ON THE ACTION OF RIBONUCLEASE*

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In an earlier investigation from this laboratory (1), prostatic phosphomonoesterase was used for the partition of the phosphomonoester and phosphodiester groups in ribonuclease digests. It was shown that the inorganic phosphate formed during incubation of ribonuclease digests with phosphatase originated practically exclusively from the acid-stable (pyrimidine) nucleotide groups of ribonucleic acids. It was concluded that the action of ribonuclease involved specifically pyrimidine nucleotide groups of yeast ribonucleic acids. Independent observations from several other laboratories likewise suggest the assumption that the action of ribonuclease involves specifically, or at least preferentially, the pyrimidine nucleotide groups of yeast ribonucleic acids. It is still unknown, however, how many of the total pyrimidine nucleotide groups of yeast ribonucleic acid are hydrolyzable by ribonuclease. This question is the problem of the present investigation.

Methods

Substrates—Sodium nucleate (Schwarz Laboratories, Inc.) was dialyzed for 21 hours in thin collodion bags against a 1 per cent solution of sodium chloride in the refrigerator, precipitated with N hydrochloric acid, and stored in the form of its sodium salt. The phosphorus content of the samples varied between 8 and 8.5 per cent. Guanylic acid and the pyrimidine compounds were prepared according to established procedures (5–7); recrystallized commercial samples of yeast adenylic acid were used in the experiments. Diphenylphosphoric acid was prepared according to Bríg and Müller (8) and isolated as the crystalline barium salt.

Enzymes—Ribonuclease was isolated from pancreas according to Kunitz (9). In many later experiments crystallized ribonuclease from the Armour Laboratories was used.

Prostate phosphatase (10) was extracted from homogenized hyperto-

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phic glands with 5 volumes of water to which a few cc. of toluene were added. After standing overnight in the refrigerator, the suspension was centrifuged and stored. The crude extracts maintained their original activity for 2 to 3 months. For experiments, suitable amounts were dialyzed in thin collodion bags in the refrigerator for 16 to 30 hours. A 1 per cent solution of acetic acid was added dropwise to the dialyzed solution until a flocculent precipitate appeared. This occurred in a range between pH 5.7 and 6. An excess of acetic acid was avoided, as it caused large losses of the enzyme. The supernatant, which contained the full original activity, kept its activity for several weeks in the refrigerator. When the activity was too weak, the solution was concentrated by precipitating it with ammonium sulfate at 0.8 saturation and redissolving the precipitate in a small volume of water.1

Analytical Methods

Inorganic Phosphate—In solutions containing nucleic acid and poly-nucleotides, the bulk of these substances and the proteins were precipitated by the addition of 5 volumes of a 1.5 per cent solution of uranium chloride in 10 per cent trichloroacetic acid (MacFadyen's reagent (11)). This technique permitted the direct colorimetric determination of the phosphate in the supernatant according to the method of Fiske and Subbarow (12). A slight turbidity which appeared on addition of the molybdate was removed by centrifugation. The determination of the acid-labile and acid-resistant phosphorus groups of the nucleotides was carried out according to a procedure described in an earlier paper (1).

Separation of Nucleosides and Polynucleotides—Two methods were used: (a) The bulk of the high molecular polynucleotides was removed by dropwise addition of 5 N hydrochloric acid to the digests and subsequent centrifugation. The phosphorus compounds of the supernatant were removed by stirring an aliquot of the solutions with the anion exchanger Ion X, 250 to 500 mesh (Microchemical Specialties Company). During the adsorption, the pH was kept below 7 by dropwise addition of glacial acetic acid. The combined filtrates and washings, which contained 96 to 100 per cent of the nucleosides according to recovery experiments with added pure nucleosides, were analyzed spectrophotometrically or by periodate oxidation. The complete removal of the phosphorus compounds required approximately 8 gm. of ion exchanger per 100 mg. of nucleate.

