THE DECOMPOSITION OF YEAST NUCLEIC ACID BY A HEAT-RESISTANT ENZYME

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(Received for publication, May 17, 1938)

In the course of a study of the action of different animal tissues upon pneumococci (1), soluble tissue extracts have been obtained which exhibit a high degree of enzymatic activity upon yeast nucleic acid (2). The method of preparation and some of the properties of the enzyme (a ribonuclease) are described in the present paper.

EXPERIMENTAL

Methods

The yeast nucleic acid used in these experiments was a commercial preparation purified by treatment with picric acid (3). A sample of yeast nucleic acid and one of thymus nucleic acid were kindly supplied by Dr. P. A. Levene to whom the author wishes to extend his heartiest thanks.

The effect of the enzyme preparations on nucleic acid was followed by determining the amount of total phosphorus rendered soluble in hydrochloric acid during the incubation of the enzyme-substrate mixture. To this end, the mixture was treated with HCl (final concentration 0.2 N), and then immediately cooled and centrifuged in the cold. The supernatant was then analyzed for phosphorus by the Youngburg and Youngburg method (4) modified in details. It was found necessary to prolong the digestion with sulfuric acid for 40 minutes in order to obtain a complete liberation of the phosphorus in the inorganic form.

MacFadyen followed the decomposition of nucleic acid by deter-
mining the amount of phosphorus soluble in a reagent consisting of uranyl chloride in trichloroacetic acid (5). This method was used in Experiment 3.

Preparation of Enzyme—Soluble extracts capable of decomposing yeast nucleic acid have been obtained from a number of animal tissues (polymorphonuclear leucocytes, liver, pancreas, spleen, and lungs) (1). The ribonuclease used in the following experiments was extracted from a commercial preparation of dried pancreatic. The method of preparation is based on the solubility of the enzyme in 50 per cent acid acetone and on its resistance to heating at high temperatures.

500 gm. of pancreatin (Parke, Davis and Company) are shaken with 750 cc. of 50 per cent acetone for 24 hours at room temperature; the pH of this material is 5.3. The soluble fraction is separated by filtration through filter paper. Addition of 250 cc. of acetone to 500 cc. of filtrate causes the precipitation of a viscous, brown liquid phase which contains the ribonuclease. The crude enzyme is diluted to 100 cc. with water, extracted three times with ether, then heated for 10 minutes in a boiling water bath. A heavy coagulum forms; it is removed by centrifugation and the ribonuclease is recovered in the clear supernatant. The enzyme solution is then dialyzed in a closed cellophane bag against distilled water maintained at pH 4.0 with acetic acid. The brown pigment dialyzes out and a new inactive precipitate separates in the dialysis bag. This precipitate is discarded and the enzyme remains in solution.

It must be pointed out that, at this stage of purification, the enzyme has become much less resistant to acetone and other precipitating agents. When, however, the aqueous solution is rapidly precipitated with 2 volumes of acetone, at slightly acid reaction (pH 3.0 to 6.0), and at low temperature, then desiccated with ether, one obtains a white feathery preparation which is readily soluble in water and which contains the ribonuclease activity of the original material. An average of 200 mg. of dried enzyme is recovered from 500 gm. of pancreatin.

If, on the other hand, the purified enzyme is added to acetone at room temperature, it becomes insoluble in water and loses its activity; the inactivation is especially rapid at neutral or alkaline hydrogen ion concentrations.
The enzyme can also be salted-out in saturated sodium sulfate; as in the case of acetone, this operation must be carried out at low temperatures.

**Protein Nature of Enzyme**—Four different preparations, desiccated with acetone and ether, have been analyzed for nitrogen and phosphorus. The results are presented in Table I.

The enzyme solutions give positive biuret, Sakaguchi, Millon, and Molisch tests. The early preparations were contaminated with large amounts of an inactive polysaccharide and it is likely that the preparations, for which analytical data are presented in Table I, also contain small amounts of this polysaccharide.

The enzyme solution gives a positive Feulgen reaction; its ultraviolet absorption spectrum exhibits a broad band in the region 2600 Å.; these findings, taken jointly with the presence of phosphorus (Table I), suggest that the preparations contain a nucleic acid of the desoxyribose type. There is, however, no evidence that this nucleic acid is associated with enzymatic activity.

The effect of proteolytic enzymes on the activity of the ribonuclease is described in the following experiment.

