Biophysical Chemistry 40 (1991) 231–239
Elsevier Science Publishers B.V., Amsterdam

BIOCHE 01574

Biophysical studies of the complexes of polyriboguanylic acid with brominated and chlorinated polyribocytidylic acids

E.M. Kogan [a], N.G. Dyatlova [a], N.S. Sidorova [a], L.M. Vilner [b], O. Vrána [c] and V. Brabec [c,*]

[a] *Institute of Macromolecular Compounds, Academy of Sciences of the USSR, Leningrad, USSR*
[b] *Institute of Poliomyelitis and Viral Encephalitis, Academy of Medical Sciences of the USSR, Moscow, USSR*
[c] *Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia*

Received 23 July 1990
Revised manuscript received 12 November 1990
Accepted 14 January 1991

Poly(rG)·poly(rC); Halogenation; Conformation; Antiviral activity

Conformation of double-stranded complexes of polyriboguanylic acid with halogenated polyribocytidylic acid [poly(C)] was studied with the aid of differential pulse polarography, terbium fluorescence and circular dichroism spectrometry. It was shown that halogenation at C(5) of cytosine residues in poly(C) disturbed the ordered structure of the double-helical complex. In addition, this halogenation does not improve antiviral activity of the polynucleotide complex studied in the system of vesicular stomatitis virus and the cell culture of chicken embryos. It was concluded that the regularity of the secondary structure of synthetic RNAs might play an important role in the mechanism of biological activity of these biomacromolecules.

1. Introduction

The double-stranded synthetic RNAs polyriboinosinic acid · polyribocytidylic acid [poly(I) · poly(C)] and polyriboguanylic acid · polyribocytidylic acid [poly(G) · poly(C)] are the most extensively studied interferon inducers or antiviral agents [1,2]. In a search for other effective compounds based on polynucleotides numerous modifications of the polynucleotide complexes have been performed in the hope that a relationship between structure and biological activity could be established. Among modifications of the polynucleotide complexes increasing their interferon inducing or antiviral capacity belong in particular those which improve the integrity of the secondary structure of these RNAs [3–8] or, in other words, decrease the extent of conformational distortions in the double-helical arrangement of these biomacromolecules.

Of the alterations which have been carried out on poly(I) · poly(C), the following modifications improve the interferon inducing activity: substitution of oxygen by sulphur at the C(2) site of cytosine [9], substitution of CH for N(7) of hypoxanthine [10], covalent binding of chlorodiethylenetriaminoplatinum(II) chloride to inosine N(7) [1], and bromination at C(5) of cytosine [10].

In the case of poly(G) · poly(C), research has been conducted to determine the effect of insertion of adenine residues in the purine or pyrimidine chain on the conformation and biological effectiveness of the complexes. The investigation of these complexes having the ratio of basic and modifying nucleosides in the range from 10 : 1 to 90 : 1 made it possible to determine the length of a continuous segment of poly(G) or poly(C), which

* To whom correspondence should be addressed.

0301-4622/91/$03.50

is necessary for the maximum biological activity of their complexes [11,12]. In contrast to the poly(I) · poly(C) complex, the effect of chemical modifications of the poly(G) · poly(C) complex on its interferon inducing or antiviral activity has not been carried out yet.

In this work preparation of the complexes of poly(G) with brominated and chlorinated poly(C) is described. We were inspired to prepare this double-stranded RNA by the results of DeClercq et al. [10] who have shown that halogenation at C(5) of cytosine in poly(C) improves antiviral activity of the poly(I) · poly(C) complex. We also compare physical and physico-chemical properties of double-stranded complexes of poly(G) and chlorinated or brominated poly(C) with their antiviral activity in order to support the view that the ordered structure of the double-helical RNA plays an important role in the mechanism of its antiviral activity.

2. Material and methods

2.1 Synthesis of 5-Br- and 5-Cl-cytidine-5′-diphosphate

Ten ml of saturated solution of chlorine or bromine in CCl_4 was added dropwise and under vigorous shaking to 10 ml of the solution of 1.2 m*M* cytidine-5′-diphosphate (CDP) in formamide. After 30 minutes of stirring 80 ml of ethylalcohol was added and the mixture was incubated at 4°C overnight. The precipitation of 5-Br-CDP or 5-Cl-CDP was collected after centrifugation, washed twice with ethylalcohol and once with ether and finally dried in vacuum. 5-Br- or 5-Cl-CDP without further purification were taken for brominated and chlorinated poly(C) synthesis. The substitution of the hydrogen atom by halogen in position 5 of cytosine was confirmed by ^{1}H NMR spectroscopy (Table 1).