1 All phosphatase preparations used in this investigation contained small amounts of ribonuclease which could be demonstrated after inactivation of the phosphatase activity by heat or by 0.01 N sodium fluoride. This did not essentially interfere with their use on ribonuclease digests, but it precluded conclusions of other than approximate nature regarding the action of phosphatase on ribonucleic acid itself.
The commercial samples of Ion X were activated in the laboratory by treatment with \( \text{NaOH} \). (b) The application of the anion exchanger is not feasible in alkaline hydrolysates of the enzymatic digests of ribonucleic acid. Owing to the absence of pyrimidine nucleotides from the phosphatase digests, a practically quantitative separation of the nucleosides from the nucleotides of the digest could be achieved by precipitation with an excess of uranium acetate in the presence of phosphate at a range between \( \text{pH} 6 \) and 6.5. In each experiment, the effectiveness of the precipitation was tested by determinations of the organic phosphorus in the supernatant. The following technique was finally adopted. An aliquot of the digest containing up to 15 mg. of total phosphorus was precipitated by the addition of 0.2 volume of 5 \( \text{N} \) hydrochloric acid. (If a subsequent precipitation with a silver salt was intended, the hydrochloric acid was replaced by sulfuric acid.) To a large aliquot of the supernatant, 1 cc. of a 0.5 \( \text{M} \) solution of monosodium phosphate and 20 cc. of a 1.5 per cent solution of uranyl acetate were added. The solution was brought to between \( \text{pH} 6 \) and 6.5 by adding an exactly measured volume of a 25 per cent solution of sodium hydroxide. After standing for 30 minutes at room temperature, the precipitate was centrifuged and the supernatant was used for the determination of the nucleosides.

**Determination of Ribonucleosides by Means of Sodium Periodate**—Since the hydrolysis conditions used in this investigation did not affect the linkages between the bases and the ribose groups, the amount of total ribonucleosides in the uranyl acetate supernatant could be determined by oxidizing their 2,3-glycol groups with sodium periodate. This technique has the advantage of giving the same value for equimolecular amounts of the nucleosides, regardless of the nitrogen content of the bases. It was found that the periodate oxidation of the 2,3-glycol group of ribose compounds proceeded very rapidly at room temperature either in alkaline or in weakly acid \( \text{pH} \) ranges (\( \text{pH} 5.5 \)). Since uranyl acetate, even in very small concentrations, interfered by precipitating periodate, it was completely removed from the supernatants by the addition of an excess of inorganic phosphate, except when the periodate reaction was carried out in the presence of sodium bicarbonate.

A sample containing approximately 5 mg. of nucleosides and ranging between volumes of 5 and 20 cc. was pipetted into a 250 cc. Erlenmeyer flask. If necessary, 0.2 volume of a 0.2 \( \text{M} \) buffer solution or 1 gm. of solid bicarbonate was added to the sample. The sample was mixed with 5 or 10 cc. of a 0.02 \( \text{M} \) sodium periodate solution (standardized against 0.01 \( \text{N} \) sodium arsenite) and left at room temperature for exactly 5 minutes, a stop-watch being used for the timing. After the addition of 1 gm. of
sodium bicarbonate and 2 cc. of a 10 per cent solution of potassium iodide, the iodine was immediately titrated with 0.01 N sodium arsenite. With this technique of timing, the losses of iodine due to secondary reactions were negligible. In more recent experiments this possibility was eliminated by adding a measured excess of a 0.01 N arsenite solution before the addition of potassium iodide and by titrating the excess of arsenite with a 0.01 N solution of iodine.

EXPERIMENTAL

Conditions of Enzymatic Hydrolysis of Yeast Ribonucleic Acid—Fig. 1 shows the approximate courses of time-activity curves of the action of ribonuclease on ribonucleic acid (RNA). Curves A, B, and C were obtained with different amounts of the enzyme. The degree of hydrolysis at each time interval was calculated from the amounts of inorganic phosphate formed by the subsequent exhaustive incubation with prostate phosphatase. It can be seen that the three curves obtained with different amounts of ribonuclease converge toward a degree of hydrolysis of 46 per cent.

Correspondingly, Fig. 2 (Curves B and B$_1$) shows time-activity curves of the action of different amounts of prostate phosphatase on digests obtained by an exhaustive incubation of sodium ribonucleate with ribonuclease. It can be seen that both curves converge toward a final degree of 46 per cent hydrolysis.

