Further Studies on the Degradation of Yeast Ribonucleic Acid by Lanthanum Hydroxide

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By applying lanthanum hydroxide, which E. Bamann[1] introduced as a phosphatase model, to ribonucleic acid (RNA), F. Egami and M. Shimomura[2,3] found that RNA was almost completely hydrolysed to nucleosides and inorganic phosphate. This finding was confirmed and extended by E. Bamann[4,5], K. Dimroth[6], F. W. Allen[7] and their coworkers. The present paper deals with further studies on the degradation of yeast ribonucleic acid by lanthanum hydroxide gel with special reference to the nature of the products and the order of their formation.

Experimental

Materials and Solvent Systems.—*Yeast ribonucleic acid.*—A commercial RNA (Schwarz Laboratories) was used as such or after purification by the chloroform gel method[8]. The phosphorus content of the purified preparation was 8.5% and that of the original RNA about 8.0%.

LaRF.—M. Shimomura[9] found that a part of RNAwas resistant to lanthanum hydroxide. He named this fraction "lanthanum hydroxide resistant fraction (LaRF)". It was prepared according to Shimomura[9].

Solvent Systems for Paper Chromatography.—Solvent system 1[10], 70% (vol.) isopropanol in concentrated aqueous ammonia in the bottom of the chamber (0.35 ml for each liter of gas space); descending for 17 to 18 hr.; suitable for fractionation of various oligo- and mononucleotides.

1) E. Bamann, *Angew. Chem.*, **52**, 186 (1939).
2) F. Egami and M. Shimomura, *Science (Japan)*, **18**, 472 (1948).
3) M. Shimomura and F. Egami, This Bulletin, **26**, 263 (1953).
4) E. Bamann, F. Fischler and H. Trapmann, *Biochem. Z.*, **325**, 413 (1954).
5) E. Bamann, H. Trapmann and F. Fischler, *Biochem., Z.*, **326**, 89 (1954).
6) K. Dimroth, L. Jaenicke and D. Heinzel, *Ann. Chem.*, **566**, 206 (1950). K. Dimroth, L. Jaenicke and D. Heinzel, *Z. Physiol. Chem.*, **289**, 71 (1952).
7) J. E. Backer and F. W. Allen, *J. Biol. Chem.*, **188**, 59 (1951).
8) M. G. Sevag, D. B. Lackman and J. Smolens, *J. Biol. Chem.*, **124**, 425 (1938).
9) M. Shimomura, This Bulletin, **27**, 29 (1954).
10) R. Markham and J. D. Smith, ibid., **52**, 552, 558, 565 (1952).

Solvent system 2, 5 ml. of concentrated aqueous ammonia added to 100 ml. of 86% (vol./vol.) aqueous *n*-butanol; descending for 17 to 18 hr.; useful for separation of the four nucleosides.

Solvent system 3[11], a mixture of saturated ammonium sulfate in water (79 ml.), water (19 ml.) and isopropanol (2 ml.); ascending for 7 hr.; used for separation of 2'- and 3'-purine nucleotides.

Solvent system 4[12], a mixture of isopropanol (170 ml.), concentrated hydrochloric acid (41 ml.) and water (total 250 ml.); ascending for 36 hr.; used for separation of adenine, guanine, cytidylic and uridylic acid.

Process of Degradation of RNA by Lanthanum Hydroxide.—The lanthanum hydroxide suspension was made by dissolving lanthanum carbonate with hydrochloric acid and then by bringing the pH of the solution to the alkaline side. When the RNA solution was mixed with the lanthanum hydroxide suspension, RNA was bound completely to lanthanum hydroxide in the presence of an excess of the latter as shown in Figs. 1, 2, 3, and in Tables II and III, where 2 g. of lanthanum carbonate was used per gram of RNA. When 0.35 g. of the carbonate was used per gram of RNA, this being the condition in experiments for Fig. 4 and Table I, some amount of RNA was left unbound. Nucleosides, which were liberated during the reaction, were not adsorbed on lanthanum hydroxide, whereas liberated inorganic phosphate was bound to lanthanum hydroxide.

Fractionation of Oligo- and Mononucleotides formed from RNA by Lanthanum Hydroxide.—At intervals, the precipitates containing nucleotides and phosphate were separated from the reaction mixture by centrifuge and washed with hot water. They were extracted with 5% sodium bicarbonate solution and fractionated by paper chromatogram using solvent system 1. Thus a chromatogram shown in Fig. 1. was obtained. The reaction was carried out under the following conditions: pH 8.5 and 60°C, 500 mg. RNA and 1g. lanthanum carbonate.

