Actual. Med. (2010) Vol. 94/2010/nº779 · Enero / Abril 2010 · Pág.047 - 057

Clásicos de la medicina

Hace 50 años

ARCHIVES OF BIOCHEMISTRY AND BIOPHYSICS 69, 119–129 (1957)

Enzymic Synthesis of Polynucleotides. III. Phosphorolysis of Natural and Synthetic Ribopolynucleotides[1]

Severo Ochoa

From the Department of Biochemistry, New York University College of Medicine, New York, New York; and the Virus Laboratory, University of California, Berkeley, California

Received January 11, 1957

Introduction

As previously reported (1), polynucleotide phosphorylase of *Azotobacter vinelandii* catalyzes the reversible Reaction (1), where

$$n\ \text{X-R-P-P} \overset{Mg^{++}}{\rightleftharpoons} (\text{X-R-P})_n + n\ \text{P} \qquad (1)$$

R stands for ribose, P-P for pyrophosphate, P for orthophosphate, and X for one or more of the following bases: adenine, guanine, uracil, cytosine, or hypoxanthine. In the direction to the left the reaction is a phosphorolysis of ribopolynucleotides leading to the formation of the corresponding nucleoside 5′-diphosphates. The enzyme was found to catalyze the phosphorolysis of a number of synthetic ribopolynucleotides and natural ribonucleic acids. However, while synthetic polynucleotides containing only one kind of nucleotide (poly A,[2] poly U, poly I) were readily phosphorolyzed, the phosphorolysis of poly AU, poly AGUC,

[1] Aided by grants from the National Institute of Arthritis and Metabolic Diseases (grant A-529) and the National Cancer Institute (grant C-2784) of the National Institutes of Health, U. S. Public Health Service; the American Cancer Society (recommended by the Committee on Growth, National Research Council); the Rockefeller Foundation; and by a contract (N6 onr 279, TO 6) between the Office of Naval Research and New York University College of Medicine.

[2] The following abbreviations are used: 5′-diphosphates of adenosine, guanosine, uridine, and cytidine, ADP, GDP, UDP, and CDP; ribonucleic acid, RNA; deoxyribonucleic acid, DNA; polyadenylic acid, poly A; polyuridylic acid, poly U; polyinosinic acid, poly I; adenylic–uridylic polynucleotide, AU; synthetic RNA, poly AGUC; aggregate of poly A and poly U, poly A + U; tobacco mosaic virus, TMV; turnip yellow mosaic virus, TYM.

or RNA was very slow (1). Further experiments, described in this paper, suggest that the low susceptibility of the latter compounds to phosphorolysis is due to the fact that they exist largely as multistranded chains which, contrary to the single-stranded chains of poly A or poly U, are rather resistant to cleavage by polynucleotide phosphorylase.

The basic observation was that the aggregate (poly A + U) formed by mixing poly A and poly U (2, 3) is phosphorolyzed very slowly. Since, in forming poly A + U, the parent polynucleotides interact through hydrogen-bonding between their adenine and uracil moieties (3) to form a double-stranded helix (4) as in DNA (5), the conclusion seems inescapable that this structure confers partial resistance to phosphorolysis. There are indications (6) that, in line with their low susceptibility to phosphorolysis, similar aggregates occur in poly AU, poly AGUC, and natural RNA from various sources. In contrast, TMV RNA was found to be phosphorolyzed fairly readily, suggesting a slight degree of chain aggregation in this compound.

The action of polynucleotide phosphorylase on oligonucleotides derived from poly A has also been examined.

Experimental

Preparations

Enzyme. Purified preparations of polynucleotide phosphorylase from *A. vinelandii* were utilized throughout. The enzyme used for the experiments of Table II and Fig. 1 (Prep. 1) was prepared as previously described (1) through step 4; its specific activity at time of use was 15. The enzyme employed for the experiments of Table III and Fig. 2 (Prep. 2) was prepared by a modified procedure (7); its specific activity was 41.4. I am indebted to Dr. Sanae Mii and Mr. Morton C. Schneider for these preparations.