**Experiment 1**—Five 0.1 mg. lots of nuclease were treated as follows: Lot 1 was adjusted to pH 2.5 with HCl. Lot 2 received 0.1 mg. of crystalline pepsin and was adjusted to pH 2.5 with HCl. Lot 3 was adjusted to pH 8.2 (borate buffer). Lots 4 and 5 received respectively 0.1 mg. of crystalline trypsin or crystalline chymotrypsin¹ and were adjusted to pH 8.2. The five prepara-

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¹ The writers are greatly indebted to Dr. J. H. Northrop and Dr. M. Kunitz for supplying them with several samples of crystalline trypsin, chymotrypsin, and pepsin.
tions were made up to a final volume of 5 cc. with distilled water, incubated at 37°, and tested for activity against yeast nucleic acid after different intervals of time.

It was found that Lot 2 had become inactive against yeast nucleic acid after 1 hour's incubation with pepsin. On the contrary the other four preparations, i.e. the one kept at pH 2.5 without pepsin and the preparations at pH 8.2 with or without trypsin or chymotrypsin, were still fully active after 48 hours.

Activity of Enzyme—The enzyme attacks ribonucleic acid between pH 5.5 and 9.5 and renders this substance soluble in mineral acids.

Experiment 2—Amounts of dried enzyme ranging from 0.01 to 0.0001 mg. were added to several 50 mg. lots of ribonucleic acid in 5 cc. of acetate buffer at pH 5.8. The mixtures were incubated at 59° for 2 hours and treated with HCl at the end of the incubation period. The amount of acid-soluble phosphorus in the different fractions is presented in Table II.

Products of Enzymatic Decomposition of Nucleic Acid—In the preceding experiment, the decomposition of nucleic acid was followed by determining the amount of total phosphorus which becomes soluble in HCl as a result of enzymatic action. The phosphorus thus released, however, is not in the inorganic form, but requires prolonged hydrolysis with sulfuric acid before being detectable by the usual colorimetric methods. It will also be shown that a large fraction of the acid-soluble phosphorus is readily precipitated by treatment with the uranyl chloride reagent (5).

<table>
<thead>
<tr>
<th>Enzyme (mg.)</th>
<th>Total phosphorus soluble in HCl mg.</th>
<th>Present in fraction mg.</th>
<th>Released by enzyme mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>1.554</td>
<td>1.463</td>
<td></td>
</tr>
<tr>
<td>0.001</td>
<td>0.419</td>
<td>1.328</td>
<td></td>
</tr>
<tr>
<td>0.0001</td>
<td>0.207</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>0.00001</td>
<td>0.145</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.091</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Experiment 3—1 mg. of enzyme was added to 250 mg. of yeast nucleic acid in 10 cc. of acetate buffer at pH 5.8. Another 250 mg. lot of nucleic acid at pH 5.8 was kept as control. Both were incubated at 59° for 2 hours in the presence of chloroform. The two solutions were then treated with 1 cc. of 1 N HCl, kept at 0° overnight, and the clear soluble fraction separated by centrifugation.

2 cc. portions of each supernatant were diluted with equal volumes of distilled water, or of uranyl chloride reagent. The heavy precipitate which formed in the latter case was discarded and the soluble fraction collected. The partition of total phosphorus in the fractions soluble in hydrochloric acid, or in uranyl chloride, is presented in Table III.

<table>
<thead>
<tr>
<th>Description of fractions</th>
<th>Total phosphorus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In solution</td>
</tr>
<tr>
<td>Control solution of nucleic acid</td>
<td></td>
</tr>
<tr>
<td>(a) Soluble in HCl</td>
<td>0.048</td>
</tr>
<tr>
<td>(b) &quot; uranyl chloride reagent</td>
<td>0.030</td>
</tr>
<tr>
<td>Products of enzymatic decomposition of nucleic acid</td>
<td></td>
</tr>
<tr>
<td>(a) Soluble in HCl</td>
<td>0.567</td>
</tr>
<tr>
<td>(b) &quot; uranyl chloride reagent</td>
<td>0.267</td>
</tr>
</tbody>
</table>

The results presented in Table III indicate that, under the conditions of Experiment 3, approximately half of the acid-soluble phosphorus released during enzymatic decomposition of yeast nucleic acid is in a form precipitable by the uranyl chloride reagent. It was also found that the phosphorus present in the fraction soluble in the latter reagent is not inorganic phosphorus. In fact, a precipitate rich in organic phosphorus appears when stannous chloride is added to this fraction.

Effect of Temperature on Nuclease—The method of purification of the enzyme depends on its resistance to high temperatures. This property is illustrated in the following experiments.

