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5S RNA MAY NOT HAVE HIGHLY ORDERED STRUCTURE LIKE 4S RNAS

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The rates of hydrolysis of the following polyribonucleotides as catalysed by RNase I, an enzyme specific for single stranded RNAs, follow the sequence shown; poly (A) > 23S RNA > 5S RNA >> 16S RNA > 4S RNA = poly (I).poly (C). The rates were measured by direct spectrophotometric as well as by trichloroacetic acid precipitation methods. The extents of inhibition of RNase I-catalysed hydrolysis of poly (A) by each of the above-mentioned polyribonucleotides follow the reverse order. Taking into account the fact that double stranded RNAs are inhibitory to RNase I it may be concluded from the above results that 5S RNA has much less ordered structure than 4S RNAs. This prediction is contrary to expectations and its validity will be known when the tertiary structure of 5S RNA will be worked out. These results also indicate that 16S RNA may have more folded structure than 23S RNA.

The enzyme RNase I of Salmonella typhimurium is being used in this laboratory in studies on the structure of E.coli ribosome (1). It is an endonuclease and specifically hydrolyses single stranded RNAs but has no base specificity (2,3). It has strong affinity for double-stranded RNAs but is not able to hydrolyse those (4). Due to this interesting property the enzyme is capable of providing useful information about the ordered structure in RNA. Some interesting observations made by using 5S RNA as substrate for the enzyme are reported here.

The kinetics of degradation of 5S RNA along with those of poly (A), 4S (mixture of tRNAs), 23S and 16S RNAs were

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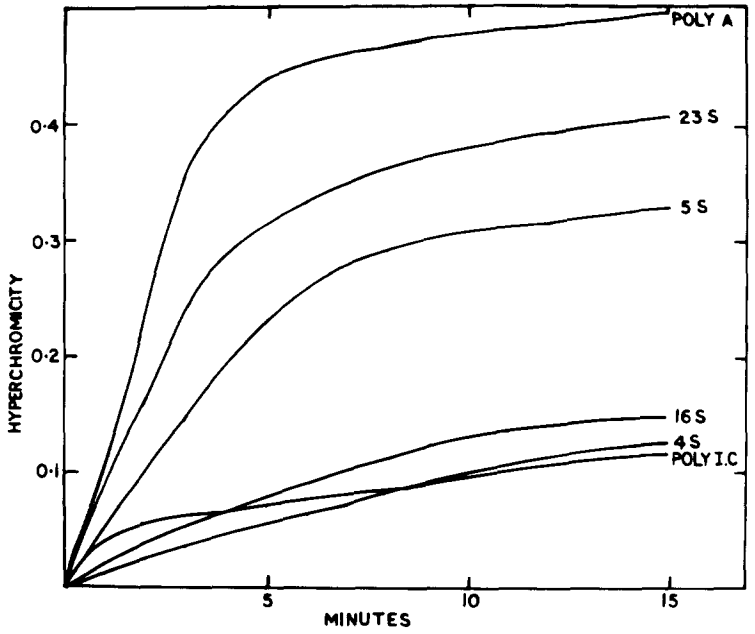

Fig.1. Kinetics of degradation of various types of polyribonucleotides by RNase I as measured by Direct spectrophotometric method

Degradation of RNAs by RNase I in presence of 0.1 mM magnesium acetate was carried out in quartz cuvette (1.0 cm path length) at 25°C in a total volume of 1 ml containing 100 µmoles of Tris-HCl pH 7, 1 A_{260} unit of each polyribonucleotide and 0.5 unit of S. typhimurium RNase I (ref.3). The enzyme was omitted in the control cuvette and the increase in absorbancy of the reaction mixture at 260 nm was followed against the control in Zeiss PMQ II Spectrophotometer.

studied in a spectrophotometer by following the increase in absorbancy (hyperchromicity) at 260 nm (Fig.1). Although the substrates are of various chain lengths and it was expected that long chains like those of 16S (~1500 nucleotides) and 23S (~ 3000 nucleotides) RNAs would be degraded more rapidly than smaller RNAs, poly (A) of very short length (~300 nucleotides), was found to be hydrolysed at a much faster rate than any of the other polyribonucleotides tested. This may be due to the fact that poly (A) has much less secondary structure in contrast to rRNAs. 23S RNA is also degraded at a very rapid rate, but somewhat slower than poly (A). 16S RNA, however, is degraded very