Synthetic Ribopolynucleotides. These were prepared with *Azotobacter* enzyme as described previously (1). The poly A (sample 1) used for the experiments of Table II had been prepared with a crude enzyme (specific activity below 8)[3] and, as indicated by dialysis, was partially degraded to oligonucleotides. The poly U in the experiments of Table II and Fig. 1 (sample 1), prepared with enzyme of specific activity 16, had a molecular weight around 70,000 (6). The poly A (sample 2) and poly U (sample 2), Table III and Fig. 2, were prepared with enzyme of specific activity about 50. No sedimentation or end-group studies are as yet available for these polymers, but their molecular weight was undoubtedly high since, at 10 mg./ml., these polymers (particularly poly A) gave highly viscous solutions in

[3] The specific activity of the *Azotobacter* polynucleotide phosphorylase used for the experiments of Fig. 5 of the first paper of this series (1) was erroneously given as 9; it was 16. The same enzyme was used for the preparation of the polynucleotides listed in Table VI of the first paper.

TABLE I

Spectral Changes on Interaction of Poly A and Poly U

Polymer	Absorption coefficient, *l*/mg.			
	280 mμ		260 mμ	
	Sample 1	Sample 2	Sample 1	Sample 2
Poly A	11.0	7.3	25.0	20.6
Poly U	10.0	9.1	27.0	24.7
Average	10.5	8.2	26.0	22.65
Poly A + U	8.0	7.3	20.6	19.4
Per cent decrease due to interaction	23.8	11.0	20.8	14.3

water. Poly AGUC sample 1 (Table II, Fig. 1) was prepared with enzyme of specific activity 18 from an equimolar mixture of ADP, GDP, UDP, and CDP. Samples 2 and 3 (Table III) were different batches prepared in the same way with enzyme of specific activity about 50. I am indebted to Dr. Sanae Mii and Miss Priscilla J. Ortiz for the polymer preparations.

Two samples of poly A + U were used, one prepared with samples 1, the other with samples 2 of poly A and poly U. Equal volumes of 10 mg./ml. solutions were mixed to prepare poly A + U. While there was no visible change in viscosity after mixing samples 1, marked increase in viscosity occurred on mixing samples 2. In the latter case, the mixture had to be diluted with an equal volume of water for convenience in handling. Warner (2, 3) has shown that interaction of poly A and poly U leads to a decrease in ultraviolet absorption. The spectral changes accompanying the formation of the above samples of poly A + U are recorded in Table I. The higher absorption of poly A sample 1, compared to poly A sample 2, is an indication of the partially degraded state of the former since hydrolysis of poly A leads to increased ultraviolet absorption (2, 3).

Polynucleotide solutions were usually kept frozen when not in use. In the case of poly A sample 2, but not any of the other polynucleotides synthetic or natural, keeping led to an increased opalescence of the solution. The polynucleotide also became increasingly insoluble in the presence of Mg^{++}, resulting in some decrease in the measured rate of phosphorolysis as the solution became older.

Oligonucleotides. The enzymic formation from poly A of adenylic acid oligonucleotides with 5′-phosphomonoester end groups has been previously described (8). Samples of 5′-ended adenylic acid trinucleotide (pApApA) and tetranucleotide (pApApApA) isolated chromatographically were kindly provided by Dr. Leon A. Heppel.

Nucleic Acids. The preparations of RNA from *A. vinelandii*; *Micrococcus pyogenes* var. *aureus* (*Staphylococcus aureus*), strain Duncan; *Alcaligenes faecalis*; and *Mycobacterium phlei*; and of DNA from *A. vinelandii* were made by Dr. R. M. S. Smellie; the procedure will be described elsewhere. Highly polymerized yeast RNA (9) was a gift of Dr. Frank W. Allen. The samples of TMV RNA were generously

provided by Dr. H. Fraenkel-Conrat. Their batch number and characteristics are indicated in the legends to Tables II and III.

All polynucleotides dissolved readily in water at concentrations of 5–10 mg./ml. and were used without prior neutralization.

