FAILURE OF DUPLEXES BASED ON POLYLAURUSIN [POLY(L), "POLYFORMYCIN B"] TO INDUCE INTERFERON

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Summary

Polylaurusin[poly(L) or "polyformycin B"] forms double-stranded complexes with polycytidylic acid (poly(C)) and with poly(5-bromocytidylic acid) [poly(br^{5}C)] with T_m's of 46.5° (0.2 M NaCl, pH 7) and 72.5° (0.15 M NaCl, pH 7), respectively. Both complexes fail to provide antiviral resistance (against vesicular stomatitis virus in primary rabbit kidney cells) or to induce interferon in "superinduced" primary rabbit kidney cells, even though they fulfill all previously recognized requirements for effective interferon inducers.

INTRODUCTION

While a number of diverse substances induce interferon [1], by far the most effective synthetic inducers are the polynucleotides. Yet within this class of polymers, there exist enormous variations in biological activity depending on the chemical nature of the synthetic nucleic acid. For instance, a variety of 2'-modified polynucleotide duplexes are 100-10,000-fold less active than their polyribonucleotide counterparts [2 and references cited therein]. Recently, it has become apparent that modification of the purine ring of poly(A)‡ or poly(I) can lead to similar dramatic changes in interferon

‡Abbreviations for polynucleotides follow CBN rules (Eur. J. Biochem. (1970) 15, 203). Unusual abbreviations are as follows: poly(L), polylaurusin or polyformycin B (the polynucleotide derived from the nucleoside 1,6-dihydro-3-β-D-ribofuranosyl-7H-pyrazolo[4,3-d] pyrimidin-7-one); poly(c^{7}I), poly(7-deazainosinc acid); poly(F), poly(formycin A); MEM, Eagle's minimal essential medium ; MID, minimum inhibitory dose; PRK, primary rabbit kidney.

inducing ability [3,4]. In this communication, we demonstrate that double-stranded complexes based on polylaurusin (which can be regarded as a modified purine polynucleotide) fail to elicit an interferon response even though they fulfill all other previously established requirements for effective interferon inducers.

MATERIALS AND METHODS

Poly(L) (s_{20} = 9.5S) was prepared by the *M. luteus* polynucleotide phosphorylase catalyzed polymerization of laurusin-5'-diphosphate. The synthesis and additional properties of the homopolymer will be described elsewhere. Poly(br^5C) (s_{20} = 11.5S) was prepared as described previously [5]. Other polynucleotides were the product of P-L Biochemicals (Milwaukee, Wisconsin) or Miles Laboratories (Elkhardt, Indiana). Poly(I) had s_{20} = 9.4S and poly(C) had s_{20} = 8.8S. Procedures employed for the determination of mixing curves, melting profiles and sedimentation constants have been reported elsewhere [6] as have the methods used to assess interferon production and ability to confer antiviral resistance [3]. Resistance of the complexes to pancreatic ribonuclease A (Worthington, Freehold, New Jersey) was determined by measuring the rate of increase in ultraviolet absorbance (hyperchromicity) when the appropriate duplex was incubated (0.15 M NaCl, pH 7, T = 37°) with the above nuclease.

RESULTS

Interaction of Poly(L) with Poly(C): Mixing curves for the interaction of poly(L) and poly(C) (Figure 1) revealed but one break (at 50 mole % poly(C)) at all wavelengths examined. Furthermore, the spectra derived from the two arms of the mixing curve could be superimposed to give distinct isosbestic points (Figure 1) providing strong evidence that in the 0-50 mole % poly(C) range, only two components were present (poly(L) and poly(L)·poly(C)) as well as in the 50-100 mole % poly(C) range (poly(L)·poly(C) and poly(C)). The melting profile (Figure 1) was monophasic with T_m = 46.5° (0.2 M NaCl, pH 7) indicating that the 1:1 complex melts directly to constituent homopolymers with no intervening rearrangements.

Interaction of Poly(L) with Poly(br^5C): In this instance also, mixing curves as a function of wavelength (Figure 2a) revealed that only one complex was