Time-activity curves of the action of different amounts of phosphatase on sodium ribonucleate itself are shown in Fig. 2, Curves A and A$_1$. The curves did not converge. The initial rates decreased strongly after respective degrees of 3 and 5 per cent$_1$ hydrolysis, but the activity continued at very slow rates for days.

Action of Prostate Phosphatase against Phosphoric Esters of Known Structure—It was found in experiments not reported in detail that the mononucleotides obtained from yeast RNA were hydrolyzed by prostate phosphatase at very similar rates and that the hydrolysis was complete within 2 hours when the incubation was carried out with 4 units$^3$ of phosphatase.

$^2$ The action of ribonuclease was slowly continuing during the incubation with phosphatase, despite the strong inhibitory effect caused by the dilution prior to the addition of phosphatase. The actual rates of ribonuclease action were, therefore, slower than those indicated by the time curves. This did not interfere with the purpose of the experiments for which only the final stages of the hydrolysis were of interest.

$^3$ 1 unit of prostate phosphatase is the amount which forms 0.1 mg. of inorganic P from 50 mg. of yeast adenylic acid within 15 minutes at 37°. Total volume 12 cc., containing 5 cc. of 0.1 N sodium acetate buffer of pH 5.5. The solution of adenylic acid is added after neutralization with sodium hydroxide to pH 7.
per mg. of nucleotide phosphorus (pH 5.5; 37°; 0.05 N sodium acetate buffer; 0.08 mg. of nucleotide phosphorus per cc.). The phosphoric diesters diphenylphosphoric acid, glycerylphosphorylcholine, glycerylphosphorylethanolamine, and the monophosphatides were completely resistant to prostatic phosphatase.

**Diagram 1. Degradation of RNA**

Analysis of RNA: total P 15 mg. (100%), inorganic P 0, 60 min. P 8.1 mg. (53.5%), uranium soluble 0, purine N 17 mg.

Digest IV. Acid phosphatase RNA

Inorganic P 1.2 mg. (8%) \(\rightarrow\) (Ribonuclease)

Digest I

\(\downarrow\) (Acid phosphatase)

Digest II. Inorganic P 6.4 mg. (42%), 60 min. P 8.3 mg. (55%)

\(\downarrow\) (NaOH, 37°, 24 hrs.)

Digest V. Acid phosphatase Digest III. Inorganic P 6.4 mg. (42%)

Inorganic P 14.7 mg. (98%) \(\rightarrow\) (Precipitation with uranium acetate)

Supernatant. Total P 0.43 mg. (2.9%), inorganic P 0

\(\downarrow\) (0.5 N sulfuric acid, 100°, 2 hrs.)

Hydrolysate. Purine N 0.4 mg. (2.4%)

Incubation with ribonuclease, 200 mg. RNA, 20 mg. ribonuclease, volume 2 cc., 24 hrs., 37°

Incubation with phosphatase, 200 mg. RNA, 20 cc. 0.05 M sodium acetate buffer (pH 5.3), 2 cc. phosphatase (600 units), 4 hrs., 37°

Specific Action of Ribonuclease on Pyrimidine Nucleotide Groups of Yeast RNA—Diagram 1 contains the results of a representative experiment on the hydrolytic degradation of yeast ribonucleic acid. After treatment of the ribonuclease digest (I) with phosphatase (Digest II), 6.4 mg. of inorganic P were formed. The organic P fraction of Digest II contained 8.3 mg. of acid-hydrolyzable P (as compared with 8.1 mg. of acid-hydrolyzable P of the original ribonucleic acid). Thus the total amount of the acid-hydrolyzable P was retained in organic linkage during the incubation with ribonuclease and phosphatase and the inorganic P formed originated exclusively from the acid-resistant fraction which was dephosphorylated to an extent of 93 per cent.