Methods

Phosphorolysis was measured either with P^{32}-labeled orthophosphate (experiments of Tables II and III and Fig. 1), making use of the fact that inorganic P^{32} is converted to organic P^{32}, or (experiments of Fig. 2) through the disappearance of orthophosphate determined chemically (10) on trichloroacetic acid filtrates. This disappearance was always accounted for by a stoichiometric increase of easily hydrolyzable phosphate, i.e., orthophosphate liberated by hydrolysis in 1.0 *N* HCl for 7 min. at 100°. This is as expected from Reaction (1), since the terminal phosphate of nucleoside 5'-diphosphates is split off as orthophosphate under these conditions.

In the P^{32} assay the amount of organically bound phosphate is given by the radioactivity remaining in the protein-free filtrate after removal of the orthophosphate through conversion to ammonium phosphomolybdate and extraction with isobutyl alcohol. Because of some modifications of the procedure previously described (1), a brief description of the method currently used follows. The reaction mixture is deproteinized with one-fifth volume of 20% trichloroacetic acid, and the precipitate is removed by centrifugation. To a suitable aliquot of the clear supernatant fluid are added 0.3 ml. of 10.0 *N* H_2SO_4[4] and 1.5 ml. of 5% ammonium molybdate; the solution is shaken and allowed to stand for 1 min. After making up the volume to 5.0 ml. with water and adding 5 ml. of isobutyl alcohol, a slow stream of air is bubbled through for 1 min. to obtain good mixing and, after separation of the liquid layers, the upper isobutyl alcohol layer is removed by aspiration and discarded. To insure complete removal of the radioactive orthophosphate, 0.01 ml. of 0.1 *M* phosphate is added to the aqueous phase followed, after brief shaking and standing, by 5 ml. of isobutyl alcohol. The solution is mixed and the isobutyl alcohol removed as above. Finally, the aqueous layer is washed with 4.0 ml. of ether, bubbling air through for 15 sec., and the ether is removed by aspiration. Aliquots of the aqueous solution, which contain the organically bound P^{32} and should be quite clear, are measured into stainless steel planchets, and their radioactivity is determined with a thin-window Geiger-Müller counter. The radioactivity of the orthophosphate is determined simultaneously on another aliquot of the protein-free filtrate.

Descending chromatography on Whatman No. 3 MM filter paper, in the isobutyric acid–ammonia–ethylenediaminetetraacetic acid solvent system described by Krebs and Hems (11) was used for identification of the nucleoside diphosphates formed by phosphorolysis of TMV RNA. The reaction mixtures were heated for 1 min. at 100°, cooled, and centrifuged, and aliquots of the clear supernatant solution were applied on the paper. To achieve good separation, the chromatograms were developed for 16 hr. (temperature, 20°); at this time the solvent front

[4] Through a typographical error, the concentration of H_2SO_4 in the previous description of this procedure (1) was given as 10.0 *M*; it should be 10.0 *N*.

had moved beyond the end of the paper sheets. After drying, the nucleotide spots were located with an ultraviolet lamp and their position, relative to that of simultaneously run ADP, was expressed as R_{ADP}. This is the ratio of the extent of movement from the origin of the unknown nucleotide to that of ADP.

Results

Polynucleotide Phosphorolysis

Inspection of Tables II and III shows, in confirmation of earlier results (1), that ribopolynucleotides can be divided into two groups according to their susceptibility to phosphorolytic cleavage: (*a*) synthetic polynucleotides containing only one kind of nucleotide residue (poly A, poly U) which are readily phosphorolyzed, and (*b*) copolymeric polynucleotides, including poly AGUC (Table II, Expts. 1 and 2, Table III, Expt. 3), yeast (Table II, Expt. 1), and bacterial ribonucleic acids (Table III, Expts. 2 and 3), which are much less susceptible to phosphorolysis. In further confirmation of earlier work, DNA was found not to

