POLYRIBONUCLEOTIDES CONTAINING A THIOPHOSPHATE BACKBONE

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Polynucleotides resistant to nucleases are of great interest to biochemists. Such polymers would allow the storage of information in a rather stable form and might be useful, e.g., as templates for polymerases and messenger-RNA. So far such polynucleotides have not been prepared. We could recently show that diuridine phosphorothioate exhibits some resistance to some nucleases [1] and we therefore tried the synthesis of polymers with a thiophosphate backbone in the hope that they might show similar properties. A polymer with alternating $\geqslant$P=O and $\geqslant$P=S groups was indeed degraded by some diesterases at a remarkably slower rate than the unmodified polymer [2]. We now wish to report the synthesis of two polymers containing thiophosphate groups exclusively.

Using DNA-dependent RNA-polymerase, poly dAT and the two substrate analogs [35S]-uridine-5'-triphosphorothioate (UTPS) and adenosine-5'-triphosphorothioate (ATPS) [3], we observed incorporation of radioactivity in the polymeric product as measured by the method already described [2]. The product was isolated by precipitation with ethanol and chrom-

atography on a Sephadex G-50 column. Alkaline degradation of the polymer yielded uridine-3'(2')-phosphorothioate and [35S]-adenosine-3'(2')-phosphorothioate in equal amounts which were separated by chromatography on Dowex 50 × 8. This nearest neighbour analysis showed that the template had been copied faithfully. The melting profile was identical with the one published for the mixed polymer [2] poly rA·US.

Fig. 1 shows the kinetics of degradation of the modified and the unmodified polymers, poly rAS·US and poly rAU by pancreatic ribonuclease. Similar kinetics have been observed with snake venom and spleen phosphodiesterase as well as micrococcal nuclease. From this figure it is quite clear that such modified polyribonucleotides, although not completely resistant to nucleases, are degraded at a considerably slower rate than the unmodified polymers.

In contrast to polynucleotide phosphorylase from *Micrococcus lysodeikticus* the enzyme from *E. coli* can use [35S]-uridine-5'-diphosphorothioate (UDPS) as substrate as shown in fig. 2. Higher concentrations
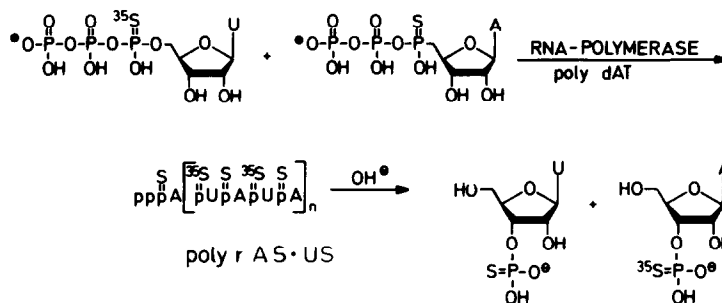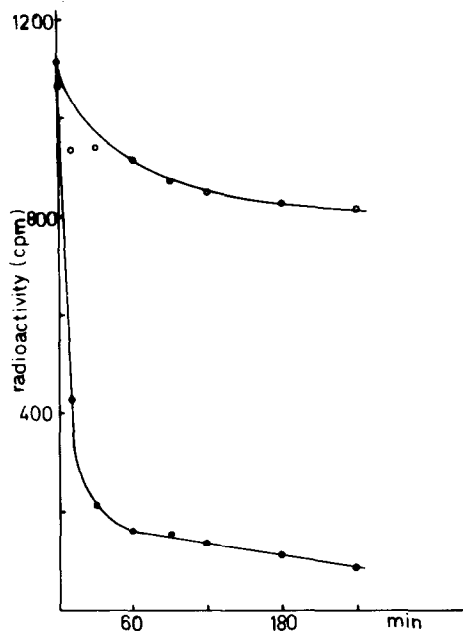
Fig. 1. Degradation of poly rAU (●) and poly rAS·US (O) by pancreatic ribonuclease at 27° as a function of time. 0.45 ml of 0.05 M Tris-HCl (pH 7.4) contained 0.37 absorbance units of polymer and 80 mμg of protein. Aliquots were taken at certain time intervals, applied on Whatman 3 MM filter disks, dipped in 5% TCA, washed with ethanol and the radioactivity counted.
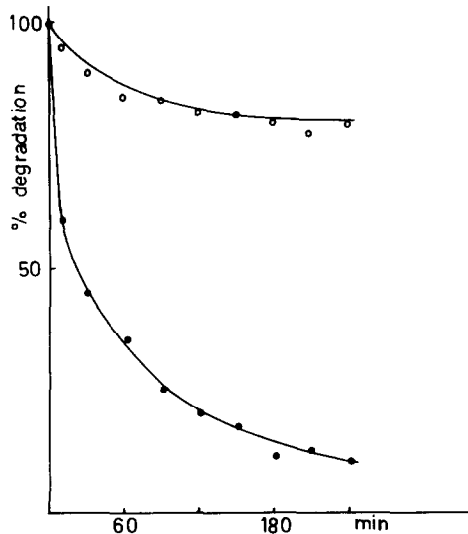