Experiment 4—5 cc. portions of the enzyme in solution were
Decomposition of Yeast Nucleic Acid

adjusted to pH 2.2, 4.2, 5.8, 6.6, and 7.4 with hydrochloric acid, acetic acid, or sodium hydroxide. They were heated at these reactions in a boiling water bath for 15 minutes, then cooled, neutralized, and tested for activity by adding 0.1 cc. of the neutralized solutions to 5 cc. of 1 per cent yeast nucleic acid. The enzyme-substrate mixtures were incubated at 37° for 24 hours and the amount of total phosphorus soluble in HCl measured at the end of the incubation period (Table IV).

The results of Experiment 4 show that the enzyme does not suffer appreciable loss of activity when heated for 15 minutes at slightly acid reactions. Complete inactivation could not be obtained even by heating the enzyme in a boiling water bath at pH 2.2 or 7.4. In view of these findings, it was of special interest to determine

<table>
<thead>
<tr>
<th>pH</th>
<th>Amount of P soluble in HCl released from nucleic acid by 0.1 cc. neutralized enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>0.213</td>
</tr>
<tr>
<td>4.2</td>
<td>0.503</td>
</tr>
<tr>
<td>5.8</td>
<td>0.567</td>
</tr>
<tr>
<td>6.6</td>
<td>0.001</td>
</tr>
<tr>
<td>7.4</td>
<td>0.240</td>
</tr>
<tr>
<td>Unheated enzyme</td>
<td>0.603</td>
</tr>
</tbody>
</table>

the optimum temperature for enzymatic activity of the nuclease. This is established in Experiment 5.

Experiment 5—5 cc. portions of a 1 per cent solution of yeast nucleic acid at pH 5.8 were brought to temperatures of 0°, 37°, 59°, 70°, and 85°. To each of these portions 0.1 cc. of nuclease was then added. The enzyme-substrate mixtures were then incubated for 2 hours at the respective temperatures. The preparations were treated with HCl at the end of the incubation period and immediately cooled. The amount of acid-soluble phosphorus is given in Table V.

The rate of enzymatic activity observed in Experiment 5 was highest at 65°; no decomposition of yeast nucleic acid took place at 85°.
Further experiments were carried out to determine more exactly the optimum temperature for enzymatic action. Although the results were somewhat irregular, this optimum appears to be at or slightly above 70°; a rapid decrease in activity takes place above 75°.

It was shown in Experiment 4 that the enzyme does not lose activity when heated at boiling temperature at slightly acid reactions. The results of Experiment 5 show, on the other hand, that the enzyme fails to decompose nucleic acid above 85°. These findings are again illustrated in Experiment 6.

Experiment 6—Two tubes each containing 5 cc. of 1 per cent yeast nucleic acid at pH 5.8 were placed at 95°; 0.1 cc. of nuclease solution was then added. The mixtures were incubated at 95° for 1 hour. At the end of this period one of the preparations (a) was cooled to 60° and kept at this temperature for 1 hour; the other preparation (b) was maintained at 95° for the whole period. Both preparations were finally treated with HCl and immediately cooled to 0°. The amount of acid-soluble phosphorus was then determined.

Preparation (a) which had been kept at 95° for 1 hour, then incubated at 60° for 1 hour, contained 0.313 mg. of acid-soluble phosphorus, whereas preparation (b) which had been maintained at 95° all the time contained only 0.089 mg.

It is therefore apparent that although the enzyme cannot decompose yeast nucleic acid during incubation at 95°, enzymatic action takes place as soon as the mixture is brought back to 60°.

<table>
<thead>
<tr>
<th>Temperature of incubation of enzyme-substrate mixtures</th>
<th>Amount of acid-soluble P released</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C.</td>
<td>mg.</td>
</tr>
<tr>
<td>0</td>
<td>0.061</td>
</tr>
<tr>
<td>37</td>
<td>0.152</td>
</tr>
<tr>
<td>59</td>
<td>0.400</td>
</tr>
<tr>
<td>65</td>
<td>0.607</td>
</tr>
<tr>
<td>74</td>
<td>0.305</td>
</tr>
<tr>
<td>85</td>
<td>0.087</td>
</tr>
</tbody>
</table>
other words, the enzyme appears to undergo at high temperatures a
form of inactivation which is reversible on cooling.