TABLE II

Phosphorolysis of Polynucleotides

The reaction mixture for each polynucleotide, in a final volume of 0.6 ml. (Expt. 1) or 0.5 ml. (Expt. 2), consisted of $MgCl_2$, 5 μmoles; potassium phosphate buffer, pH 7.4 (contuining P^{32}, 300 × 10^3 counts/min.), 5 μmoles; polynucleotide, 0.5 mg.; and *Azotobacter* enzyme (Prepn. 1, specific activity, 15) with 0.14 mg. of protein. The mixture of poly A + poly U contained 0.25 mg. of each polynucleotide. Incubation 2 hr. at 30°. The samples of TMV RNA used were as follows: HR1, biologically inactive; (*A*), 287×, biologically inactive; (*B*), 66B (freshly prepared); (*C*) 58B drainage (high sedimentation fraction); (*D*), HR-266 cs. Samples *B*, *C*, and *D* were biologically active at time of experiment.

1		2	
Polynucleotide	P^{32} incorporation	Polynucleotide	P^{32} incorporation
	counts/min. × 10^{-3}		counts/min. × 10^{-3}
None	0	None	0.1
Poly A	10.4	Poly U	58.1
Poly U	61.4	Poly AGUC	9.0
Poly A + U	8.2	Poly A + U	9.4
Poly AGUC (sample 1)	8.9	TMV RNA (*A*)	17.4
Yeast RNA	11.1	*Do.* (*B*)	35.0
TMV RNA (HR1)	23.8	*Do.* (*C*)	23.8
		Do. (*D*)	38.9

TABLE III

Phosphorolysis of Polynucleotides

The reaction mixture for each polynucleotide, in a final volume of 0.5 ml., consisted of $MgCl_2$, 5 μmoles; potassium phosphate buffer, pH 7.2 (containing P^{32}, from 194×10^3 to 470×10^3 counts/min.), 5 μmoles; polynucleotide, 0.5 mg. unless otherwise noted; and *Azotobacter* enzyme (Prepn. 2, specific activity, 41.4) with 0.074 mg. of protein. The mixture of poly A + poly U (Expt. 4) contained 0.25 mg. of each polynucleotide. The TMV RNA (82A) was biologically active. Incubation, 2 hr. at 30°. For comparison of individual experiments the results are expressed as counts/min. P^{32} "exchange" per 100×10^3 counts/min. orthophosphate-P^{32}.

1		2		3		4	
Polynucleotide	P^{32} incorporation	Polynucleotide	P^{32} incorporation	Polynucleotide	P^{32} incorporation	Polynucleotide	P^{32} incorporation
None	0.11	None	0.4	None	0.13	Poly A	18.0
Poly A[a]	27.5	Poly A[a]	13.5	Poly A[b] (no enz.)	0.03	Poly U	21.6
pApApA[c]	3.7	TMV RNA (82A)	7.4	Poly A[b]	12.3	Poly A + U	3.2
pApApApA[d]	3.1	*S. aureus* RNA	1.7	*A. vinelandii* RNA	0.8	*A. vinelandii* DNA	0.0
		A. faecalis RNA	1.5	AGUC (sample 2)	1.2		
		M. phlei RNA	4.0	AGUC (sample 3)	1.5		
				A. faecalis RNA	1.2		

[a] 0.45 mg.

[b] 0.4 mg.

[c] Adenylic acid trinucleotide with 5′-phosphomonoester end group about 0.67 μmole adenine.

[d] Adenylic acid tetranucleotide with 5′-phosphomonoester end group, about 0.6 μmole adenine.

be attacked (Table III, Expt. 4), The poly A, sample 1, of Table II appears to make an exception to the rule. However, as pointed out in the section on *Preparations*, this polymer was partially degraded and, as shown in Table III, Expt. 1, small 5′-ended adenylic acid polynucleotides have but low susceptibility to phosphorolytic cleavage.

