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Research Article

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BIOLOGIC PROPERTIES OF POLYNUCLEOTIDES. I. THE ANTI-COMPLEMENTARY ACTIVITY OF POLYNUCLEOTIDES *

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The biologic role of nucleic acids is, under normal circumstances, concerned with protein synthesis and cell replication; however, instances may be found where polynucleotides participate in biologic phenomena not directly concerned with such synthetic processes. For example, certain deoxyribonucleic acids (DNA) have the capacity to elicit specific antibody formation in experimental animals (2); in the human, the sera of patients suffering from lupus erythematosus have been shown to contain antibodies specific for DNA (3); and under conditions of rapid cellular destruction, the release of large amounts of DNA into the circulation has been thought to cause small vessel occlusion and death from widespread pulmonary infarction (4).

Recent evidence suggests (5–7) that it is possible to effect transformation of mammalian cells by exogenous nucleic acids; thus it is possible that these materials may become useful as therapeutic agents in genetic disease, a prospect that might entail the administration of large amounts of nucleic acids to humans. For these reasons, a systematic investigation of the effects of polynucleotides on various biologic systems has been undertaken. In addition to studies with naturally occurring nucleic acids, the biologic properties of synthetic ribopolynucleotides have been examined because these substances offer the opportunity to observe the effects of variations in base composition and secondary structure on the participation of nucleic acids in such phenomena.

In our previous work on the potential antigenicity of homoribopolynucleotides in experimental animals (8), we noted that one of these compounds, polyinosinic acid (poly I)[1] exhibited strong anticomplementary (anti-C′) activity. The present report describes this phenomenon in detail. The data deal with quantitative as well as qualitative aspects of the poly I-complement (C′) interaction. In addition, evidence is presented that will define some of the structural requirements necessary for the endowment of ribopolynucleotides in general with anti-C′ properties.

MATERIALS AND METHODS

The synthetic polynucleotides used were synthesized by polynucleotide phosphorylase isolated from *Micrococcus lysodeikticus*. Purification of the enzyme was carried through an acetone fractionation, and the protein precipitating between 40 to 65% acetone (vol/vol) was used (9). The 280 mμ/260 mμ absorbancy ratio of this enzyme preparation was on one occasion as high as 1.77, indicating a low degree of contamination by nucleic acid material. This paucity of potential primer might explain the difficulty encountered in synthesizing not only poly G but also those mixed GU copolymers containing more than 50% G. In synthesizing the GU copolymers, it was necessary to add a small amount of pApApA primer (10).[2] Even then the synthesis of copolymer 2 (66% G, 34% U) required 72 hours to approach equilibrium, and copolymer 1 (85% G, 15% U) required 215 hours, with intermittent replenishment of enzyme, for synthesis of polymer in relatively low yield. The ribonucleoside diphosphates used as substrates in these syn-

* A preliminary report of this work has been published (1).

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[1] The following abbreviations are used: biosynthetic homopolymers of adenylic, guanylic, uridylic, cytidylic, and inosinic acids: poly A, poly G, poly U, poly C, and poly I, respectively; copolymer of inosinic and uridylic acids: poly IU; copolymer of guanylic and uridylic acids: poly GU; 5′-monophosphates of guanosine, uridine, or inosine: GMP, UMP, or IMP. Oligonucleotides are indicated as follows: a phosphate is designated by p; when placed to the right of a nucleoside symbol, the phosphate is esterified at C-3′ of the ribose moiety; when placed to the left of the nucleoside symbol, the phosphate is esterified at C-5′ of the ribose moiety.

[2] Kindly supplied by Dr. Maxine Singer, National Institutes of Health, Bethesda, Md.

