Studies on soluble ribonucleic acid of rabbit liver. Action of polynucleotide phosphorylase

It has recently been established that specific amino-acid-activating enzymes catalyze both the formation of amino acid adenylates and the transfer of the amino-acyl moiety to RNA to form an amino acid-RNA compound[1,2]. Only cytoplasmic or soluble RNA appears to function as an acceptor in this way and there is evidence that there exist different *s*-RNAs for different amino acids[2-5]. The amino acid presumably is bound to the polynucleotide chain through the unesterified 2'- or 3'-hydroxyl group of the terminal nucleotide (nucleoside end)[3,6,7].

Both venom phosphodiesterase[8] and polynucleotide phosphorylase[9] act by stepwise removal of mononucleotide units starting from the nucleoside end of a polynucleotide chain. GRUNBERG-MANAGO observed that *s*-RNAs from different sources are slowly and incompletely phosphorolyzed by polynucleotide phosphorylase whereas other RNAs are degraded to completion[10]. PREISS *et al.*[3] reported that *s*-RNA from *Escherichia coli* loses its ability to accept amino acids after minimal degradation (less than 5 %) with venom diesterase. We have confirmed this observation with rabbit liver *s*-RNA. It was of interest, therefore, to investigate more extensively the action of polynucleotide phosphorylase on *s*-RNA, particularly with respect to its effect on amino acid acceptor ability.

As shown in Table I the phosphorolysis of rabbit liver *s*-RNA* stops at 20–30 % of completion. The cessation of the reaction is not caused by enzyme inactivation or inhibition since (1) addition of fresh enzyme after 5 h produces no additional breakdown; (2) polyadenylic acid[11] added after 5 h is rapidly phosphorolyzed; and (3) the acid-soluble reaction products are not inhibitory. The extent of phosphorolysis

TABLE I

PHOSPHOROLYSIS OF *s*-RNA

Expt.	*Time h*	*μmole $^{32}P_i$ incorporated*	*% phosphorolyzed*
A	0	0.00	—
	5	0.12	28
	6	0.12	28
B	3	0.09	16
	6	0.11	20
	22	0.10	18

The reaction mixtures (0.20 ml) contained 0.1 *M* tris(hydroxymethyl)aminomethane buffer, pH 8.2, 10 m*M* $MgCl_2$, 0.2 m*M* ethylenediaminetetraacetate and potassium [^{32}P]phosphate buffer, pH 7.4 (23 m*M* in Expt. A and 10 m*M* in Expt. B). The amounts of *s*-RNA, in μmole organic P, were 0.43 and 0.56 in Expts. A and B respectively. 90 and 210 μg of a preparation of *A. agilis* polynucleotide phosphorylase of specific activity 23 (ref. 11) were used in Expts. A and B respectively. After incubation at 37°, 0.05 ml aliquots were added to 1.00 ml cold 2.5 % $HClO_4$ and the precipitate was removed by centrifugation. The supernatant fluids were treated with charcoal to determine ^{32}P in acid-soluble nucleotides[12]. The results are given as μmole $^{32}P_i$ incorporated into acid-soluble nucleotides per 0.2 ml reaction mixture.

Abbreviations: RNA, ribonucleic acid; *s*-RNA, RNA from the soluble portion of a tissue homogenate; P_i, inorganic orthophosphate.

* The *s*-RNA used in these experiments, prepared from rabbit liver by NaCl extraction, contained less than 0.01 μmole of bound amino acids per 25 μmoles of *s*-RNA ribose.

is not dependent on the concentration of *s*-RNA and is unaffected by mild alkaline treatment of *s*-RNA.

The portion of the *s*-RNA that remains undigested after treatment with polynucleotide phosphorylase retains its ability to accept activated amino acids. The specific activity* of *s*-RNA, with respect to proline or leucine, was exactly the same before and after phosphorolysis, namely, 914 ± 58 and 906 ± 48 for proline, 140 ± 20 and 128 ± 24 for leucine; with respect to valine, on the other hand, the specific activity showed a small decrease, 96 ± 6 and 76 ± 8.