Of particular significance is the fact that the susceptibility to phosphorolysis of poly A + U is the same as that of compounds of group (*b*) (Table II, Expts. 1 and 2, Table III, Expt. 4). As pointed out in the

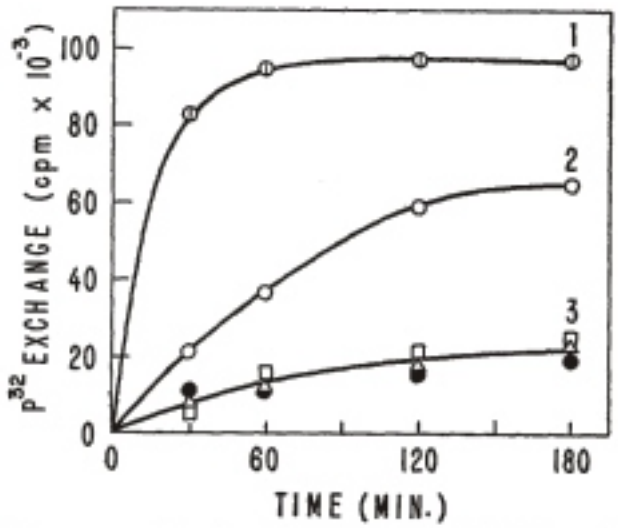

FIG. 1. Time course of phosphorolysis measured with P^{32}. The reaction mixture for each polynucleotide, in a final volume of 1.0 ml., consisted of $MgCl_2$, 10 μmoles; potassium phosphate buffer, pH 7.4 (containing P^{32}, 577 × 10^3 counts/min.), 10 μmoles; polynucleotide, 1.0 mg.; and *Azotobacter* enzyme (Prep. 1, specific activity, 15) with 0.27 mg. of protein. Incubation at 30°; 0.15-ml. aliquots were withdrawn for analysis at the indicated times. Polynucleotides: Curve 1, Poly U; curve 2, TMV RNA (*D*, Table II); curve 3, A + U (containing 0.5 mg. of each poly A and U) (●), poly AGUC (△), or yeast RNA (□).

Introduction, this result may now be correlated with the occurrence of multistranded chains in poly A + U and the polynucleotides of group (*b*).

Further inspection of the tables reveals that, as regards phosphorolysis, TMV RNA occupies an intermediate position between groups (*a*) and (*b*). A number of different samples have been examined (Table II; Table III, Expt. 2), and all proved to be fairly easily phosphorolyzed although not as easily as compounds of group (*a*). L. A. Heppel and J. D. Smith[5] have observed a similar behavior of TYM RNA. It thus appears that TMV (and probably TYM) RNA belongs to a separate group of ribonucleic acids with intermediate susceptibility to phosphorolysis.[6]

[5] L. A. Heppel, personal communication.

[6] The biological activity of TMV RNA is rapidly destroyed on incubation with *Azotobacter* polynucleotide phosphorylase, in the presence of phosphate and Mg^{++} (H. Fraenkel-Conrat and S. Ochoa, unpublished observations).