TABLE I

*Selected properties of polynucleotides**

Polynucleotide	Base composition (%) A	U	G	C	I	ϵ_M(P)†‡ ×10⁻³	S‡§
I. RNA							
Guinea pig liver RNA	18.2	20.5	34.5	26.8		7.61	
Rat liver RNA	18.9	16.3	38.1	26.7		7.85	
E. coli S RNA	19.2	20.3	32.1	28.4		7.69	
E. coli M RNA	26.7	19.0	30.8	23.5		7.82	
II. DNA							
Calf thymus DNA‖	27.4	29.1¶	21.4	21.0		6.44	
Calf thymus apurinic acid		58¶		42		4.21	
III. Synthetic polyribonucleotides							
Poly A	100					8.78	5.4
Poly U		100				9.18	3.8
Poly C				100		5.64	3.9
Poly I					100	6.13	5.6
Copolymer 1, poly GU		15	85			8.22	
Copolymer 2, poly GU		34	66			10.1	
Copolymer 3, poly GU		68	32			9.68	
Copolymer 4, poly IU		17			83	6.73	
Copolymer 5, poly IU		44			56	7.71	
Copolymer 6, poly IU		69			31	8.26	

* Abbreviations: poly A, poly G, poly U, poly C, and poly I = biosynthetic homopolymers of adenylic, guanylic, uridylic, cytidylic, and inosinic acids, respectively; poly GU = copolymer of guanylic and uridylic acids; poly IU = copolymer of inosinic and uridylic acids; S and M = *Escherichia coli* soluble and microsomal RNA, respectively.

† Molar absorbancy expressed in terms of P concentration.

‡ Tris saline buffer, pH 7.4.

§ Beckman model E ultracentrifuge, UV optics.

‖ Base composition from The Nucleic Acids, E. Chargaff and J. N. Davidson, Eds. New York, Academic Press, 1955, vol. 1, p. 354.

¶ Thymine.

theses and the 5′-monophosphates used in certain of the experiments were obtained commercially.[3]

Calf thymus DNA was a commercial product.[4] Varieties of mammalian ribonucleic acid (RNA) were prepared by the method of Laskov, Margoliash, Littauer, and Eisenberg (11). In general, after the phenol denaturation step, the RNA was precipitated by ethanol and consisted of total cellular RNA. However, in one instance (rabbit liver RNA), precipitation of RNA was accomplished by 1 M NaCl, a procedure that favors the isolation of high molecular weight material. *Escherichia coli* soluble (S) and microsomal (M) RNA were prepared by ultrasonication, differential centrifugation in a sucrose medium, phenol extraction, DNose digestion, and a second phenol treatment. The M RNA was then precipitated with 1 M KCl and the S RNA with ethanol.[5] Apurinic acid was prepared from calf thymus DNA by the method of Tamm, Hodes, and Chargaff (12).

The synthetic polymers used were virtually protein-free, whereas the naturally occurring nucleic acids contained a maximum of 2% protein. Two batches of synthetic homoribopolynucleotides were used, and no essential differences were found between them in their effects on the complement system. A list of all the polynucleotides used is given in Table I together with some of their properties. Stock solutions of polynucleotides containing 4 μmoles P per ml were prepared in 0.15 M NaCl, 0.01 M Tris buffer, pH 7.4, and were stored at −20° C. Because of the difficulty encountered in dissolving poly I at this pH it was usually dissolved in Tris-saline, pH 9.2, and subsequently titrated to pH 7.4 with small amounts of HCl.

Protein determinations were done by the method of Hewitt (13), and phosphorous determinations by the method of Fiske and SubbaRow (14). Base compositions of ribopolynucleotides were determined by alkaline hydrolysis followed by paper electrophoresis in 0.025 M citrate buffer, pH 3.5, for 20 hours at 400 v (15). Because separation of IMP and UMP could not be accomplished by electrophoresis, the alkaline hydrolysates of poly IU were subjected to descending paper chromatography in an isopropanol: concentrated HCl: water solvent system (680:132:188, vol/vol). In this system the R_f values of IMP and UMP are 0.39 and 0.77, respectively. Although the base compositions of the IU and GU copolymers were roughly proportional to the concentrations of the respective nucleoside diphos-

[3] Pabst Laboratories, Milwaukee, Wis.

[4] Sigma Chemical Co., St. Louis, Mo.

[5] Kindly supplied by Dr. William Robinson, Argonne Cancer Research Hospital.

phates used in the synthetic step, significant differences were noted.

