Studies on the Degradation of Yeast Ribonucleic Acid by Lanthanum Hydroxide. On the Existence of a Resistant Fraction

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Ribonucleic acid (RNA) was shown in the previous paper[1] to be hydrolysed almost completely into nucleosides and inorganic phosphate by the action of lanthanum hydroxide. However, it was still ambiguous as to whether the nucleic acid is completely dephosphorylated or not, since it is a compound of high complexity. In the case of the enzymatic degradation of RNA by ribonuclease (RNase), a non-dialysable residue has been known to remain which comprises 15 to 50 per cent of the original[2)3)], and this fact has offered a clue for the research on the ribonucleic acid structure[4)5)6)].

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In the work to be described it was found that the action of lanthanum hydroxide can not lead to the perfect dephosphorylation of RNA, but that a resistant fraction (LaRF) remains. Some properties of the resistant fraction were studied and compared with those of the ribonuclease resistant fraction (RNaseRF).

Materials and Analytical Methods

Yeast Ribonucleic Acid A commercial sample of RNA prepared from brewer's yeast by the method of Clark and Schryver[7] (Kirin Institute) was used after purification by the usual chloroform method.[8] The phosphorus content of the purified preparation was 8.0 per cent.

Lanthanum Carbonate The purification procedure of the carbonate has been described previously.

Ribonuclease The enzyme was extracted from calf. spleen and partially purified by the method of McDonald[9] up to the step of B Fraction. The preparation was freed from salt by washing with aqueous ethanol and then with absolute ethanol and dried in vacuum. Yeast RNA liberated no inorganic phosphate by the action of this preparation.

Ribonuclease Resistant Fraction 200 mg. of RNA were digested with 10 mg. of RNase in 15 cc. of M/100 magnesium sulfate. The digestion proceeded at 37° for 3 hours and occasional addition of dilute alkali maintained the mixture at *p*H 7—8. A 5 cc. aliquot was then removed and the residual 10 cc. of digested nucleic acid solution was dialysed through cellophane first against 500 cc of 2M solution of sodium chloride[10] then against 1 litre of distilled water for 24 hours, respectively. The estimation of the phosphorus content of the dialysis residue showed that 80 per cent of the original material had been removed.

Analytical Methods The same methods as those in the previous paper[1] were used.

Methods and Results

Estimation of LaRF The reaction mixture, similar to that described in the previous paper[1], was constructed as follows: 200 mg. of lanthanum carbonate were suspended in distilled water in a 50 cc. Erlenmeyer flask and dissolved by adding hydrochloric acid. On adjustment of the solution to *p*H 8.5 by the addition of aqueous ammonia, lanthanum hydroxide was precipitated. To this suspension a RNA solution containing 10.8 mg. of the purified material, 10 cc. of M/5 borate buffer solution at *p*H 8.5 and then water were added till the final volume was 40 cc. The reaction proceeded at 80°.

Preliminary experiments showed certain difficulties in the estimation of undephosphorylated RNA from the difference between the total and the inorganic phosphorus of the reaction mixture, since the difference was small. Fortunately, however, it was found that the liberated nucleosides are not adsorbed on lanthanum hydroxide, a powerful precipitating agent of nucleic acid. Therefore, the measurements were carried out in the following manner. A 4 cc. sample was withdrawn at intervals and centrifuged. The precipitates were washed twice with 8 cc. of dilute lanthanum hydroxide suspension respectively in order to remove nucleosides, and analysed for RNA by the orcinol method. It is a well known fact that in this colorimetric method the color is given by purine-bound ribose. The results of the experiment, which are presented in Table, 1, have been calculated on the assumption that the purine/pyrimidine ratio of the undephosphorylated fraction is the same as that of the original material.

TABLE I

ESTIMATION OF UNDEPHOSPHORYLATED RNA

Time (hrs.)	Undephosphorylated RNA (%)
40	7.8
56.5	7.3
99	5.9

It can be seen in TABLE I that some amounts of RNA remain undephosphorylated, although they are not completely stable to the action of lanthanum hydroxide. At any rate, they are remarkably resistant in view of the fact that 99.9 per cent dephosphorylation might be expected in 40 hours from the calculation based on the first order reaction constant of the reaction up to 85 per cent dephosphorylation.