Fig. 2. Polymerisation of [³H]-UDP (●) and [³⁵S]-UDPS (O) by polynucleotide phosphorylase at 37° as a function of time. 0.1 ml of incubation solution contained 0.25 absorbance units of substrate, 1.05 units of enzyme, 5 μl of 1 M Tris-HCl (pH 9.2), 0.5 μl of 1 M MgCl₂, 1 μl of 0.04 M EDTA and 0.2 μl of 1 M MnCl₂. Aliquots were taken at certain time intervals, applied on Whatman 3 MM paper, eluted with 0.3 M ammonium formate and the radioactivity of the starting zone counted.

Fig. 3. Degradation of poly U (●) and poly US (O) by pancreatic ribonuclease at 27° as a function of time. Conditions as described for fig. 1, except that 6 mμg of enzyme were used. Samples were treated as described for fig. 2.
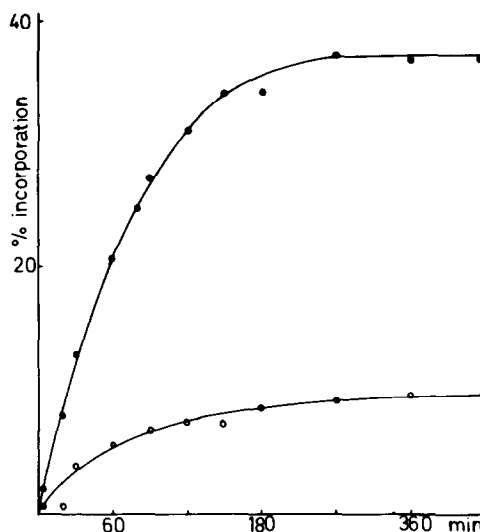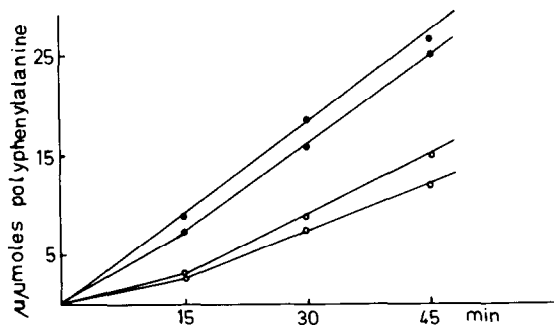

Fig. 4. Polymerisation of phenylalanine in the presence of poly U (●) and poly US (O). 1 ml of reaction mixture contained 80 μmoles Tris-HCl, pH 7.8; 4 μmoles (NH₄)₂SO₄; 18 μmoles KCl; 14 μmoles MgCl₂ in the presence of poly U and 16 μmoles with poly US; 600 μg ³H-Phe-tRNA (5 mC/ μmole); 1 mg ribosomes; 270 mμmoles GTP; 25 μmoles PEP; 1.5 μg pyruvate kinase; and 25 γ of a partially purified T factor fraction containing saturating amounts of G factor. The volume of a single assay was 75 μl. After the induction time, when the reaction was proceeding linearly, aliquots for each sample were withdrawn, placed on GF/A filters (Whatman) and immediately dipped in cold 10% TCA. After 15 min in 5% TCA at 95°, the filters were washed, dried and counted [5]. Each experiment was carried out twice.

of substrate lead to complete inhibition of polymerisation. The isolation of the product was carried out by precipitation with ethanol, removal of protein by extraction with phenol and chromatography on Sephadex G-25 and G-100.

Fig. 3 represents the kinetics of degradation with pancreatic RNase. Similar results were obtained with snake venom and spleen phosphodiesterase. Again there is no complete resistance but a remarkable slowdown in rate. In collaboration with Dr. Parmeggiani we could show that this modified polymer, poly US, can serve as messenger for the polymerisation of phenylalanine in an *in vitro* protein-synthesizing system at an efficiency which is about 45% of poly U.

Thus it is clear that it is possible to synthesize polyribonucleotides which show some degree of resistance to nucleases but which are nevertheless biologically active.

At present work is in progress to extend these studies to polydesoxynucleotides, in particular the synthesis of poly dAS·TS. Since we had found dithymidine phosphorothioate to be resistant to snake venom and spleen phosphodiesterase [4] we hope this modified polymer might also turn out to be completely resistant to nucleases.

References

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