All C′ studies were carried out in barbital buffered saline, pH 7.4, containing optimal Ca^{++} and Mg^{++} at 37 ± 0.1° C. C′ titrations were carried out by the 50% hemolytic unit ($C'H_{50}$) assay technique. The procedure was that outlined by Kabat and Mayer (16). Single batches of pooled human (45 $C'H_{50}$ per ml) and guinea pig (295 $C'H_{50}$ per ml) serum stored in portions at −80° C were used throughout. The C′ titer of these reagents was assayed periodically and remained constant during these studies. The quantitation of anti-C′ activity was a modification of the method described by Taylor (17). A known amount of C′ sufficient to cause 100% lysis was used, usually 3 or 4 $C'H_{50}$. After a preliminary titration to define the approximate range of activity of the substance under study, a titration was performed with amounts of the test substance that would span the range of 10 to 90% hemolysis. The concentration of the test material was then plotted against percentage of hemolysis on logarithmic probability paper, and the amount giving 50% lysis was read from a line fitted to the points by inspection. From this, the amount of material needed to inhibit 1 $C'H_{50}$ could be calculated. For example, if the test system contained 4 $C'H_{50}$, and 0.6 μmole of test substance yielded 50% lysis, then 0.2 μmole (0.6/3) of the substance would inhibit 1 $C'H_{50}$. Although the order of addition of reagents was not critical, the following sequence was adopted: sensitized cells, buffer, test substance, C′. Most often, the standard 7.5-ml test system was used, but where the test material was available in very small amounts, a 1.5-ml system was employed, with all the reagent volumes reduced by $\frac{1}{5}$. In these instances, the supernatant fluid was diluted 1:10 and read at 413 mμ instead of the usual 540 mμ wave length. The anti-C′ potency of all substances, however, is expressed in the 7.5-ml system.

Reagents lacking each of the four C′ components (R1, R2, R3, and R4) were prepared by established techniques (16a). Samples of a 1:10 dilution of each reagent were stored at −80° C. At this concentration, none of these reagents would support hemolysis in the standard test system, nor did any of them display anti-C′ activity when tested with 4 $C'H_{50}$. Titration of these reagents for each of the 4 C′ components, with 50% hemolysis as the end point, was performed.

Sheep cells preserved in Alsever's solution and ambocepter were commercial preparations.[6] Since the anti-C′ effect of heparin has been extensively studied (17, 18), several preparations of heparin[7] were assayed for their anti-C′ potency to allow comparison with the polynucleotides under study. The anti-C′ potency of polynucleotides

[6] Markham Laboratories, Chicago, Ill.

[7] Heparin HWD (Hynson, Westcott, and Dunning, Baltimore, Md., lot no. 262, 13% S); heparin U (Upjohn Company, Kalamazoo, Mich., lot no. MK-449-HL, 13.18% S); heparin T (Testagar and Co., Detroit, Mich., lot no. 12778, 9.5% S).

TABLE II

Titration of the inhibition of complement (C′) by polyinosinic acid (poly I)

Tube	Sensitized cells	C′(4 $C'H_{50}$)	Buffer	Poly I	A_{540}	A_{540} (av.) − A_{540} blank	Lysis
	ml	*ml*	*ml*	*μmole* P			%
1	1.0	1	5.5	0.06	0.024	0.002	0.3
1′	1.0	1	5.5	0.06	0.023		
2	1.0	1	5.5	0.05	0.027	0.008	1.2
2′	1.0	1	5.5	0.05	0.031		
3	1.0	1	5.5	0.04	0.035	0.020	2.9
3′	1.0	1	5.5	0.04	0.047		
4	1.0	1	5.5	0.03	0.122	0.106	15.3
4′	1.0	1	5.5	0.03	0.131		
5	1.0	1	5.5	0.025	0.188	0.167	24.2
5′	1.0	1	5.5	0.025	0.188		
6	1.0	1	5.5	0.020	0.456	0.433	62.8
6′	1.0	1	5.5	0.020	0.452		
7	1.0	1	5.5	0.015	0.661	0.648	94
7′	1.0	1	5.5	0.015	0.675		
C′ control	1.0	1	5.5		0.713	0.692	100
C′ control	1.0	1*	5.5		0.382	0.361	52.3
Cell blank	1.0		6.5		0.021		
Lysis, 100%	1.0		6.5†		0.711	0.690	

* 1 $C'H_{50}$.