An explanation of the above results was suggested by the observation that prostatic phosphatase acted as a phosphomonoesterase in the experiments with mononucleotides and with phosphodiesters. If it is assumed
that the phosphodiester groups in polynucleotides are resistant to phosphatase action, it follows from Diagram 1 that the phosphomonoester groups formed by the action of ribonuclease on yeast ribonucleic acid

![Graph](image1.png)

**Fig. 1.** Approximate time curves of ribonuclease action. Portions of 500 mg. of sodium ribonucleate (total P 37.0 mg.) incubated with 50 mg. (Curve A), 10 mg. (Curve B), and 2 mg. (Curve C) of crystallized ribonuclease respectively. Volume 5 cc.; pH 5.5; no buffer was added. At various time intervals aliquots of 1 cc. of the incubation mixtures were mixed with 10 cc. of 0.05 n acetate buffer of pH 5.5 and 1 cc. of prostate phosphatase (40 units; see foot-note 3) and incubated for 3 hours. The amounts of inorganic P formed based on the original hydrolysis mixture are plotted as ordinates. In the experiment for Curve A, 300 mg. of yeast sodium ribonucleate were added to a 1 cc. aliquot of the incubation mixture at a time (arrow) when the ribonuclease action on the original substrate had approached its maximum. The hydrolysis curve after the addition of fresh substrate is represented by Curve A1.

**Fig. 2.** Action of prostate phosphatase on sodium ribonucleate (500 mg.) before (Curves A and A1) and after (Curves B and B1) exhaustive digestion with ribonuclease (21 hours). (600 mg. of RNA; total P 43 mg.; 60 mg. of ribonuclease; total volume 6 cc.) Total volume during incubation with phosphatase in both experiments 66 cc.; 0.05 n acetate buffer, pH 5.5. Curves A and B, 4 units of phosphatase per mg. of nucleotide P; Curves A1 and B1, 40 units of phosphatase per mg. of nucleotide P. (Inorganic P based on original hydrolysis mixtures.) In the experiment for Curve B, 4 cc. of a neutralized 1.25 per cent solution of yeast adenylic acid were added to an aliquot of 40 cc. of the digest at a time when the action of phosphatase on the original substrate solution had approached its maximum (arrow).

were exclusively attached to pyrimidine nucleoside residues, and that exhaustive treatment with ribonuclease led to the transformation of at least 93 per cent of the phosphoryl groups attached to pyrimidine nucleoside residues into phosphomonoester groups. Since no measurable part of the purine nucleotide groups was affected by ribonuclease, the results
suggest that two types of split-products were formed during the exhaustive action of this enzyme: (a) pyrimidine mononucleotides, and (b) purine polynucleotides (13) containing pyrimidine residues whose phosphate is predominantly attached in the form of monoester groups. These polynucleotides will be designated as limit polynucleotides in analogy to the nomenclature used for the hydrolysis of polysaccharides.

**Determination of Purine Nucleosides after Hydrolysis with Ribonuclease, Phosphatase, and Alkali and Fractionation with Uranium Acetate (Digest III)**—It was desirable to have an independent identification of the acid-labile phosphorus groups with the purine nucleotide residues. If the inorganic phosphate formed during the incubation with ribonuclease and phosphatase originated exclusively from pyrimidine nucleotide residues, the nucleoside fraction of the digest should not contain more than negligible amounts of purine ribosides. Digest II was incubated overnight with sodium hydroxide (Digest III). The hydrolysis was quantitative, since subsequent incubation of an aliquot of Digest III with phosphatase at pH 5.3 (Digest V) resulted in the transformation of all phosphorus groups into inorganic phosphate. The nucleotides formed were separated from the nucleosides by precipitation with uranyl acetate as described under “Analytical methods.” An aliquot of the supernatant was hydrolyzed by refluxing with 0.5 N sulfuric acid for 2 hours, and the purines were quantitatively precipitated from the hydrolysate with silver sulfate. The amount of purines found was 2.4 per cent of the total purines in the original ribonucleic acid. Since an amount of organic phosphorus corresponding to 2.9 per cent of the total nucleic acid phosphorus remained in the uranium acetate supernatant, the small quantities of purines found may be accounted for by the purine nucleotide groups which were not precipitated by the uranium reagent. A similar amount of purines was found in the nucleoside fraction of digests obtained by the incubation of yeast RNA with phosphatase and by subsequent hydrolysis with alkali without preliminary incubation with ribonuclease.