Specificity of Enzyme—The results of Experiment 3 indicate
that the nuclease described in the present paper is not a phos-
phatase. This is confirmed by the fact that the enzyme does not
hydrolyze any of the phosphoric esters tested. Other substrates
have also been tested; namely, egg albumin, hemoglobin, Witte’s
peptone, mucoproteins,\(^2\) a number of animal, plant, and bacterial
polysaccharides, ethyl acetate, tributyrin, and an ether-soluble
fraction extracted from pneumococci. The enzyme does not
attack any of these substances, nor could any action be detected
against thymus nucleic acid. In fact, of all soluble substrates
tested, yeast nucleic acid was the only one to be decomposed.
The enzyme also attacks the killed cells of pneumococci, changing
them from the Gram-positive to the Gram-negative state. This
change in staining behavior is not accompanied by any disintegra-
tion of the cell body. Only a small fraction of the cellular material
goes into solution as a result of enzymatic action; the chemical
nature of this phenomenon will be described in a later publication.

DISCUSSION

The present paper deals with an enzyme (ribonuclease) from
animal tissues which decomposes yeast nucleic acid and changes
pneumococci from the Gram-positive to the Gram-negative state.
The most active preparations (still contaminated with an inactive
polysaccharide) were found to contain 11 to 12 per cent nitrogen
and to give the usual protein reactions. The enzyme is salted-out
by sodium sulfate; it is soluble in 50 per cent acid acetone, but
rapidly becomes insoluble and inactive in neutral or alkaline ace-
tone. It is also completely inactivated by small amounts of
crystalline pepsin. All these properties, when taken jointly, leave
no doubt as to the protein nature of the enzyme. This protein,
however, is completely resistant to trypsin and chymotrypsin and
also retains all its enzymatic activity after prolonged heating at 95\(^\circ\).

The rate of enzymatic decomposition of yeast nucleic acid in-
creases with temperature up to about 70\(^\circ\); it then decreases sharply

\(^2\) The authors are greatly indebted to Dr. K. Meyer of the Department
of Ophthalmology of the Presbyterian Hospital, New York, for supplying
them with samples of different mucoproteins.
and no appreciable action can be detected at 85°. When, however, the enzyme-substrate mixture, previously heated to boiling temperature, is cooled to a temperature compatible with enzymatic action (60° for instance), the nucleic acid is rapidly decomposed. It appears possible that the protein which constitutes the ribonuclease is denatured at a high temperature, and is inactive in the denatured state; reversal of denaturation, accompanied by recovery of activity, would then take place at lower temperatures. It may be recalled that reversal of protein denaturation has already been established in the case of other enzymes, trypsin for instance (6).

In 1912 Jones (7) observed in a preparation of digested pancreas the existence of a principle capable of breaking down yeast nucleic acid. Although the same author stated later that, “it has been found difficult to repeat this experiment” (8), it appears that the enzyme described in the present paper may be the same as that discovered by Jones.

The unusual properties of the nuclease render it easy to concentrate and purify; the purified preparations do not attack any of the soluble substrates tested except yeast nucleic acid. These preparations, however, change pneumococci from the Gram-positive to the Gram-negative state and at the same time inactivate the type-specific antigen of encapsulated cells. It appears likely that the same principle which decomposes yeast nucleic acid also attacks the bacterial cells; in fact it will be shown elsewhere that it is possible to extract from pneumococci a soluble fraction which reacts like nucleic acid and is readily decomposed by the nuclease described in the present paper.

**SUMMARY**

An enzyme capable of decomposing yeast nucleic acid has been obtained from many animal tissues; the method of purification is described.

The enzyme is a protein which is rapidly inactivated by pepsin, but completely resistant to trypsin and chymotrypsin; it does not lose its activity when heated at boiling temperature over a wide range of pH.

The rate of enzymatic decomposition of yeast nucleic acid increases with temperature up to 70°; it then decreases and no
Decomposition of Yeast Nucleic Acid

action can be detected at 85°. The inhibiting effect of high temperature on the enzyme-substrate mixture is completely reversible on cooling.

The action of the ribonuclease on nucleic acid gives rise to decomposition products which are soluble in mineral acids. There is, however, no inorganic phosphorus liberated, and approximately half of the total phosphorus is recovered as an organic complex precipitable by uranyl chloride.

The ribonuclease does not decompose any of the other soluble substrates tested, in particular not thymus nucleic acid. It is capable, however, of attacking the killed cells of pneumococci, changing them from the Gram-positive to the Gram-negative state, without causing any disintegration of the cell structure.

BIBLIOGRAPHY

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