By analysing the base constituents and comparing the spots with those of mononucleotides, it was suggested that fraction A contained pyrimidine nucleotides and adenylic acid, fraction B guanylic acid and oligonucleotides and fractions C, D and E polynucleotides. Under more drastic conditions, at 80°, a few hours were sufficient to degrade RNA to fractions A and B. When 10—20% of the original RNA was dephosphorylated, most of products were composed of fractions A and B.

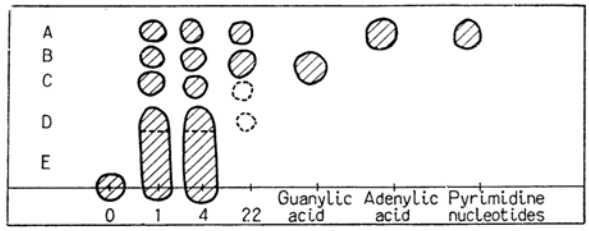


Fig. 1. Fractionation of lanthanum hydroxide hydrolysates by paper chromatography.

On the other hand, degradation of RNA by a less amount of lanthanum hydroxide (lanthanum carbonate 70 mg. to RNA 200 mg., that is, the atomic ratio of lanthanum to RNA phosphorus was 1/4), proceeded as shown in Table I.

Although the supernatant contained RNA, dephosphorylation did not occur in this fraction, but only in the precipitate fraction. On the other hand, paper chromatography with solvent system 1 showed that RNA in both the supernatant and the precipitate was similarly degraded to fractions A and B within 12 hr.

TABLE I

DEPHOSPHORYLATION OF RNA BY A LESS AMOUNT OF LANTHANUM HYDROXIDE. THE REACTION WAS CARRIED OUT AT 60° FOR 9 HR. AND CONTINUED AT 80°. THE WHOLE REPRESENTS THE ENTIRE REACTION MIXTURE AND, AT INTERVALS, WAS DIVIDED INTO THE SUPERNATANT CONTAINING UNBOUND RNA AND THE PRECIPITATE

Fraction	Time (hr.)			
	5	12	27	51
Supernatant	0%	0%	0%	0%
Whole	ca. 0	3.3	18	31
Precipitate	ca. 0	12	49	74

Production of 2'- and 3'-Purine Nucleotides.—The precipitates, which were separated from the reaction mixture by centrifuge and washing, were extracted with 5% sodium carbonate solution. By two dimensional paper chromatography with solvent system 1 and 3, 2'- and 3'-adenylic, and 2'- and 3'-guanylic acid were detected in the extracts.

According to this chromatogram, 2'-purine nucleotides were produced less than the corresponding 3'-nucleotides, and

11) R. Markahm and J. D. Smith, ibid., **49**, 401 (1951).
12) G. R. Wyatt, ibid., **48**, 584 (1951).

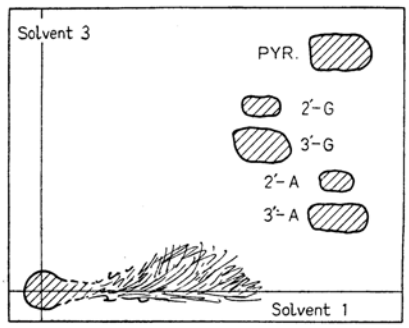


Fig. 2. Two dimensional paper chromatogram

A proviso: RNA 500 mg. lanthanum carbonate 1 g. at pH 8.5 and 80, after 1 hr.

Spots: PYR., 2'-G, 3'-G, 2'-A, 3'-A, are abbreviations for pyrimidine nucleotides, 2'- and 3'-guanylic, 2'- and 3'-adenylic acid, respectively.

this tendency was still more markedly observed in the precipitate fraction under the same conditions as in Table I. Besides we were not successful in detecting 5'-mononucleotides by the Boeseken reaction and the periodate oxidation method[13].

Liberation of Nucleosides.—Purine-bound ribose was measured by the orcinol reaction on the assumption that the color intensity was given quantitatively and only by purine-bound ribose. The molar ratio of purine-bound ribose/RNA-phosphorus of the original RNA preparation was taken as 0.5, assuming that equimolar proportions of purine and pyrimidine nucleotides were present.