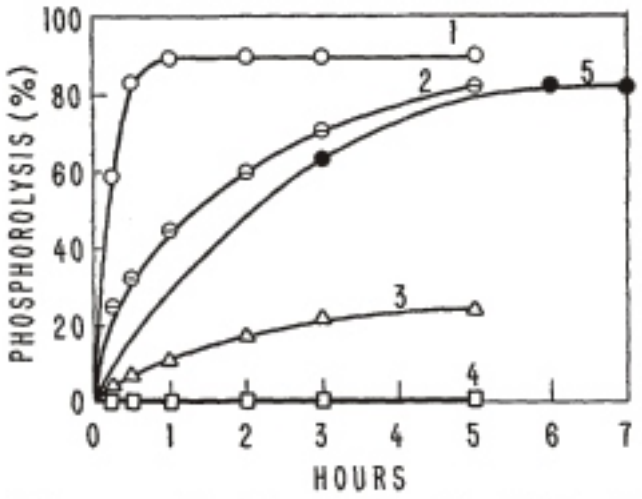

FIG. 2. Time course of phosphorolysis followed by orthophosphate disappearance and formation of easily hydrolyzable phosphate. The reaction mixture for each polynucleotide, in a final volume of 0.5 ml., consisted of $MgCl_2$, 5 μmoles; potassium phosphate buffer, pH 7.2, 6.5 μmoles; polynucleotide, 1.0 mg. (except TMV RNA, 0.54 mg.), and *Azotobacter* enzyme (Prep. 2, specific activity, 41.4) with 0.148 mg. of protein. Incubation at 30°; 0.05-ml. aliquots were withdrawn for analysis at the indicated times. Polynucleotides: Curve 1, poly U; curve 2, poly A; curve 3, poly A + U (containing 0.5 mg. of each poly A and poly U); curve 4, poly U, no enzyme; curve 5, TMV RNA (82 *A*, Table III). The ordinate gives the per cent phosphorolysis calculated from orthophosphate disappearance and formation of easily hydrolyzable phosphate; these two values were identical at each time interval.

The rates of phosphorolysis of representative compounds of the above three groups of ribopolynucleotides are illustrated in Figs. 1 and 2. In the experiments of Fig. 1, phosphorolysis was followed by P^{32} incorporation. The rapid cleavage of poly U contrasts with the very slow breakdown of poly A + U, poly AGUC, or yeast RNA, all of which are attacked at about the same rate, and with the intermediate rate of phosphorolysis of TMV RNA. The experiments of Fig. 2, in which phosphorolysis was followed by the removal of orthophosphate and formation of easily hydrolyzable phosphate, substantiate the above results for poly A, poly U, poly A + U, and TMV RNA. It will be seen that equilibrium [cf. Reaction (1)] was rapidly attained in the case of poly U.

Oligonucleotide Phosphorolysis

As previously noted by Heppel,[5] small adenylic polynucleotides with 5′-phosphomonoester end groups, produced by partial hydrolysis of poly A with an enzyme from liver nuclei (8), show slight but definite susceptibility to phosphorolysis by *A. vinelandii* polynucleotide phosphorylase (Table III, Expt. 1). Similar results have been obtained, in unpublished experiments of M. Singer and L. A. Heppel,[5] with purified polynucleotide phosphorylase of *Escherichia coli* (12). On the other hand, oligonucleotides consisting of one or more adenylic acid residues with one terminal uridine 3′-phosphate unit, obtained by Heppel by exhaustive digestion of poly AU with pancreatic ribonuclease (6), are quite resistant to the action of either the *A. vinelandii* or the *E. coli* phosphorylase.[5] It may be that a 5′-phosphomonoester end group is essential for cleavage by polynucleotide phosphorylase, but this point requires further investigation.

Phosphorolysis Products of TMV RNA

A sample of TMV RNA was incubated with *Azotobacter* enzyme, Prepn. 2, under conditions similar to those in Fig. 2. Aliquots were withdrawn at zero time and after incubation for 6 hr. and chromatographed as outlined in the section on *Methods*. At zero time, the only ultraviolet-absorbing spot visible was at the origin, corresponding to undegraded RNA. After 6 hr., the intensity of the RNA spot was reduced and three other spots were visible at a distance from the origin. The R_{ADP} values of the nucleoside 5′-diphosphate markers were, ADP, 1.0; CDP, 0.80; GDP, 0.57; UDP, 0.57. The R_{ADP} values of the experimental spots were, 1.04, 0.82, and 0.58, corresponding to ADP, CDP, and GDP or UDP, respectively. The ammonium isobutyrate solvent system does not separate GDP and UDP.

Discussion

The low susceptibility of the poly A + U aggregate to phosphorolysis, which contrasts sharply with the rapid rate of cleavage of its component polynucleotides poly A and poly U, would seem to reflect an intrinsic resistance of double-stranded polynucleotide chains to attack by phosphorylase. It is, therefore, justified to assume that the analogous resistance to phosphorolysis exhibited by poly AGUC, as well as by yeast

and bacterial RNA, is due to their existing largely as multistranded structures. Evidence for the occurrence of aggregates in these compounds is provided by the observation that the number-average molecular weight of poly AU and poly AGUC, based on chain-length determinations, is much smaller than the weight-average molecular weight, based on sedimentation measurements (6). Similar discrepancies have been observed with samples of natural RNA (6, 13).