It is apparent, therefore, that although venom diesterase and polynucleotide phosphorylase are thought to act in analogous fashions, the results of their action on *s*-RNA are quite different. The most likely interpretation of these findings suggests that polynucleotide phosphorylase degrades only a fraction of the polynucleotide chains present in the preparation and these to completion. 70–80% of the chains may be entirely resistant to attack and the determining factor could be the extent of secondary structure (hydrogen bonding) in the resistant polynucleotide chains. The experiments of GRUNBERG-MANAGO[10] and OCHOA[13] have shown that multistranded polyribonucleotide chains are resistant to phosphorolysis. This hypothesis is supported by the observation that treatment of *s*-RNA with polynucleotide phosphorylase has essentially no effect on either the modal or the weight-average sedimentation coefficient (Table II).

TABLE II

EFFECT OF POLYNUCLEOTIDE-PHOSPHORYLASE TREATMENT ON SEDIMENTATION OF *s*-RNA*

	Sedimentation coefficient	
	Weight average	*Mode*
s-RNA (untreated)	4.0	4.0
s-RNA (undigested)	3.9	4.1

* The absorbancy of the *s*-RNA solution was 1.0.

Measurements at 59,780 rev./min were made in 0.2 *M* NaCl, using the Spinco Model E Ultracentrifuge with u.v. optics. Values were computed from tracings obtained from the photographic images using the Spinco Model RA Analytrol, equipped with a microanalyzer attachment[14].

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* Specific activity is expressed as counts/min/absorbancy in a hot-$HClO_4$ digest of *s*-RNA.

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Peptides isolated from peptic hydrolysate of diisopropylphosphoryl-trypsin

In the comparative studies on protein structures[1] which our laboratory has been working on we have been concerned above all with chymotrypsinogen and trypsin. A survey of the peptide sequences in these proteins so far known was published by us earlier[2].

The present paper gives a preliminary report on results obtained by studying peptides isolated from a peptic hydrolysate of DIP-trypsin. This study permits us to ascertain mutual bonds in the vicinity of the residues of lysine and arginine, which are split on tryptic hydrolysis.

DIP-trypsin (1000 mg) was hydrolyzed by pepsin (enzyme to substrate ratio, 1:50) at 37° and pH 2.0 for 2.5 h. A small quantity of non-dialyzing material was removed from the peptic hydrolysate by dialysis against distilled water. The freeze-dried dialysate was fractionated on a Zerolit 225 X2 ion-exchange resin (100–200 mesh) column of 2.2 × 150 cm at 40° using an elution gradient. For this purpose volatile buffers composed of pyridine and formic acid or pyridine and acetic acid proved satisfactory as in the case of the separation of a peptic hydrolysate of chymotrypsinogen[3].

Elution was initiated with the 0.1 *M* buffer (with respect to pyridine) and the gradient developed by gradually introducing solutions of increasing molarity and pH-value into a mixing vessel (volume, 2200 ml). The solutions used were as follows: 0.2 *M*, pH 3.1; 1 *M*, pH 5.1; 2 *M*, pH 5.2; 2 *M*, pH 7.0 and 2 *M* ammonia. The eluates were collected in 20-ml fractions at a flow rate of 1 ml/min.

The course of the separation on the column was checked by submitting dry residues of 0.4-ml aliquots taken from each fraction to descending paper chromatography[3] in the system *n*-butanol–pyridine–acetic acid–water (15:10:3:12)[4]. According to the chromatograms, individual fractions obtained from the column were pooled and fractionated further by preparative chromatography on Whatman No. 1 or No. 3 paper in the system[4] mentioned above. The zones obtained were purified by paper electrophoresis in the system formic acid–acetic acid of pH 1.9 at a potential gradient of 65 V/cm.

A majority of the zones isolated comprised individual peptides as was proved by calculating the molar ratios of amino acids by comparing the peptide hydrolysate

Abbreviation: DIP-, diisopropylphosphoryl-.