Preparation of LaRF An RNA solution containing 15 g. of the material was mixed with a lanthanum hydroxide suspension which was prepared from 30 g. of the carbonate. The final volume was about 1 litre. This mixture was adjusted at *p*H 8.5 by the addition of aqueous ammonia and maintained at 80° for 40 hours. The occasional addition of ammonia prevented the lowering of *p*H of the mixture during the hydrolysis. The precipitate was subsequently filtered while warm, washed with hot water to remove nucleosides and suspended in about 150 cc. of 5 per cent solution of sodium bicarbonate which had previously been adjusted at *p*H 8 with dilute hydrochloric acid. After standing for about 2 hours, lanthanum carbonate was removed by centrifugation. The clear supernatant, which contained RNA, was made slightly acid (*p*H 5) with acetic acid, freed from the excess of bicarbonate by a current of air under reduced pressure and neutralized with ammonia.

The above procedure was repeated once more with lanthanum hydroxide prepared from 10 g. of the carbonate. LaRF solution thus obtained contained a small amount of inorganic phosphate, which was removed by the addition of a small amount of lanthanum hydroxide and subsequent centrifugation. The final yield of LaRF was calculated on the basis of its phosphorus yield to be about 1.5 per cent of the original material.

Analysis of LaRF The LaRF solution prepared as described contained about 15 per cent of

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DNA (TABLE II); it may be explained by the facts that DNA is not dephosphorylated by lanthanum hydroxide, and that yeast RNA preparation is generally contaminated by a small amount of DNA. Its purine-bound ribose/RNA-P was much greater than that of the original RNA.

TABLE II

COMPARISON OF ANALYTICAL FIGURES OF VARIOUS FRACTIONS

	DNA-P	Purine-bound Ribose
	Total P	RNA-P
	%	mol./mol.
LaRF	15	1.08
RNaseRF		0.80
RNA		0.50 (Standard)

DNA-P was measured by the diphenylamine method on the assumption that the purine/pyrimidine ratio was 1. Purine-bound ribose was calculated from the color intensity of the orcinol test on the assumption that the original RNA preparation contained equimolecular purine-and pyrimidine-bound riboses. RNA-P was taken as the difference between total P and DNA-P.

Dephosphorylation of Various Fraction by Lanthanum Hydroxide The dephosphorylation of LaRF by lanthanum hydroxide was very slow (Curve 1), and this was also the case even after digestion by RNase (Curve II), while RNaseRF (Curve III) and the whole digest of RNA by RNase (Curve IV) were dephosphorylated as easily as RNA itself.

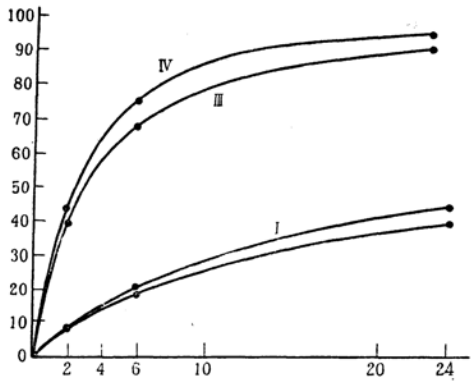

Fig. 1. Dephosphorylation of various fractions by lanthanum hydroxide. The preparation of RNaseRF and the whole digest of RNA by RNase are described in the text. The digestion of LaRF by RNase; LaRF solution containing about 400 μg. of phosphorus was incubated with 1 mg. of RNase at *p*H 7.0—7.5 and 37° for 3 hours. The incubations with lanthanum hydroxide were carried out at *p*H 8.5 and 80°. (The methods were described in the previous paper.)

Discussion

In the present experimental conditions, LaRF was not completely resistant to the action of lanthanum hydroxide. Anyhow, the observations presented here indicate that the structure of a certain specific portion of RNA is different from the rest.

The recent study in this laboratory showed that RNA becomes, by methylation of the hydroxyl group at C_2' of the sugar residue, as lanthanum hydroxide resistant as DNA, which has no hydroxyl group at C_2', and that the methylated RNA is not attacked by RNase[11]. In this connection, it is interesting that LaRF itself and LaRF treated with RNase were not easily dephosphorylated by lanthanum hydroxide, although the resistibility of LaRF to lanthanum hydroxide was not so great as that of the methylated RNA. It may be supposed that the hydroxyl group at C_2' might be masked in some way in LaRF. Anyhow this fact will offer a new clue for the elucidation of the structure of RNA.

Summary

1. Yeast ribonucleic acid is, as reported in the previous paper, hydrolysed almost completely into nucleosides and inorganic phosphate by lanthanum hydroxide, but the remaining fraction is considerably resistant to the action of the hydroxide.

2. Purine-bound ribose/RNA-p ratio of the resistant fraction is about 1.

3. Even after the treatment with ribonuclease, dephosphorylation of the resistant fraction by lanthanum hydroxide is not easy.

4. The ribonuclease resistant fraction is dephosphorylated by lanthanum hydroxide as easily as ribonucleic acid itself.

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