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THE ENZYMATIC DEGRADATION OF RIBONUCLEIC ACID BY CRYSTALLINE RIBONUCLEASE

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THE ENZYMATIC DEGRADATION OF RIBONUCLEIC ACID BY CRYSTALLINE RIBONUCLEASE

C. E. Carter and W. E. Cohn

The action of ribonuclease upon yeast nucleic acid is characterized by the appearance of dialyzable products, the liberation of acid groups, and by the isolation of mononucleotides which are presumably primary products of the enzymatic reaction.[1,2,3] On the latter point there is disagreement as to the identity of the mononucleotide product. Loring and Carpenter[3] isolated and identified the four mononucleotides composing yeast ribonucleic acid, while Schmidt et al.,[4] by a combination of enzymatic and chemical analyses, advanced evidence which indicates that the pyrimidine nucleotides are the principal, if not exclusive, mononucleotide products.

By a combination of ion-exchange analysis[5] and paper chromatography[6], we have analyzed the acid-soluble products of the ribonuclease degradation of yeast ribonucleic acid and have found, in agreement with Schmidt[4], that the principal mononucleotide products are the pyrimidine nucleotides, uridylic and cytidylic acids. From approximately 220 mg. of acid-soluble nucleic acid degradation products, 25 mg. of cytidylic acid and 26 mg. of uridylic acid were obtained. In addition to the pyrimidine nucleotides, very small amounts of adenylic (1-2 mg.) and guanylic acids (0-1 mg.) were identified. The latter nucleotide was found in fractions of the eluate subsequent to mononucleotide removal and is probably a degradation product of labile polynucleotides arising during the separation procedure. Also four fractions were isolated from the column effluents which are apparently low molecular weight polynucleotides of differing compositions. Two of these fractions (B-3 and C-2) exhibited constant spectrophotometric ratios throughout the elution peak

and all chromatographed as single components. Regarding the purine nucleotides and the polynucleotide fractions, it cannot be definitely stated that these components are not artifacts arising during the analytical procedure. In the case of the purine nucleotides, this is almost certainly true, because small amounts appear in anomalous regions in the ion-exchange elution diagram.

Aside from the interest attendant upon the identification of pyrimidine nucleotides as the mononucleotide product and the significance this may hold for structural considerations in nucleic acid chemistry, the ability to isolate small molecular weight polynucleotide fractions from the digest also opens an avenue of approach to the study of the subgroup structure of nucleic acids. Although the evidence presented (i.e., single component by ion exchange and paper chromatography) does not establish the molecular homogeneity of any one of the polynucleotide fractions, and only tentative statements regarding subgroup structure can be made, it is apparent that the polynucleotide components, isolated by ion-exchange methods from the nuclease digest, exhibit strikingly different composition following degradation to their constituent mononucleotides in alkali (Table I). If the difference of composition is a reflection of intramolecular order of nucleotides in yeast ribonucleic acid then, as shown in Fraction C-2, some polynucleotide segments are probably completely lacking in cytidylic acid subsequent to nuclease degradation and this may be an indication of "branching" in the parent molecule.

This latter problem, which deserves more extensive investigation is undergoing further study in this laboratory. However, it seems worthwhile to report at this time our preliminary studies and present the evidence in support of Schmidt's findings that pyrimidine nucleotides are the principal mononucleotide products of the ribonuclease degradation of yeast ribonucleic acid. (It has also been found[7], that the degradation of calf spleen ribonucleic

TABLE I

MONONUCLEOTIDES RECOVERED BY ION-EXCHANGE SEPARATION AFTER ALKALINE HYDROLYSIS OF POLYNUCLEOTIDE FRACTIONS

Fraction	Mg.	% Recovery as Mononucleotide	Cyt.	Ad.	Ur.	Gu.
			mg.	mg.	mg.	mg.
B-2	8	ca 70	0.75	2.5*	1.1	1.1**
B-3	22	-	0.3-0.5	6.5-9.8*	4.2-7.8	1.3-4.5
B-4	5	ca 90	0.8	2.0*	0.65	1.0**
C-2	59	ca 100	~0	10	14	35**

* Adenylic a and b both present.