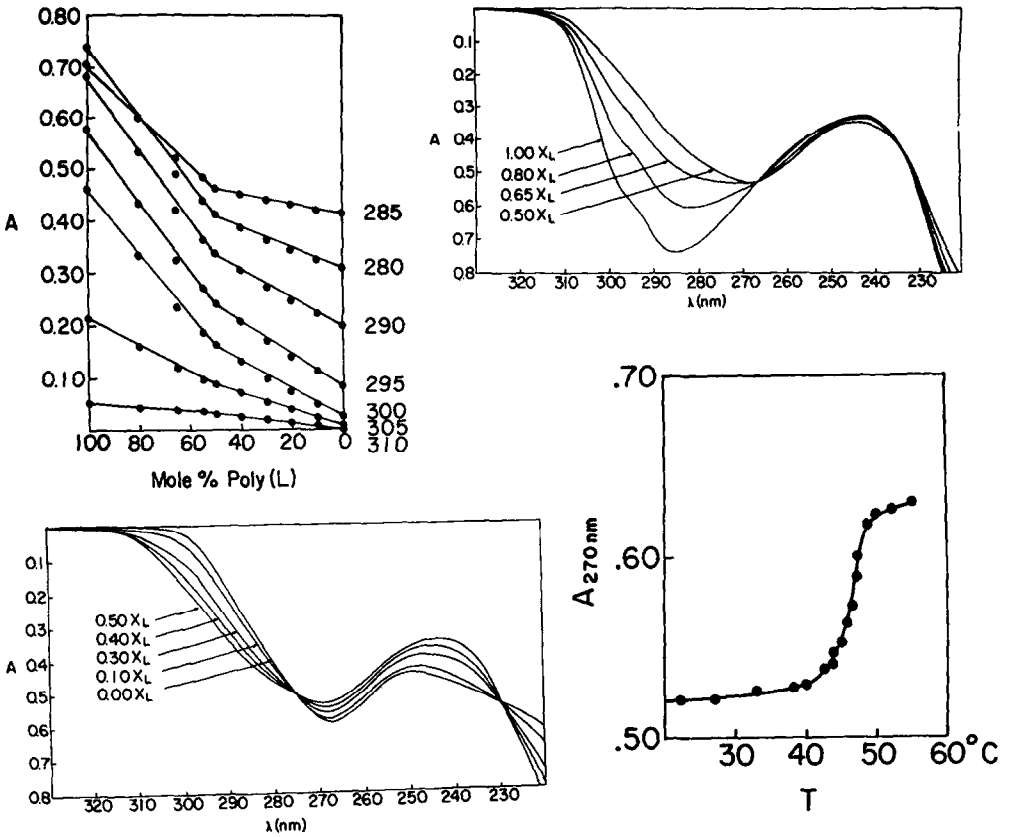

Figure 1 (upper left). Mixing curve (method of continuous variation) for the interaction of poly(L) with poly(C) in 0.20 M NaCl, 0.01 M sodium cacodylate, pH 7. Wavelengths (nm) are indicated to the right of each mixing curve. After mixing the solutions were allowed to equilibrate for 48 h at 20-25° and then for 1 week at 4°. (upper right) Isosbestic points generated from spectra derived from the 1.0-0.5 mole fraction poly(L) (X_L) solutions of Figure 1a. Isobestic points are 267 ± 0.2 nm and 233.5 ± 0.5 nm. (lower left) Same as upper right but representing the 0.5-0.0 X_L solutions. Isosbestic points are 275 ± 0.2 nm and 230 ± 0.3 nm. (lower right) Melting profile of the 50 mole % poly(L) solution (poly(L)·poly(C)) in 0.20 M NaCl, 0.01 M Na cacodylate, pH 7.

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formed, namely, poly(L)·poly(br^{5}C). This duplex also underwent a cooperative monophasic transition with a T_m of 72.5° (0.15 M NaCl, pH 7), an elevation to be expected due to the presence of the 5-bromo substituent (Figure 2b).

Ribonuclease Sensitivity of the Poly(L) Complexes as Compared to Poly(I)·Poly(C): Under the same conditions, poly(I)·poly(C), poly(L)·poly(C), poly(L)·poly(br^{5}C), poly(C) and poly(I) were degraded at rates of 0.008, 0.04, 0.009, >>0.3 and 0.00 μmoles/min/μg RNase, respectively.

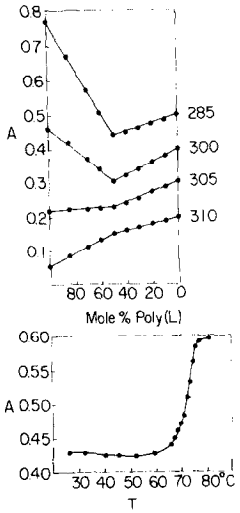

Figure 2 a. Mixing curve (continuous variation) for the interaction of poly(L) with poly(br^{5}C) in 0.15 M NaCl, 0.01 M sodium cacodylate, pH 7. Wavelengths (nm) monitored are indicated to the right of each mixing curve. Time of reaction was as in Figure 1. b. Melting profile of poly(L). poly(br^{5}C) in 0.15 M NaCl, 0.01 M Na cacodylate, pH 7.

Biological Evaluation of the Modified Duplexes: Table I shows the results obtained when these polynucleotide duplexes were evaluated for their ability to confer antiviral resistance (against vesicular stomatitis virus in PRK cells) and to induce interferon in "superinduced" [7] PRK cell cultures. Table II gives the results when the polymers were added in sequential order [8]. It is obvious that no matter what assay is employed, these poly(L) complexes fail to provide any antiviral resistance or fail to induce interferon even at relatively high concentration (10µg/ml).