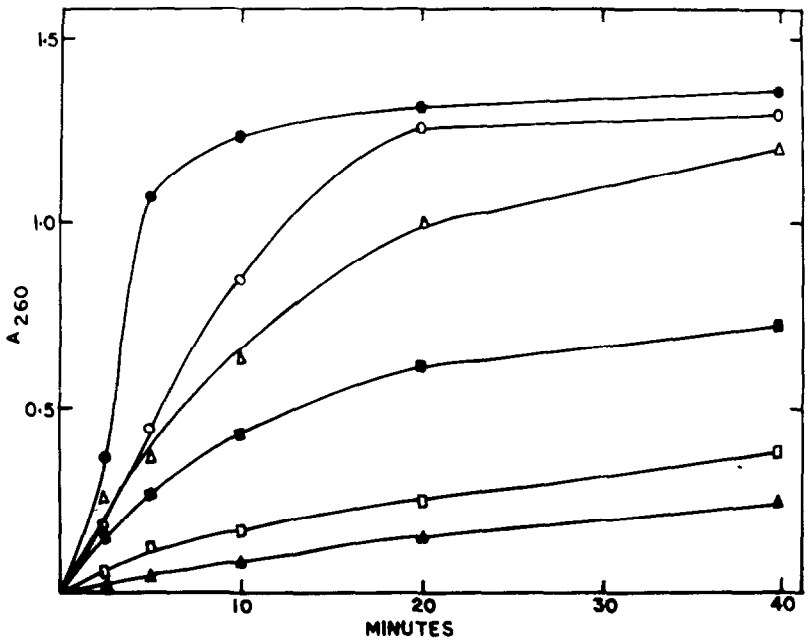

Fig.2. Kinetics of degradation of various types of polyribonucleotides by RNase I as measured by trichloroacetic acid precipitation method

The incubation was carried out in a total volume of 3 ml containing 100 µmoles of Tris-HCl, pH 7, 300 µg of polyribonucleotide, 240 mg of bovine serum albumin, 0.1 mM magnesium acetate and 0.36 unit of S.typhimurium RNase I. The incubation was carried out at 35°C and 0.5 ml of aliquot was removed at requisite time intervals. The reaction was stopped by the addition of 0.1 ml of 50% trichloroacetic acid containing 0.38% uranyl acetate. After half an hour in ice the precipitate was removed by centrifugation. The release of acid-soluble material was measured at 260 nm in Zeiss PMQ II Spectrophotometer after suitable dilution. The blank against which the reading was taken contained trichloroacetic acid and uranyl acetate in the same proportions as in the diluted reaction mixture.

● Poly (A); ○ 23S RNA; △ 5S RNA; □ 4S RNAs
■ 16S RNA; ▲ Poly (I).Poly (C)

slowly. The most interesting observation, however, was made with 5S RNA, which is degraded much more rapidly than 16S RNA. As expected, 4S RNAs and poly (I).poly(C) are degraded very slowly, because of their highly ordered structure.

Somewhat similar observations were made by measuring the absorbancy of the trichloroacetic acid-soluble materials

released (Fig.2). While the spectrophotometric assay depends on the cleavage of phosphodiester linkages, independent of the chain lengths produced, the assay based on release of acid-soluble mononucleotides and oligonucleotides depends on the size of the fragments produced. Again it is observed that poly (A) is degraded most rapidly and poly (I).poly (C) at the slowest rate. The rate of degradation of 5S RNA is comparable to the rates observed with poly (A) and 23S RNA, at least in the initial stage; it is, however, degraded much more rapidly than 16S RNA and behaves quite differently from 4S RNAs. Very little difference was observed between the A and B forms (5,6) of 5S RNA as substrates for RNase I (results not presented) indicating the absence of a gross change in the three-dimensional structure in the switch from one form to the other.

The results of a third method of assay, the inhibition assay, are shown in Fig.3. RNAs with highly ordered structure are known to inhibit the hydrolysis of a polynucleotide, poly(A) in this case, as catalysed by RNase I. Using [^{3}H]-poly (A) as substrate the hydrolysis of poly (A) was found to be inhibited by some polyribonucleotides. The 4S RNAs are strongly inhibitory; about 2 µg are capable of producing 50% inhibition. Poly (I).poly(C) was also found to be strongly inhibitory. To produce 50% inhibition more than 20 µg of 23S RNA are necessary whereas about 4 µg of 16S RNA are capable of producing the same amount of inhibition. About 9 µg of 5S RNA are required to produce 50% inhibition. These results also clearly indicate that 5S RNA may not have highly ordered structure as 4S RNAs.