**Amount of Mononucleotides Formed by Action of Ribonuclease on Yeast Ribonucleic Acid**—The partition of the phosphoric monoester groups between free mononucleotides and terminal groups of limit polynucleotides after the combined action of ribonuclease and phosphatase was determined by measuring the periodate uptake of the resulting nucleoside fraction. Table I contains the results of such experiments as well as of similar tests on mononucleotides, on an alkaline hydrolysate of yeast RNA, and on polynucleotide and nucleotide fractions prepared after ribonuclease action only.

The mononucleotides of yeast nucleic acid (prepared by the procedures
mentioned), as well as dialyzed yeast ribonucleate and the products of its hydrolysis by ribonuclease, did not consume more than negligible amounts of periodate. The results of periodate determinations of the

**Table I**

*Action of Sodium Periodate on Nucleotides and on Alkaline and Ribonuclease Digests of Yeast RNA after Incubation with Phosphatase*

<table>
<thead>
<tr>
<th>Fraction No.</th>
<th>Substrate</th>
<th>Inorganic P formed, per cent of total P</th>
<th>0.005 N periodate consumed per mg. inorganic P</th>
<th>Ratio, nucleosides inorganic P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adenylic acid</td>
<td>98</td>
<td>7.2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Guanylic acid</td>
<td>97</td>
<td>7.5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Cytidylic acid</td>
<td>98</td>
<td>7.3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Uridylic acid</td>
<td>100</td>
<td>7.0</td>
<td>1</td>
</tr>
</tbody>
</table>

Alkaline hydrolysate of yeast RNA

| 5            | Total digest    | 96                                     | 7.2                                           | 1                              |
| 6            | Nucleoside fraction (Method b) | 7.3†                                    | 1                              |

Ribonuclease digest of yeast RNA

| 7            | Total digest    | 42                                     | 6.8                                           | 0.96                           |
| 8            | Nucleoside fraction (Method a) | 4.2                                    | 0.61                           |
| 9            | "              | 4.2†                                   | 0.61                           |

Acid-insoluble polynucleotide fraction (Fraction A)$

| 10           | Total digest    | 16.3                                   | 7.0                                           | 1                              |
| 11           | Nucleoside fraction (Method a) | 1.2                                    | 0.17                           |
| 12           | "              | 1.3†                                   | 0.19                           |

Acid-soluble nucleotide fraction (Fraction B)

| 13           | Total digest    | 78.5                                   | 7.0                                           | 1                              |
| 14           | Nucleoside fraction (Method b) | 6.0†                                    | 0.86                           |

* Conditions of incubation with ribonuclease, phosphatase, and alkali, respectively, as indicated in Diagram 1.

† Ratio of periodate to inorganic P calculated from inorganic P present before treatment with uranium.

‡ See foot-note 4.

Nucleoside fractions of the four mononucleotides after complete enzymatic dephosphorylation, Table I, Fractions 1 to 4, show a periodate uptake between 7.0 and 7.5 cc. of 0.005 N periodate per mg. of inorganic P formed. (The theoretical value for an equimolecular ratio would have been 6.5
cc.) In the case of guanylic acid, a very small but measurable reduction with Somogyi's reagent was found after phosphatase hydrolysis, indicating the presence of a small amount of nucleoside phosphorylase in this enzyme preparation and accounting for the higher periodate titration.

The results of a similar control experiment carried out on an alkaline hydrolysate of yeast nucleic acid and on the nucleoside fraction prepared from it are shown in Fractions 5 and 6. The periodate consumption of the hydrolysate amounted to 7.2 cc. per mg. of inorganic phosphorus formed. It may be noted that the periodate consumption of the uranium supernatant (the nucleoside fraction) was practically identical with that of the total digest. This shows that no losses of nucleosides occurred during the separation of the inorganic phosphate and the nucleoside fraction.