† 0.1% Na_2CO_3.

is expressed in μmoles P and that of heparin in μmoles S needed to inhibit 1 $C'H_{50}$.

Pancreatic ribonuclease (5 × crystalline)[8] and *Crotalus adamanteus* venom[9] were obtained commercially. Digestion of poly GU was accomplished by incubating 1 μmole P poly GU and 50 μg ribonuclease in 1 ml barbital buffered saline, pH 7.4, at 37° C for 2 hours.

A Beckman Zeromatic pH meter was used for pH measurements. All spectrophotometric measurements were made with a Zeiss PMQII spectrophotometer in cuvettes with a 1-cm light path.

RESULTS

The results of a typical poly I titration are shown in Table II and are plotted graphically in Figure 1. The latter shows that 0.022 μmole P poly I yields 50% lysis. Since there were 4 $C'H_{50}$ present in this system, 0.0073 μmole P poly I will inhibit the hemolytic activity of 1 $C'H_{50}$. Table III shows the results of a series of similar titrations using both human and guinea pig C′ that were performed throughout the course of this study. The amount of poly I needed to inhibit 1 $C'H_{50}$ is similar for the human and guinea pig reagent. The following polynucleotides were tested for anti-C′ activity in both human and guinea pig C′ systems and were found to be completely inactive in amounts as high as 1 μmole P polynucleotide: calf thymus DNA, both native and heat denatured; apurinic acid; guinea pig liver RNA; rat liver RNA; *E. coli* S RNA; *E. coli* M RNA; poly A; poly U; and poly C. Oxalate ion in amounts up to 1 μmole had no effect on C′ activity. 5′-Ribonucleotides, alone or in combination, were also devoid of anti-C′ activity. The anti-C′ activity of the several heparins studied is shown in Table IV. In comparison with heparin, poly I is an extremely potent anti-C′ substance, being approximately 300 times more potent (S : P molar ratio).

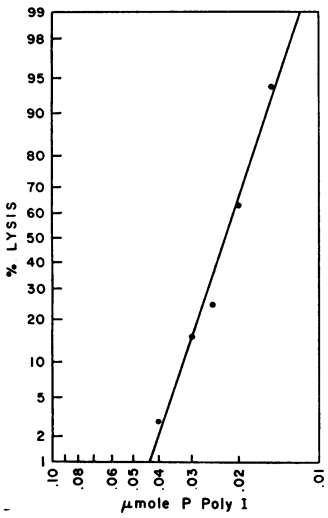

FIG. 1. TITRATION OF THE INHIBITION OF COMPLEMENT (C′) BY POLYINOSINIC ACID (POLY I). A graphic representation of the experiment outlined in Table I. P poly I, 0.022 μmole, yields 50% lysis. Since the test system contained 4 $C'H_{50}$, 0.0073 μmole P poly I inhibits 1 $C'H_{50}$.

[8] Worthington Biochemicals Corp., Freehold, N. J.

[9] Ross Allan Snake Farm, Silver Springs, Fla.

TABLE III

*The amount of poly I (μmole P) needed to inhibit 1 $C'H_{50}$ of human and guinea pig C′**

Determination no.	Guinea pig C′	Human C′
1	0.0073	0.0086
2	0.0073	0.0110
3	0.0082	0.0065
4	0.0075	0.0068
Mean ± SD	0.0076 ± 0.0004	0.0082 ± 0.0018

* A series of titrations using single batches of each C′ were performed during this study.

The experiment shown in Table V was to establish whether poly I was capable of preventing the attachment of amboceptor to sheep red cells. P poly I, 0.08 μmole, completely inhibits lysis when added during the second phase of the experiment, but does not interfere with the sensitization of sheep cells to lysis if present only during the first phase. In common with other anti-C′ substances (17), however, increasing the amount of hemolysin in the test system, even while keeping C′ constant, overcomes the inhibitory effects of poly I (Figure 2, A).