** Guanylic a and b both present.

acid by crystalline ribonuclease liberates only pyrimidine mononucleotides.)

Experimental

Schwarz sodium ribonucleate (yeast) was dialyzed against distilled water with frequent changes for three days. From the nondialyzable fraction an aliquot containing 300 mg. nucleic acid was removed and the volume adjusted to 10 ml. with distilled water and the pH to 7.6 with sodium hydroxide. This solution was then placed at the glass electrode and 5 mg. of crystalline ribonuclease (Armour) added. Stirring was accomplished with a magnetic flea and the digest was intermittently titrated with 0.1 N sodium hydroxide from a microburette to maintain the pH at 7.6. At the end of approximately 90 minutes, there was no further liberation of titratable acid groups, 7×10^{-7} eq. OH^- per mg. of nucleic acid having been added to this point. Hydrochloric acid was then added to pH 3.0 and the digest centrifuged to remove acid insoluble material. The supernatant solution, estimated by spectrophotometric assay to contain 220 mg. of nucleic acid derivatives, was adjusted to pH 9.0 and a volume of 100 ml. with ammonium hydroxide and put on an anion-exchange column (Dowex-1) which had been converted to the chloride form. The dimensions of the resin bed were 12 cm. by 0.74 cm^2. The column was then washed with water, then with 100 ml. of 0.01 N sodium chloride.

The procedure from this point, described by Cohn[5], consisted in removing the mononucleotides from the column with 0.003 N hydrochloric acid. The nucleotides have been shown[5] to appear in the following order: Cytidylic acid, adenylic "a"[5,6], adenylic "b"[5,6], uridylic acid, and guanylic acids. The effluent was analyzed spectrophotometrically[5] and, after concentration, by paper chromatography[6], showed the following:

1. Effluent before acid elution: less than 1 mg. free base and riboside.

2. 0 - 110 ml. - A single peak containing 25 mg. cytidylic acid.

3. Next 250 ml. - Two small peaks identified as adenylic acid "a" + "b" (not completely resolved) containing approximately 0.5 mg. of each component.

4. Next 250 ml. - A single small peak, not characteristically found in the elution diagram of mononucleotides[5] shown by paper chromatography to contain both of the two adenylic acids and a small amount of cytidylic acid. The total nucleotide content was less than 1 mg.

5. Next 1000 ml - A single peak, the maximum occurring at 500 ml. of this volume. The central portion of the fraction (400-600 ml.) was shown to be at least 95% uridylic acid; 14 mg. of uridylic acid was found in this fraction. Chromatographic analysis of the ascending portion of the peak (200-400 ml.) showed small amounts of adenylic and cytidylic acid, which were also found in the descending portion of the peak (600-800 ml.). In these fractions, another 12 mg. of uridylic acid was identified. The next 800 ml. of the tail of this peak contained 4 mg. of nucleotide material; this was shown to be adenylic acids "a" + "b" and cytidylic acid in roughly equivalent amounts. Again, as in 4 above, finding adenylic and cytidylic in this region of the elution diagram is anomalous and indicates that these compounds result from a slow degradation of a polynucleotide still on the column.

In the guanylic acid region of the elution diagram, employing 0.003 N hydrochloric acid, no ultraviolet absorbing material was found. Previous experiments[5] had shown that all mononucleotides are off the column at an effluent volume of 2400 ml. In the nuclease experiments, no guanylic acid had been

found in the effluent by the time this volume was reached.

From the above data, it is concluded that the mononucleotide product of the nuclease degradation of ribonucleic acid was, in terms of spectrophotometric units (260 mμ. density x volume of solution), approximately 27% of the acid-soluble nucleic acid derivatives, which had been put on the column, and consisted mainly of uridylic acid (26 mg.) and cytidylic acid (25 mg.).

The subsequent fractions in the column effluent, which were obtained by increasing the acidity and chloride concentration of the eluting solutions, are not clearly defined chemical entities. Some of these fractions, when examined by paper chromatography, were found to contain small amounts of components which appeared in the positions characteristic of mononucleotides. However, since these particular fractions either were or contained mixtures of mononucleotides and were not characterized by constant spectrophotometric ratios (280 mμ./260 mμ.), it is believed that the mononucleotide content was due to continuous degradation of higher molecular weight compounds during the separation. In addition, all of these fractions showed the presence of a substance or substances which chromatographed as single components but could not be identified as mononucleotides. It is believed that these may be polynucleotides.