As shown in Fig. 3, the ratio increased with reaction time. It seems to indicate that there is an increase in the amount of purine-bound phosphorus in the undephosphorylated RNA; it is evident that pyrimidine nucleosides were liberated faster than purine nucleosides on the whole (Fig. 3).

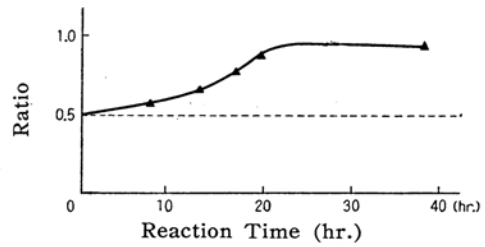


Fig. 3. Change in the purine-bound ribose/RNA-phosphorus ratio of the undephosphorylated RNA with time under the same conditions as used in Fig. 2.

Interrelation between purine nucleosides and also between pyrimidine nucleosides was observed as in the following Tables IIa and IIb. Adenosine was liberated faster than guanosine, and cytidine faster than uridine as expected from the dephosphorylation of mononucleotides[5] (Table III). However unexpected liberation of nucleosides was observed at the initial stage of reaction. Namely, at the initial stage liberation of adenosine was extraordinarily more than that of guanosine, and that of uridine was faster than that of cytidine, and increased later again during the reaction.

TABLE IIa

LIBERATION OF PURINE NUCLEOSIDES AT VARIOUS INTERVALS, THE MOLAR RATIO TAKING GUANOSINE AS UNITY

Nucleosides	Time (hr.) 0 to 0.5	0.5 to 6	6 to 20	20 to 40
Guanosine	1	1	1	1
Adenosine	2.5	1.7	0.5	0.3

TABLE IIb

LIBERATION OF PYRIMIDINE NUCLEOSIDES AT VARIOUS INTERVALS, THE MOLAR RATIO TAKING URIDINE AS UNITY

Nucleosides	Time (hr.) 0 to 0.5	0.5 to 6	6 to 20	20 to 40
Uridine	1	1	1	1
Cytidine	0.6	1.3	0.9	0.6

TABLE III

DEPHOSPHORYLATION OF MONONUCLEOTIDES UNDER THE FOLLOWING CONDITIONS: pH 8.5 AND 80°, EMPLOYING MONONUCLEOTIDES 30 mg. EACH AND LANTHANUM CARBONATE 200 mg.

Nucleotide	Time (hr.) 5	20	40
Guanylic acid	39%	90%	100%
Adenylic acid	55	94	100
Uridylic acid	40	93	100
Cytidilic acid	70	97	100
5'-Adenylic acid	12	54	100

The preferential liberation of uridine in the beginning was clearly observed on the chromatogram of the nucleosides in the supernatant of the reaction mixture under the same conditions as in Table I. In this experiment, the atomic ratio of lanthanum to RNA phosphorus was about 1/4 (Fig. 4). For the first 5 hr., uridine was detected as the only nucleoside on

13) K. Makino, *J. Japan. Biochem. Soc.*, **27**, 489 (1955).

the chromatogram; it was observed that, at this time, little of RNA was dephosphorylated and only a small amount of pyrimidine nucleotides was detected on the chromatogram with solvent system 3.

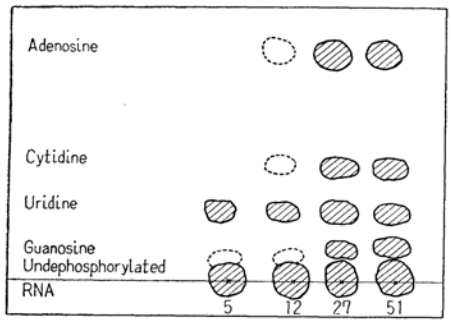


Fig. 4. Nucleosides chromatogram with solvent system 2

On the Nature of LaRF.—After dialysis of LaRF against distilled water through a cellophane bag for 2 days, the dialysed fraction showed the following broad absorption curve with maximum about 260 mμ; however the nondialysable fraction had no absorption maximum at 260 mμ (Fig. 5). The latter fraction did not give the orcinol reaction either.