To discover which components of C′ were in-

TABLE IV

The amount of heparin (μmole S) needed to inactivate 1 $C'H_{50}$ of human and guinea pig C′

Heparin*	Guinea pig C′	Human C′
HWD		2.64
U	2.22	2.48
T	2.23	2.32
Mean	2.23	2.48

* See footnote 7.

TABLE V

*The effect of varying the stage at which poly I is added to the hemolytic system**

	Constituents		
	Tube 1	Tube 2	Tube 3
Sensitization step			
Sheep cells	0.5 ml	0.5 ml	0.5 ml
Hemolysin	0.5 ml	0.5 ml	0.5 ml
Saline		1.0 ml	1.0 ml
Poly I, 0.08 μmole/ml	1.0 ml		
Incubate 37°, 30 minutes.	Wash cells 3 times. Add following:		
Hemolysis step			
Saline	6.5 ml	5.5 ml	6.5 ml
Poly I, 0.08 μmole/ml		1.0 ml	
4 $C'H_{50}$/ml	1.0 ml	1.0 ml	1.0 ml
Incubate 37°, 60 minutes.			
Hemolysis, %	100	2	100

* When added during the amboceptor-red cell sensitization step, and removed by washing the cells before C′ addition, poly I will not inhibit red cell lysis.

TABLE VI

*The capacity of R reagents to restore hemolysis in a poly I inhibited C′ system**

Amount of poly I present	R reagent added	Hemolysis
		%
None	None	100
0.02 μmole	None	8.6
	R1	24.9
	R2	33.8
	R3	94.2
	R4	45
0.03 μmole	None	3.2
	R1	10.9
	R2	14.2
	R3	29.8
	R4	18

* Total reaction volume, 7.5 ml. All tubes contained 3 $C'H_{50}$ human C′. Poly I and R reagents, 1.0 ml, were added as indicated.

volved in the inhibition of C′ activity by poly I, the various R reagents were added to a system containing C′ and optimal inhibitory amounts of poly I, and the restoration of hemolytic potency was measured (Table VI). All four reagents caused partial restoration of hemolytic activity, the relative degree of restoration being maintained at both poly I concentrations. The order of magnitude of this hemolysis-restoring ability was R3 > R4 > R2 > R1.

Under the conditions of ionic strength and pH used in these experiments, poly I exists largely as a random coil (19). Poly I, however, will react stoichiometrically with both poly C and poly A to form ordered hydrogen-bonded helical structures (9a). The experiment shown in Figure 3

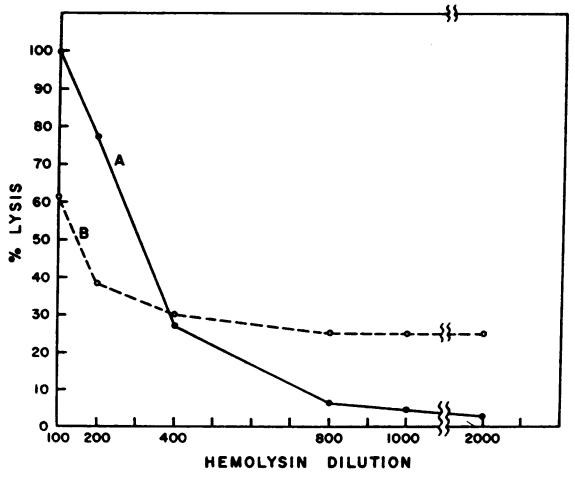

FIG. 2. A. THE EFFECT OF INCREASING HEMOLYSIN CONCENTRATION UPON THE ANTICOMPLEMENTARY ACTIVITY OF POLY I. Each tube contained 0.5 ml sheep cells, 0.5 ml hemolysin dilution, 4 $C'H_{50}$, and 0.04 μmole P poly I in a total volume of 7.5 ml. B. THE EFFECT OF INCREASING HEMOLYSIN CONCENTRATION UPON THE HEMOLYTIC POTENCY OF A C′ LIMITED SYSTEM. There is no poly I present. Each tube contained 0.5 ml sheep cells, 0.5 ml hemolysin dilution, 0.75 $C'H_{50}$, and buffer to a total volume of 7.5 ml.

