Preliminary Communications

The Presence of \(N^6-(N\text{-formyl-}\alpha\text{-aminoacyl})\text{adenosine in an Enzymatic Hydrolysate of Yeast Soluble Ribonucleic Acid}\)

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A recent report (1) from this laboratory described the isolation of an enzymatic hydrolysate of yeast soluble ribonucleic acid of a group of nucleosides which was assigned the common structure \(N^6\text{-}(\alpha\text{-aminoacyl})\text{adenosine. The original characterization from an enzymatic hydrolysate of yeast soluble ribonucleic acid has not been excluded completely (1).}

Although attachment to the \(N^1\)-position as an alternate possibility that the acyl bond is attached to the \(N^6\)-position of adenosine N-formylaminoacyl-N6-adenosine bond is cleaved under very mild alkaline conditions, no free amino acids can be detected. These results have led to the finding that these products contain a formyl residue attached to the \(\alpha\)-amino group of the amino acid. Thus, the tentative structure for these compounds is \(N^6\text{-}(N\text{-formyl-}\alpha\text{-aminoacyl})\text{adenosine (Fig. 1).}

The assumption is made here that almost a quantitative yield of the \(N\text{-formylamino acid was isolated.}

No-(\(N\text{-formyl-}\alpha\text{-aminoacyl})\text{adenosine appears to consist of 1 mole of amino acid per mole of adenosine as shown by the following experiments. A solution of } N^6\text{-(N-formyl-}\alpha\text{-aminoacyl})\text{adenosine (1.0 }\mu\text{mole per ml) served as a stock solution. An aliquot of this solution was made 0.1 }\mu\text{mole with respect to hydrochloric acid and heated at 100° for 20 hours. Quantitative analysis by the ninhydrin method (4) gave a value corresponding to 0.95 }\mu\text{mole per ml of stock solution. When 1 }\mu\text{mole of adenosine was digested under similar conditions, it produced approximately 1 }\mu\text{mole of ninhydrin-reacting material.}

In another determination of the amino acid to adenosine ratio, an aliquot of the stock solution was made 0.1 N with respect to sodium hydroxide and the solution was heated for 2 hours at 100°. This solution contained an amount of ninhydrin-positive material corresponding to 0.95 }\mu\text{mole per ml. Under these conditions, adenosine released approximately 5 mole % of ninhydrin-positive material. These experiments show that there is very little, if any, polypeptide material in this sample.

The formyl group in } N^6\text{-(N-formyl-}\alpha\text{-aminoacyl})\text{adenosine was characterized in the following series of experiments. } N^6\text{(N-formyl-}\alpha\text{-aminoacyl})\text{adenosine (1.0 }\mu\text{mole) in 0.8 ml of 0.25 N ammonium hydroxide was heated for 2 hours at 100° in a sealed tube. (Examination of this reaction mixture by means of paper chromatography in Solvent System A showed that at least 95% of the starting material had been degraded to adenosine.)}

The solution was lyophilized and the residue was dissolved in 1.5 ml of water. This solution was passed through a column containing 0.2 ml of Dowex 50 resin (H+ form, 200 to 400 mesh). The column was washed with water and the combined effluent (4.0 ml) served as a stock solution. The mild hydrolytic conditions used here were not sufficient to break the formyl bond, so that almost a quantitative yield of the } N\text{-formylamino acid was obtained.}

When the stock solution was analyzed directly for amino acid by the ninhydrin method, a value of zero was obtained. The total amino acid in the stock solution was determined by analyzing an aliquot which had been made 0.1 N with respect to sodium hydroxide and heated for 3 hours at 100°. This procedure gave a value of 1.05 }\mu\text{mole of amino acid. The amount of formic acid in the stock solution was determined colorimetrically by the chromotropic acid method (5). Analysis was performed on an aliquot which had been made 0.1 N with respect to sodium hydroxide and heated for 2 hours at 100°. The result corresponded to 1.01 }\mu\text{mole of formic acid.}