The order of elution and analysis of fractions, following mononucleotide removal as described above, follows: (The amounts estimated for each fraction are based on absorption of the solution at 260 mμ. and, in mixture of unknown composition, may be in error by 10-20%.)

A. Elution with 0.01 N hydrochloric acid gave a single peak, with a maximum at 250 ml. The 280/260 ratio rose from a value of 0.57, constant during the first 100 ml. of this elution, to a maximum of 0.99 at the 300-ml. mark and then fell to 0.65 at the 550-ml. mark. This was taken as evidence

of further removal of cytidylic acid, which was confirmed by paper chromatograms, as well as of more than one component. The chromatograms showed two additional spots, both in the adenylic acid region; at least one of these seemed to be polynucleotide in nature, similar to those eluted later (see B and C below). The amounts eluted were: 0-100 ml., 2 mg.; 100-550 ml., 17 mg.; 550-950 ml., 2 mg. (280/260 ratio of 0.65).

B. Elution with 0.02 N hydrochloric acid gave two peaks with maxima at about 30 ml. and at 200 ml. The first 90 ml., containing 7 mg. of total nucleotide and with a 280/260 ratio of 0.65, chromatographed as a mixture of components, one of which seemed to be adenylic acid; the high 280/260 ratio would thus indicate the presence of cytidylic acid in this fraction. The second 100 ml. (Fraction B-2), essentially the ascending portion of peak two, contained 8 mg. with an average (but falling) 280/260 ratio of 0.48. Alkaline hydrolysis of this, followed by ion-exchange separation of the mononucleotides, yielded the data in Table I.

The third fraction, of 410 ml. (B-3), containing the second peak had 22 mg. of material with a constant 280/260 ratio of 0.35 throughout its removal from the column and chromatographed as a single component. The next 400 ml. (B-4), containing 5 mg., showed a continually rising 280/260 ratio which went from 0.36 to 0.6 as the nucleotide content fell. These fractions were also degraded by alkali and analyzed by ion-exchange separation[5] of the derived nucleotides; the data are given in Table I.

C. Elution with 0.02 N HCl + 0.08 N NaCl also gave a prompt peak in the first 20 ml. followed by one at 100 ml. The first 30 ml. contained 5 mg. of material giving two spots on the paper chromatogram in the adenylic acid region but with a 280/260 ratio of 0.63, too high for free adenylic acid.

About 60 mg. were removed in the next 650 ml. (C-2), with a relatively constant 280/260 ratio of 0.47. This chromatographed as a single component and was analyzed by the same procedure employed for the fractions B-2, B-3 and B-4 (see Table I).

D. Elution with 0.02 N HCl + 0.2 N NaCl yielded 16 mg. of material, with a constant 280/260 ratio of 0.52, which chromatographed as two components. No further identification was made. It is not to be concluded that this material differs in composition from that removed in C.

E. Elution with 0.1 N NaOH yielded nothing, but subsequent elution with 100-ml. portions of 0.5 N NaCl and of 0.1 N HCl reclaimed 12 mg. of material with a 280/260 ratio of 0.6. This brought the total recovery to 94% (in terms of 260 mμ. absorption); further treatment of the column with 1 N sodium hydroxide and acid failed to produce the remainder.

Summary

The products of the enzymatic separation of yeast ribonucleic acid by crystalline ribonuclease have been analyzed by ion exchange and paper chromatography. The mononucleotide product is almost exclusively uridylic and cytidylic acids in approximately equal amounts. The small quantity of adenylic and guanylic acid appearing as mononucleotides seems to arise from higher molecular weight compounds during the analytical separation on the ion-exchange resin. Several discrete fractions, which were bound more strongly to the anion exchanger than are mononucleotides, were analyzed and each was found to have a different polynucleotide composition. These latter fractions may be artifacts resulting from the analytical procedure or polynucleotide subgroups arising from the action of nuclease on nucleic acid or degradation products of such polynucleotides.

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