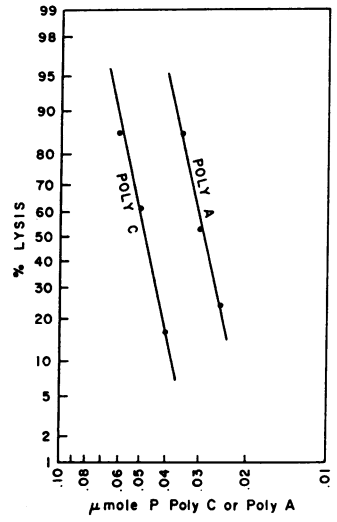

FIG. 3. THE EFFECT OF HELIX FORMATION ON THE ANTI-C′ POTENCY OF POLY I. Each tube contained 1 ml sensitized sheep cells, 4 $C'H_{50}$, and 0.08 μmole P poly I together with the indicated amount of either polyadenylic acid (poly A) or polycytidylic acid (poly C). Fifty per cent hemolysis occurs when either 0.029 μmole P poly A or 0.048 μmole P poly C is added to the test system.

TABLE VII

The anticomplementary (anti-C′) potency of a series of IU and GU copolymers

	Polymer no.	Base composition I	G	U	Amount of polymer needed to inhibit 1 $C'H_{50}$	Anti-C′ potency compared with poly I (based on % I or G content)
		%			*μmoles P*	
Poly IU						
	4	83		17	1.23	1/134
	5	56		44	*	
	6	31		69	*	
Poly GU						
	1		85	15	0.25	1/27
	2		66	34	†	
	3		32	68	*	

* No anti-C′ activity with 3 μmoles P polymer.
† Slight anti-C′ activity with 3 μmoles P polymer.

demonstrates that the formation of such helices destroys the anti-C′ activity of poly I. The amount of poly C necessary to restore 50% hemolysis was 1.7 times the amount of poly A required. This is consistent with the known structures of the respective helices which in the case of poly C is two-stranded (I + C), whereas poly I + poly A yields a triple-stranded helix (2I + A) (9a). In this experiment, the helices were preformed before their addition to the test system. Even if C′ and poly I are first mixed, however, the inhibitory effect of poly I can be abolished by subsequent immediate addition of poly A or poly C. Poly U, which cannot react with poly I, has no effect on the latter's anti-C′ activity even at poly U : poly I ratios of 6 : 1. The anti-C′ activity of poly I was destroyed by digestion with snake venom (*Crotalus adamanteus*) phosphodiesterase.

Table VII shows that the anti-C′ potency of the IU copolymers is very much less than that of poly I. In addition, poly GU (85% G, 15% U) is 5 times more potent than poly IU of similar base ratio. Digestion of the GU copolymer with ribonuclease did not destroy its anti-C′ properties.

DISCUSSION

These studies demonstrate that certain synthetic polyribonucleotides possess powerful anti-C′ activity, poly I being the most potent. Although the phosphate groups of polynucleotides are known to bind Ca^{++} and Mg^{++} on an equimolar basis (9b), it is clear from the data presented that this cannot explain the anti-C′ effect of poly I, since *1)* the amounts of poly I used were much too small to bind either of these cations completely, *2)* other polynucleotides lacking anti-C′ activity are known to have similar chelating potential, and *3)* oxalate ion in 130 times the concentration of poly I had no inhibitory effect.