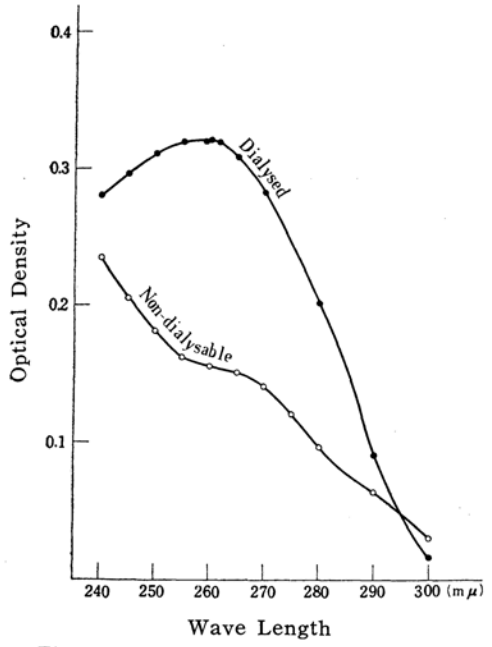


Fig. 5. Absorption curve after dialysis

LaRF at the concentration of 4 to 5 mg./cc. was not precipitated with uranium reagent (0.25% uranyl acetate in 2.5% aqueous trichloroacetic acid solution; this is a powerful precipitant of oligo- and polynucleotides) and by lowering pH to 1 by hydrochloric acid, either.

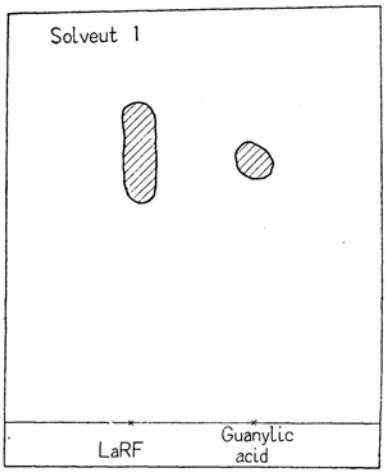


Fig. 6. Paper chromatogram of LaRF

Paper Chromatography of LaRF.—Fig. 6 shows the paper chromatogram of LaRF with solvent system 1. The R_f value of LaRF was close to that of guanylic acid and no spots were detected near the origin. So LaRF seems to contain no large polynucleotides. The spot of LaRF on this chromatogram showed fluorescence by hydrogen chloride fume. Molar ratios of bases in LaRF:

TABLE IV
MOLAR RATIOS OF BASES IN LaRF WHERE GUANINE IS UNITY

Guanine	Adenine	Uracile	Cytosine
1	0.8	ca. 0.2	ca. 0.3

According to this analysis of the base constituents, LaRF contained predominantly guanine, and adenine and a much smaller amount of uracil and cytosine as already suggested by Shimomura[9].

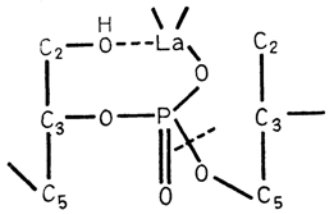
Discussion

The formation of four mononucleotides at the early stage of reaction (10~20% dephosphorylation) and the parallelism between the liberation of nucleosides and the dephosphorylation velocity of mononucleotides seem to indicate that most of the RNA was degraded to mononucleotides, and the latter were in turn dephosphorylated.

However it should be noted that the liberation of uridine and adenosine was unexpectedly fast at the beginning and

that the liberation of uridine increased later again. These findings seem to suggest that lanthanum hydroxide binds more easily with certain specific positions of RNA, and uridine exists in two different positions.

As intermediate reaction products, 2′- and 3′-purine nucleotides were detected. 2′-Nucleotides were less in amount than corresponding 3′-nucleotides, especially in the experiments with a small quantity of lanthanum hydroxide. These facts may be explained by Bamanns inner complex hypothesis[5].



Concerning LaRF, its chemical nature could not be finally elucidated. However it was concluded that it is a low molecular weight compound with high guanylic and adenylic acid content. It is not yet resolved, on what its resistance to lanthanum hydroxide depends.

Summary

The degradation of yeast ribonucleic acid by lanthanum hydroxide was reinvestigated. The results may be summarized as follows:

1) Most of the RNA seems to be degraded through mononucleotides to nucleosides and inorganic phosphate.

2) 2′- and 3′-purine nucleotides were detected as intermediate products. 2′-Nucleotides were produced in less amounts than the corresponding 3′-nucleotides.

3) On the whole, pyrimidine nucleosides were liberated faster than purine nucleosides.

4) Liberation of uridine was characteristic. It was preferencially liberated at the beginning and its liberation predominated again at the last stage of reaction.

5) "Lanthanum hydroxide resistant fraction (LaRF)" was dialysable, not precipitated with uranium reagent and had R_f value close to that of guanylic acid.

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