr>
<tr>
<td></td>
<td>Acetylated (5 equivalents ketene)</td>
<td>42</td>
<td>40</td>
<td>200 (2.2, &quot; 4)</td>
</tr>
<tr>
<td></td>
<td>Acetylated and hydrolyzed</td>
<td>45</td>
<td>4</td>
<td>2 (4.8, &quot; 3)</td>
</tr>
<tr>
<td></td>
<td>Control for hydrolysis</td>
<td>0</td>
<td>0</td>
<td>&gt;150 (&lt;2.7, Dog 2)</td>
</tr>
<tr>
<td>B</td>
<td>Original protein</td>
<td>0</td>
<td>0</td>
<td>200 (1.5, Dog 1)</td>
</tr>
<tr>
<td></td>
<td>Acetylated (3 equivalents ketene)</td>
<td>39</td>
<td>19</td>
<td>160 (1.5, &quot; 2)</td>
</tr>
</tbody>
</table>

* The nitrogen content of the injected dose is indicated in mg. by the first figure in parentheses.

81 mg. of protein nitrogen in 35 ml. of 0.03 M phosphate buffer of pH 6.0. The pH was maintained constant during the acetylation by the addition
of 0.1 N sodium hydroxide. After the reaction was complete, the product was dialyzed against running water for 4 hours to remove much of the inorganic matter present, and finally made to 100 cc., after the pH was adjusted to 4.0, which effected almost complete solution. This solution was analyzed for total nitrogen and for amino nitrogen by the Van Slyke manometric procedure. The semicolloidal nature of the suspensions encountered here, and later, presented no problem in sampling for analysis. The determination of free and acetylated phenolic hydroxyls by the methods of Herriott (8) at pH 8 and 11 gave the per cent of phenolic hydroxyls covered. The protein was kept in solution during the color development by the addition of urea to give a final concentration of 4 M (4). This solution was also assayed for its biological activity (7).

The hydrolysis of O-acetyl linkages within this product was accomplished by digestion for 4.5 hours at 37° in 0.1 M borate buffer of pH 10.0 (see Table I). The bulk of the product dissolved upon acidification to pH 4.0, and the resulting cloudy solution was dialyzed overnight against running water. A complete analysis was carried out as outlined for the acetyl hormone before hydrolysis.

A sample of the original unacetylated hormone was also subjected to the same conditions, pH 10.0 and 37°, for 4.25 hours. This was dialyzed and assayed.

The data obtained from these experiments are assembled in Table II.

**DISCUSSION**

Acetylation with ketene results in complete inactivation of the parathyroid hormone. In the light of present knowledge this must be attributed to alteration of either the free amino groups or the phenolic hydroxyls, or both. It is not felt that the exposure to bodily conditions of pH and temperature during the assay period is sufficiently long to cause an appreciable hydrolysis of O-acetyl linkages. It has been demonstrated that acetic acid bound to phenolic hydroxyls is slowly liberated at pH 7 and 37° (9). Since, however, hydrolysis of oxygen-bound acetyl residues, by which the phenolic groups are restored to the molecule, does not effect reactivation, it must be concluded that the amino groups are an essential part of the biologically active molecule. No statement can be made on the basis of our data regarding the dispensability of the phenolic hydroxyls.

The colorigenic value of the unacetylated hormone is 10 to 15 per cent higher than that obtained when the pH 11 method is applied to the acetylated material. Since presumably all of the O-acetyl groups in the latter are liberated under the conditions of the pH 11 method, additional colorigenic groups of unknown nature have been acetylated and such acetyl linkages are relatively stable at high pH values. No information may be gained from the present data regarding the nature of these groups.
The parathyroid hormone is therefore like diphtheria toxin (10), certain of the pituitary hormones (11, 12), and the gonadotropic hormone of pregnant mare serum (11) in its dependence upon unaltered amino groups, and differs from pepsin (3), insulin (4), and human chorionic gonadotropin (11), which are active regardless of the presence of these groups.

**SUMMARY**

1. Acetylation of the parathyroid hormone with ketene is accompanied by complete biological inactivation.

2. Since liberation of the phenolic hydroxyls by alkaline hydrolysis does not restore activity, it is concluded that the hormone is dependent upon the presence of free amino groups.

**BIBLIOGRAPHY**

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