DISCUSSION

All evidence gathered in this study indicates that poly(L) forms only 1:1 stoichiometric complexes with both poly(C) and poly(br^{5}C). Above 20° at moderate ionic strength, only the duplexes are capable of existence and these would be expected to persist under physiological conditions. These poly(L)-derived complexes meet all requirements previously established for effective polynucleotide interferon inducers [2,3 and references cited therein]; *i.e.*, double-strandedness, sufficiently high T_m (60° in 0.15 M salt, pH 7), high

Table I

Antiviral Activity and Interferon Inducing Ability of Polynucleotides*

Complex or Homopolymer	Induction of Cellular Resistance to Virus Infection Minimum Inhibitory Concn+	Interferon Production++ in "Superinduced" PRK Cells Polynucleotide dose (μg/ml) 0.1	1.0	10
Poly(L)	>10	<3	<3	<3
Poly(L)·Poly(C)	>10	<3	<3	<3
Poly(L)·Poly(br^{5}C)	>10	<3	<3	<3
Poly(I)	>10	--	--	<3
Poly(I)·Poly(C)**	0.003	20	200	2000
Poly(I)·Poly(br^{5}C)	0.003	300	1000	2000
Poly(C)	>10	--	--	3
Poly(br^{5}C)	>10	--	--	20
Poly(I)·Poly(C)†	0.001	--	--	2500

* Procedures as described previously [3].
\+ In μg/ml.
++ In units/ml.
** Source: constructed from the homopolymers used to prepare the above complexes.
† Source: P-L Biochemicals

molecular weight, adequate resistance to nucleases (*e.g.*, pancreatic RNase) and presence of 2'-OH groups. The T_m of poly(L)·poly(C) is definitely lower than the value usually considered optimal; nonetheless, poly(c^{7}I)·poly(C) [4] (T_m 49°, 0.2 M salt, pH 7) is an interferon inducer albeit a less effective one than poly(I)·poly(C). Poly(L)·poly(br^{5}C) and poly(I)·poly(C) are equally resistant to RNase A; poly(L)·poly(C) is more sensitive probably due to its lower T_m. These considerations not withstanding, both poly(L)·poly(C) and poly(L)·poly(br^{5}C) are at least 10,000 times less active than poly(I)·poly(C) or poly(I)·poly(br^{5}C).

What is the basis for this dramatic change in biological activity? We have previously demonstrated that replacement of purine N-7 of poly(A) by

Table II

Induction of Cellular Resistance to Virus Infection. Complementary Homopolynucleotides Added Sequentially

Sequence of Addition		Minimum* Inhibitory Concn (μg/ml)
First	Second	
Poly(I)	Poly(C)$_5$	0.0006
Poly(I)	Poly(br^{5}C)	0.003
Poly(L)	Poly(C)$_5$	>1
Poly(L)	Poly(br^{5}C)	>1
Poly(C)	Poly(I)	0.01
Poly(C)	Poly(L)	>1
Poly(br^{5}C)	Poly(I)	0.003
Poly(br^{5}C)	Poly(L)	>1

* Refers to the homopolymer that was added second to the PRK cell cultures in tubes. The first homopolymer was add at 1 μg/ml in MEM (1 ml/tube) for 1 hr at 37°. The cells were then washed (3X) with minimal Eagle's medium and further incubated with varying concentrations of the second homopolymer (1,0.1, 0.01,...μg/ml) for 20 hr at 37°. The cells were then processed as described previously [3].

CH leads to duplexes which are totally devoid of interferon inducing ability [3]; however, this same modification of the purine ring of poly(I) gives rise to a duplex (poly(c^{7}I)·poly(br^{5}C)) with somewhat greater activity than poly(I)·poly(C) (in PRK cells) [4]. These and related findings led to the suggestion that such modifications may result in significant changes in the conformation of the polynucleotide double helix which may prevent effective binding to the cellular receptor site for interferon inducers, or which, although permitting binding, may fail to trigger the necessary message for interferon synthesis. Poly(L) and its constituent nucleoside, formycin B, as well as the adenine analog (formycin A) possess a number of unusual properties which might substantially alter the conformational properties of helices derived therefrom. Laurusin is a C-nucleoside with a significantly greater glycoside bond distance than occurs in normal nucleosides. This property leads to a lower energy

barrier to rotation about the glycoside bond resulting in a displacement of the *syn-anti* equilibrium seen in most C-N glycoside nucleosides, and is reflected in the anomalous biochemical and physical properties of poly(F) and poly(L) as well as in the nucleosides themselves [9,10 and references therein]. It is probable that these properties may also influence the duplexes based on poly(L), especially since it has been demonstrated that polynucleotide helices may exist even when one component strand is locked in the *syn* conformation [11].

Finally, we must point out that Ikehara and Tezuka (M. Ikehara and T. Tezuka, personal communication, 1974) have found that mixing curves of the interaction of poly(L) and poly(C) (T = 2.5°, 0.1 M NaCl, pH 7) show 1:2 and 2:1 complexes but no 1:1 complex. Their data are difficult to reconcile with those presented herein, although it may be possible that at 2.5° such complexes are formed and then rearrange to the 1:1 complex at higher temperatures. Alternatively since mixing curves were determined after 10 hrs equilibration in the experiments of Ikehara and Tezuka, whereas in this work they were determined after at least one week, the 2:1 and 1:2 complexes may form initally but eventually rearrange at equilibrium to the duplex.

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