The periodate uptake in a representative experiment with a digest obtained by incubation of ribonucleic acid with ribonuclease and phosphatase and the resulting nucleoside fraction is shown in Fractions 7 to 9. In the nucleoside fraction (Nos. 8 and 9), the periodate consumption per mg. of inorganic phosphorus was 4.2 cc. or 61 per cent of the value found for the total digest shown for Fraction 7.

Behavior of Limit Polynucleotides against Sodium Periodate Before and after Dephosphorylation by Prostate Phosphatase—It has been mentioned that the limit polynucleotides did not consume significant amounts of periodate. It was found, however, that their enzymatic terminal dephosphorylation led to the formation of polynucleotides which consumed periodate in amounts which were approximately equimolecular with the amounts of their dephosphorylated end-groups (cc. of 0.005 N periodate per mg. of inorganic phosphate = 6.8, Fraction 7). Further evidence for the presence of periodate-consuming groups in partially dephosphorylated polynucleotides was obtained by analyses of the separated mono- and polynucleotide fractions, (Table I, Fractions 10 to 14). According to a previous report (13), a crude separation of the limit polynucleotides and of the mononucleotides could be achieved with barium acetate. The barium salts of the limit polynucleotides are much less soluble in water than those of the pyrimidine nucleotides, and the free limit polynucleotides are precipitated by hydrochloric acid from concentrated (5 to 10 per cent) solutions. Accordingly, the two fractions were prepared by adding to the ribonuclease digest of 1 gm. of ribonucleate 0.3 volume of a 25 per cent solution of barium acetate. The copious precipitate (Fraction A) containing approximately 50 per cent of the total phosphorus was centrifuged and washed once with a 10 per cent solution of barium acetate. The supernatant was combined with the washing and represented
the mononucleotide fraction (Fraction B) contaminated with unprecipitated polynucleotides. Fraction A was washed with alcohol and finally dried with alcohol and ether. The barium salt was decomposed by suspending it in 2 to 3 cc. of water and by adding dropwise under stirring approximately 0.2 volume of 5 N hydrochloric acid. During the addition of the acid, the free polynucleotides settled out in the form of a gummy precipitate which was centrifuged and washed twice by stirring it thoroughly with 2 to 3 cc. of 0.5 N hydrochloric acid. A considerable amount of polynucleotide was lost in the washings. The final precipitate, which was practically free of barium, was suspended in 5 cc. of water and dissolved by neutralizing it cautiously with sodium hydroxide. This solution was used for the enzymatic dephosphorylation. The mononucleotide fraction was freed from barium by sulfuric acid and neutralized with sodium hydroxide. The conditions of the enzymatic dephosphorylation were those described in Diagram 1.

It can be seen (Table I, Fraction 10) that the dephosphorylation of Fraction A resulted in the liberation of 16.3 per cent of its total P as inorganic phosphate. The total digest required 7.0 cc. of 0.005 N periodate per mg. of inorganic P liberated. Of the total consumption only 17 to 19 per cent was accounted for by the nucleoside fraction (Fractions 11 and 12).

The mononucleotide fraction (Fraction B) (Fractions 13 and 14) behaved very differently. It was dephosphorylated to an extent of 78.5 per cent, the periodate consumption was equimolecular to the formation of inorganic phosphate, and 86 per cent of the total periodate consumption took place in the nucleoside fraction.

**DISCUSSION**

*Action of Prostate Phosphatase*—The application of prostate phosphatase for the specific hydrolysis of phosphomonoester groups in ribonuclease digests was suggested by the observation that all mononucleotides of yeast ribonucleic acids were rapidly and completely hydrolyzed by this enzyme, whereas "model" diesters of phosphoric acid such as diphenyl phosphate, glycerylphosphorylcholine, and glycerylphosphorylethanola-

4 The nucleosides originated from a contamination of the polynucleotide fraction with barium salts of pyrimidine nucleotides. This contamination can be largely avoided by direct precipitation of the polynucleotide fraction from the ribonuclease digests with 0.5 N hydrochloric acid. Exhaustive digestion of such preparations with phosphatase resulted in the liberation of 17 per cent of the total phosphorus as inorganic phosphate. The formation of nucleosides accounted maximally for 5 per cent of the inorganic phosphate present after the incubation.
mine were completely resistant to prostate phosphatase. The transfor-
mation by phosphatase of at least 93 per cent of the acid-resistant phos-
phoryl groups of ribonuclease digests into inorganic phosphate, despite
the practically complete preservation of the acid-hydrolyzable phosphoryl
groups associated with purine nucleotides, suggests that the action of
ribonuclease consists in the specific hydrolysis of interlinking phosphoryl
groups between pyrimidine nucleotides to phosphomonoester groups.