The fact that poly I is primarily single stranded under the conditions of pH and ionic strength used in these experiments is not unique, for poly U and poly A are also single stranded under these conditions. Molecular size is not the critical factor in explaining the anti-C′ property of poly I, since the poly A and poly I used had very similar sedimentation coefficients (5.4 and 5.6, respectively). If we assume that the anti-C′ activity of poly I depends upon its ability to interact with one or more of the serum proteins that constitute the C′ system, such an interaction must depend primarily upon a structural feature of the polymer bestowed by the constituent base. This contrasts with the interaction of DNA and ribopolynucleotides with bovine serum albumin, where all the nucleic acids are capable of combining with the protein in an interaction that is primarily electrostatic in nature, involving the negatively charged phosphate groups (20). Although we have had no success as yet in synthesizing poly G, it seems reasonable to conclude from the data on the anti-C′ potency of the IU and GU copolymers that it may be at least as potent, or indeed even more potent, than poly I[10] in anti-C′ activity. We may

[10] A theoretical objection to this assumption may be found in the recent work of Fresco and Massoulié (21), who have found that in 0.15 M NaCl, pH 7.4, poly G

conclude that the anti-C′ activity of polynucleotides depends in part upon the presence of a purine ring possessing an OH group at the 6 position. In the case of poly I, involvement of the OH group in hydrogen-bond formation in the helical structures (I + C) and (2I + A) stoichiometrically abolishes anti-C′ activity.

Uridylic acid was chosen as the second base in the synthesis of the IU and GU copolymers to eliminate the possibility of intermolecular hydrogen-bond formation. Such copolymers probably exist in solution largely as random coils. Since IMP and GMP are lacking in anti-C′ activity, we may conclude that polymerization of these molecules is critical in endowing them with this property. Since poly U was incapable of preventing the anti-C′ activity of poly I even when present in excess (6:1), the marked drop in anti-C′ potency of the IU copolymers cannot be explained on the basis of active reversal by the presence of polymerized U within these molecules. If all that was necessary to endow an I residue with anti-C′ properties was its presence in a polymer, then the anti-C′ potency of the I molecules in the IU copolymers should remain unchanged. The most reasonable explanation for the precipitous decline in the anti-C′ potency of polymerized I residues in these copolymers is that, in order for I residues to possess anti-C′ activity, they must occur in a repetitive extended sequence uninterrupted by another base.

An estimate of the critical chain length, k, may be deduced from the fact that the I residues in the copolymer (83% I + 17% U) possess only 0.75% of potency of poly I. Statistical methods may be used to estimate k, since the incorporation of base into polymer by polynucleotide phosphorylase occurs in a random fashion (22). Such an analysis [11] utilizing the base composition of this copolymer reveals that a k of 43 I residues is necessary for a sequence of I to equal poly I in anti-C′ potency. If we assume that poly G = poly I in anti-C′ activity, then the k for G sequences is 28 (Figure 4). Chains of this length are maximal estimates, and it is likely that shorter k's of I or G possess graded anti-C′ activity.

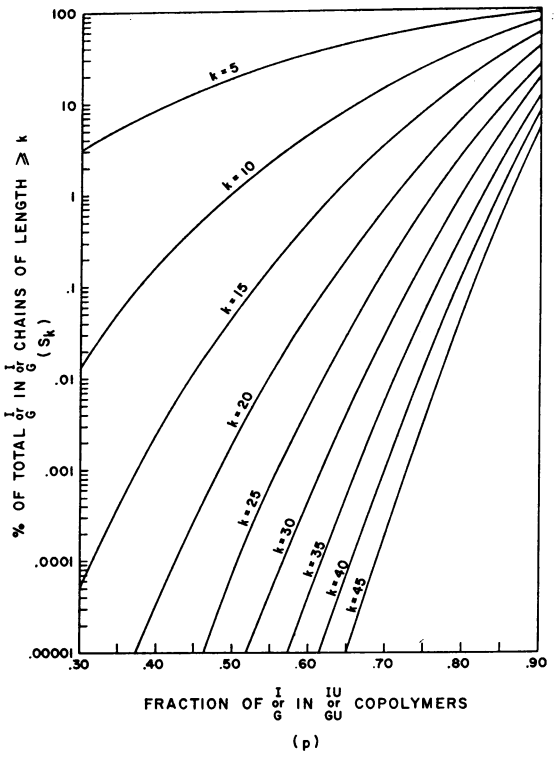

FIG. 4. THE PROBABILITY OF VARIOUS CHAIN LENGTHS, k, OF INOSINIC ACID (I) OR GUANYLIC ACID (G) OCCURRING IN INOSINIC AND URIDYLIC ACID (IU) OR GUANYLIC AND URIDYLIC ACID (GU) COPOLYMERS. See appendix for equations used in deriving these curves.