The standard curve used in this analysis was prepared by analyzing a standard solution of } N\text{-formyl-dL-valine, first subjected to alkaline hydrolysis under the above conditions. The points on this curve coincided exactly with those of a curve prepared by analyzing a standard solution of formic acid.}

The rate of hydrolysis of the formyl group was studied by making aliquots of the stock solution of the released } N\text{-formylamino acids 0.1 N with respect to sodium hydroxide and heating the solution at 100°. Synthetic } N\text{-formyl-dL-valine, } N\text{-formyl-dL-phenylalanine, and } N\text{-acetyl-dL-alanine were subjected to the same conditions.}

The natural product and the model } N\text{-formylamino acids exhibit similar behavior toward alkaline hydrolysis and this behavior contrasts sharply with that of } N\text{-acetyl-dL-alanine.}

An attempt was made to prepare the hydrazide derivative of the formyl group for use in paper chromatography. The } N\text{-formylamino acid isolated from } N^6\text{-(N-formyl-}\alpha\text{-aminoacyl})\text{adenosine was treated under anhydrous conditions with an excess of hydrazine for 12 hours at 100°. The hydrazine was removed by lyophilization in a vacuum of 10^{-4} \text{mm of Hg.}

The flask remained at room temperature. Examination of the residue by means of paper chromatography showed a small spot (about 5 to 10% of the total) corresponding to the move-
The amount of amino acid released was determined by the ninhydrin method and is plotted as the mole percentage of the total N-acylamino acid. X, N-acetyl-o,L-alanine; Q, N-formyl-DL-valine; A, N-formylamino acid isolated from the aminoacyladenosine nucleoside; C, N-formyl-DL-phenylalanine.

**TABLE I**

Paper chromatography of hydrazides

Whatman No. 1 paper was used. The papers were developed for 20 hours in the descending manner. Solvent systems: A, isopropyl alcohol-water-concentrated ammonium hydroxide (7:2:1); B, isopropyl alcohol-1% aqueous ammonium sulfate (2:1); C, isopropyl alcohol-collidine-water (10:2:1); D, n-butyl alcohol-water-concentrated ammonium hydroxide (86:14:5). The hydrazides of formic acid and acetic acid were synthesized according to the method of Narita (9). Solvent System C is particularly definitive for separation of the hydrazides of fatty acids (7). The hydrazides were visualized by spraying the developed chromatograms with n-butyl alcohol saturated with a 10% aqueous solution of ammoniacal silver nitrate (7).

<table>
<thead>
<tr>
<th>Hydrazide</th>
<th>Rf values in solvent systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic acid hydrazide</td>
<td>0.70 0.72 0.14 0.37</td>
</tr>
<tr>
<td>Formic acid hydrazide</td>
<td>0.61 0.64 0.076 0.23</td>
</tr>
<tr>
<td>Formic acid hydrazide from N(3)-(N-formyl-o,L-aminoacyl)adenosine</td>
<td>0.61 0.64 0.076 0.23</td>
</tr>
<tr>
<td>Formic acid hydrazide from N-formyl-NH2-phenylalanine</td>
<td>0.61 0.64 0.076 0.23</td>
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Regeneration of Active Enzyme from the Mixed Disulfide of Egg White Lysozyme and Cystine*

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Hopkins (1) originally reported that reaction may occur between sulfhydryl-containing proteins and low molecular weight disulfides. Since this observation, it has been demonstrated that reactions of this type proceed reversibly by disulfide interchange as follows (2, 3).


A number of mixed disulfides of proteins which normally contain free thiol groups have been described (4–6). Complete regeneration of the biologically active, thiol-containing protein could be achieved on treatment of each of these derivatives with a low molar concentration of free thiol (4). This possibility is of considerable biological interest especially in view of the demonstration that protein—S–S–R + R–S–

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‡ On leave of absence from the Laboratoire de Chimie Générale, Faculté des Sciences, Université Libre de Bruxelles, Belgium.

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