This interpretation is supported by the independent isolation of pyrimi-
dine mononucleotides after ribonuclease action (4). In our experiments,
this fraction accounts for 61.5 (±5) per cent of the total pyrimidine
groups. It is likewise very probable4 that the pyrimidine residues which
remained attached to the limit polynucleotides also contain phosphate
in monoester linkage because the associated acid-resistant groups were
likewise hydrolyzed by phosphatase, whereas no appreciable amounts of
inorganic phosphate originated from the purine nucleotide groups.

Structure of Yeast Ribonucleic Acid—The preservation of all purine
groups in the form of polynucleotides during an enzymatic hydrolysis
which leads to the dephosphorylation of at least 93 per cent of all pyrimi-
dine groups excludes a regular alternating sequence of purine and pyrimi-
dine groups in the ribonucleic acid molecule. Chains of several pyrimidine
groups must instead be linked to chains of several purine groups. The
fact that approximately 60 per cent of the pyrimidine groups are liberated
by ribonuclease as mononucleotides shows that, in the ribonucleic acid
molecule, the remaining pyrimidine groups are linked to purine groups or
are combined in such a way as to be resistant to further action by this
enzyme.

The relatively large number of pyrimidine groups present in limit polynu-
ucleotides has a bearing on the possible average length of the pyrimidine
chains. A detailed discussion of this problem will only be fruitful when
it is possible to establish the location of an additional number of pyrimi-
dine groups in the ribonucleic acid molecule. Experiments concerning
this problem are in progress in our laboratory.

Nature of Some Interlinkages between Pyrimidine Nucleotide Groups in
Ribonucleic Acid Molecule—The observation that the action of phospha-
tase on limit ribopolynucleotides results in the formation of polynucleotides
which reduce periodate strongly suggests that at least a part of the in-
terlinkages between the pyrimidine nucleotide residues involves positions
other than the 2'- or 3'-carbon atoms of the ribosyl groups. Such posi-
tions could be either the 5' position of the ribosyl groups or certain posi-
tions of the pyrimidine rings.
ACTION OF RIBONUCLEASE

SUMMARY

1. The combined action of ribonuclease and of prostate phosphatase results in the transformation of at least 93 per cent of the acid-stable phosphorus groups of yeast ribonucleic acid into inorganic phosphate, whereas the amount of acid-labile organic phosphorus groups remains unchanged during the digestion. The evidence presented supports the explanation that ribonuclease specifically hydrolyzes the phosphoryl linkages between nucleoside residues of yeast ribonucleic acid, converting those attached to pyrimidine nucleosides to phosphomonoester groups.

2. The hydrolysis products formed during the exhaustive action of ribonuclease on yeast nucleic acid are (a) pyrimidine mononucleotides amounting to approximately 60 per cent of the total pyrimidine residues and (b) limit polynucleotides in which the purine to pyrimidine ratio is much higher than that of yeast ribonucleic acid.

3. Partial enzymatic dephosphorylation of the limit polynucleotides results in the formation of polynucleotides which react with periodate, in contrast to ribonucleic acid and to the limit polynucleotides. The periodate consumption is approximately equimolecular with the amount of inorganic phosphate formed. This suggests that at least a part of the pyrimidine nucleoside residues of ribonucleic acid is linked to the neighboring nucleotide groups on positions other than the 2' or 3' positions of their ribosyls.

4. The bearing of these observations on the structure of yeast ribonucleic acid is discussed. A regular alternating sequence between purine and pyrimidine groups in the yeast ribonucleic acid molecule has been excluded.

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