Experiments to test this hypothesis will be reported subsequently. The requirement for an extended base sequence for anti-C′ activity resembles the antigenic determinants in DNA that react with DNA antibodies in lupus sera, where an extended sequence of thymine base plays a critical role (23).

This concept explains the lack of discernible anti-C′ activity in the synthetic copolymers containing 60% or less of I or G, and also explains the lack of anti-C′ activity in naturally occurring RNA, where the G content ranges between 30 and 40%; in the latter case, base distribution is not random, and there is a large amount of inter- and intramolecular base pairing (24).

The fact that ribonuclease digestion of the copolymer (85% G, 15% U) did not destroy its anti-C′ properties is compatible with the hypothesis outlined, since it means that the products of digestion which consist predominantly of oligonucleotides of the general structure $G(pG)_nUp$ re-

exists as a multistranded helix. Ordered structure of polynucleotide may be incompatible with anti-C′ activity.

[11] See appendix.

tain as much anti-C′ activity as was originally present in the intact molecule.

The ability of increasing hemolysin concentrations to reverse the anti-C′ activity of poly I reflects the fact that the hemolysin used is one which, in a limited C′ system, increases the relative hemolytic potency of whatever C′ is free to act (Figure 2, B). The exact nature of poly I-C′ interaction, in terms of the 4 C′ components, is not clear. The experiments with the R reagents suggest that C′1 may be the most vulnerable factor, but if one examines the relative C′1 titers of the C′1-containing reagents, this concept breaks down, since R3, which was most effective in restoring hemolytic potency in a poly I inhibited C′ system, had the lowest C′1 titer. Clearly, as other authors have concluded (25), the use of the R reagents is of limited value in studies of this kind. Investigations presently under way are aimed at pinpointing the site of poly I-C′ inhibition by the use of the kinetic analysis methods of Mayer and others and by a study of its effects on C′1 esterase activity.

These experiments define a heretofore unrecognized biologic property of certain polyribonucleotides. Apart from the inherent interest of this phenomenon, the potency of poly I as an anti-C′ substance, surpassing as it does the activity of other anti-C′ substances such as heparin and the dextran sulfates (17), may warrant its trial in situations where the participation of C′ in immune reactions leads to cellular injury or death. It seems reasonable to assume that a potent anti-C′ substance may prove useful in the treatment of certain immunologic diseases of man where C′, in association with other factors, leads to tissue injury.

SUMMARY

Polyinosinic acid has been found to be a potent anticomplementary substance; 0.0073 μmole P of this material will inhibit 1 50% hemolytic U of complement. A wide variety of mammalian and bacterial RNA, as well as calf thymus DNA, polyadenylic acid, polyuridylic acid, and polycytidylic acid, are devoid of anticomplementary activity. Mononucleotides also are devoid of anticomplementary activity. Polyinosinic acid does not interfere with the uptake of hemolysin by sheep red cells. The anticomplementary activity of polyinosinic acid can be abolished stoichiometrically by polyadenylic or polycytidylic acid in ratios consistent with the known structure of these hybrid helices (I + C, 2I + A). Mixed copolymers of inosinic and uridylic acids, or guanylic and uridylic acids having high guanylic or inosinic acid contents, possess anticomplementary activity that is not destroyed by pancreatic ribonuclease digestion. The anticomplementary properties of polynucleotides depend upon base composition and secondary structure.

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APPENDIX

Given an infinite molecule of the form ABBABBBAAB . . ., where A and B are incorporated in a random fashion in the proportions p for A and q for B. Let k = any given length of uninterrupted A molecules. Let T_k = fraction of A molecules in chains of length k. Then $T_k = k_q{}^2\, p^{k-1}$.

Let S_k = fraction of A molecules in chains of length $\geqslant k$. Then $S_k = p^{k-1}(p + kq)$.

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