Since formation of multistranded chains must occur during synthesis of RNA, the resulting diminished susceptibility to phosphorolytic cleavage must favor RNA synthesis by decreasing the rate of the reverse reaction [cf. Reaction (1)]. This, in effect, shifts the equilibrium in favor of polymerization.

If the degree of resistance to phosphorolysis is mainly a reflection of the degree of aggregation of the polynucleotide chains, it would appear that the relatively high rate of phosphorolysis of TMV RNA reflects a small tendency of this compound to assume a multistranded structure, although there might be other reasons for its behavior. It may be pointed out in this connection that the nucleotide composition of TMV and TYM RNA differs markedly from that of other ribonucleic acids and might not permit adequate pairing of complementary bases. It is possible that whatever properties of TMV RNA are responsible for its sensitivity to phosphorolysis, they might be of significance with regard to its biological activity.

Acknowledgments

I am greatly indebted to Dr. Wendell M. Stanley for the hospitality of the Virus Laboratory, Dr. Arthur B. Pardee for generously sharing the facilities of his laboratory, Dr. H. Fraenkel-Conrat for the samples of tobacco mosaic virus RNA, and Dr. Frank W. Allen for a sample of highly polymerized yeast RNA. I am also indebted to Miss Priscilla J. Ortiz for help in some of this work.

Summary

1. Ribopolynucleotides can be classified into three groups according to their susceptibility to cleavage by polynucleotide phosphorylase of *Azotobacter vinelandii*: (*a*) rapidly phosphorolyzed; this group includes synthetic polynucleotides, such as polyadenylic or polyuridylic acid, containing only one kind of nucleotide; (*b*) slowly phosphorolyzed; this group includes synthetic (poly AGUC), yeast, and bacterial RNA, as well as the aggregate (poly A + U) formed by mixing solutions of polyadenylic and polyuridylic acid; (*c*) phosphorolyzed at an intermediate

rate; this is the case with tobacco mosaic virus RNA. The slow phosphorolysis of polynucleotides of group (*b*) can be related to the fact that, contrary to those of group (*a*), they consist largely of multistranded rather than single-stranded chains. In view of this, the intermediate rate of phosphorolysis of tobacco mosaic virus RNA might reflect a slight tendency of this compound to assume a multistranded structure.

2. Adenylic acid tri- and tetranucleotides with 5′-phosphomonoester end groups, obtained by hydrolysis of polyadenylic acid with an enzyme from liver nuclei, are slowly phosphorolyzed by *Azotobacter* polynucleotide phosphorylase.

3. The nucleoside 5′-diphosphates produced by phosphorolysis of tobacco mosaic virus RNA have been identified chromatographically.

References

1. Grunberg-Manago, M., Ortiz, P. J., and Ochoa, S., *Biochim. et Biophys. Acta* **20**, 269 (1956).
2. Warner, R. C., *Federation Proc.* **15**, 379 (1956).
3. Warner, R. C., *Ann. N. Y. Acad. Sci.*, in press.
4. Rich, A., and Davies, D. R., *J. Am. Chem. Soc.* **78**, 3548 (1956).
5. Watson, J. D., and Crick, F. H. C., *Nature* **171**, 737 (1953).
6. Ochoa, S., *Federation Proc.* **15**, 832 (1956).
7. Ochoa, S., *Ann. N. Y. Acad. Sci.*, in press.
8. Heppel, L. A., Ortiz, P. J., and Ochoa, S., *Science* **123**, 415 (1956).
9. Crestfield, A. M., Smith, K. C., and Allen, F. W., *J. Biol. Chem.* **216**, 185 (1955).
10. Lohmann, K., and Jendrássik, L., *Biochem. Z.* **178**, 419 (1926).
11. Krebs, H. A., and Hems, R., *Biochim. et Biophys. Acta* **12**, 172 (1953).
12. Littauer, U. Z., and Kornberg, A., *J. Biol. Chem.*, in press.
13. Ochoa, S., and Heppel, L. A. *in* "Chemical Basis of Heredity" (McElroy, W. D., and Glass, B., eds.), Baltimore, p. 615, 1957.