The clover-leaf model of tRNA was proposed long before its three-dimensional structure was worked out by X-ray crystallography (7,8). Although there are differences in detail

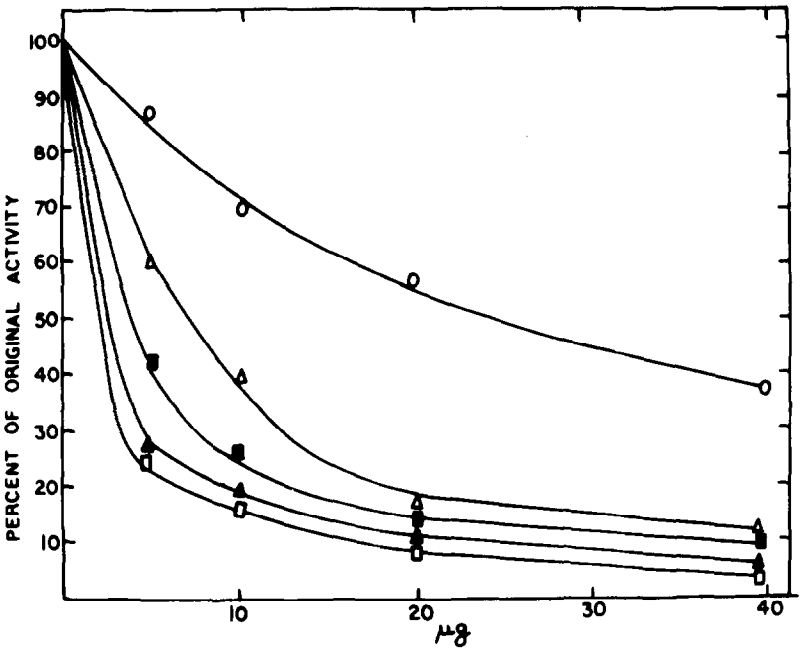

Fig.3. Inhibition of RNase I-catalysed hydrolysis of [^{3}H] - poly (A) by different polyribonucleotides

The incubation was carried out in a total volume of 0.25 ml containing 25 µmoles of Tris-HCl, pH 7, 0.1 µmole of Mg^{++}, 200 µg of serum albumin, 50 µg (9,000 counts/min) of [^{3}H]-poly (A) and 0.06 unit of RNase I. Varying amounts of nonradioactive polyribonucleotides were added to the incubation mixture, as indicated. A control incubation without RNase I was run in each case. The reaction was stopped by the addition of 0.05 ml of cold 50% trichloroacetic acid. After 10 min in ice the precipitate was removed by centrifugation and 0.15 ml of the supernatant was added to 5 ml of Bray's solution (15) and counted in the Liquid Scintillation Counter of Beckman Instrument Co. The counts released in absence of any added nonradioactive polyribonucleotide were 6,400 and taken as 100 per cent activity. Symbols as in Fig.2.

the folded tertiary structure of tRNAs is based on the secondary structure according to the clover leaf model. The primary structure of E.coli 5S RNA is known (9). Several models based on base pairing have been proposed for its secondary structure. Of these, the model proposed by Fox and Woese (10) is most widely accepted. The tertiary structure of 5S RNA, however, remains unknown despite vigorous attempts to unravel it (11). Kinetic studies with an enzyme are not expected to provide unequivocal

evidence for the secondary and tertiary structures but the preliminary results obtained with RNase I lead us to believe that 5S RNA does not have the highly ordered structure found in 4S RNAs. The validity of the prediction will be known only when three-dimensional structure of 5S RNA will be worked out. It is interesting to note that ethidium bromide binding (12) as well as tritium exchange (13,14) studies of 5S RNA showed some interesting differences from 4S RNAs. It should also be noted that 16S RNA is quite resistant to the hydrolytic action of RNase I, as compared with 23S RNA ; thus 16S RNA may have more folded structure than 23S RNA. It should also be noted that 30S ribosome is more resistant to RNase I than 50S ribosome (3).

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