2.2 Synthesis of single-stranded poly(5-Br-C), poly(5-Cl-C) and copolymers containing various amounts of 5-halogen-cytosine

The synthesis was performed with the aid of *Escherichia coli, Thermus thermophilus* polyribonucleotide phosphorylase (PNP) containing no admixtures of other nucleases in particular ribonuclease A. PNP was kindly provided by Drs. Glazunov and Timkovsky from the Laboratory of Biopolymers of the Institute of Nuclear Physics, Academy of Sciences of the USSR, Gatchina. The conditions of synthesis and extraction of polymers were the same as those described earlier in the case of poly(C, A) synthesis [12]. In order to get copolymers with different contents of 5-halogen-cytosine, a mixture of CDP and 5-halogen-CDP of

Table 1

The characteristics of the derivatives of cytidine-5′-diphosphate

Nucleotide	Reaction conditions		Yield (%)	R_f [a]	NMR spectroscopy [b]			UV absorption pH 7.0	
	T (°C)	time (h)			C′ (1)	C(5)	C(6)	λ_{max} [c]	ε [d]
Cytidine-5′-diphosphate	–	–	–	0.55	5.20	5.85	7.75	272	9250
5-Br-cytidine-5′-diphosphate	20	2	92	0.62	5.20	–	8.60	289	6460
5-Cl-cytidine-5′-diphosphate	20	1	92	0.64	5.20	–	8.60	286	7100

[a] The system for thin layer chromatography was dioxan × ammonia × water (6 : 1 : 4).

[b] Chemical shifts of downfield peaks (in ppm) observed in the 60 MHz ^{1}H NMR spectra at pH 7.0. Chemical shifts are reported relative to the internal standard TSP.

[c] Maximum wavelength (nm).

[d] Molar extinction coefficient at λ_{max} (l mol^{-1} cm^{-1}).

the molar ratio in the range from 2 : 1 to 10 : 1 was employed. The molecular mass of polynucleotides was determined with the aid of Sephadex G-200 using standard conditions [13]. Two sets of preparations were synthesized: poly(5-Br-C, C) (containing 33, 67 and 100% of 5-Br-C) and poly(5-Cl-C, C) (containing 10, 25 and 100% of 5-Cl-C).

2.3 Formation of double-stranded complexes

The double-stranded complexes were prepared by the incubation of equimolar mixtures of single-stranded polynucleotides in 0.005 *M* phosphate buffer (NaH_2PO_4/Na_2HPO_4), pH 7.0 plus 0.1 *M* NaCl. The details of this procedure can be found elsewhere [8]. The stoichiometry of the complexes was estimated by measuring the mixing curves (a continuous variation experiment) [14,15]. The optical density was measured by means of a Beckman spectrophotometer, Model 25. The molecular mass of the polynucleotide complexes was determined by gel chromatography using Sepharose 2B; the details have already been published [13]. If not stated otherwise, the polynucleotide concentrations given in this paper are related to the monomer content.

2.4 Methods of physical and physico-chemical characterization of polynucleotide complexes

Differential pulse polarographic (DPP) measurements were carried out on a PAR polarographic analyser, Model 174A, with a three-electrode system. The working electrode was a PAR static mercury drop electrode, Model 303A, medium size. The reference electrode was a saturated calomel electrode and the auxiliary electrode was a coiled platinum wire. Measurements were performed at 25°C. The solutions were deoxygenated by passing a slow stream of pure argon through them. Argon was allowed to pass over the solution during the measurement to maintain an inert atmosphere. DPP measurements were carried out with the following apparatus settings: pulse amplitude −50 mV, voltage scan rate 2 mV/s, drop time 1.0 s.

Terbium fluorescence measurements were performed as follows: terbium was added to 8 μg of polynucleotide at a final concentration equivalent to twice the monomeric nucleotide content. The fluorescence intensity was measured after equilibration for 60 minutes at 25 °C in the dark. The fluorescence intensity was measured using a 1 cm quartz cell in a Shimadzu RF 1540 spectrofluorophotometer equipped with a high-pressure xenon lamp. An excitation wavelength of 290 nm and an emission wavelength of 546 nm were used. The entrance and exit slit widths were maintained at 5 nm. $TbCl_3 \cdot 6\ H_2O$ (Wako Pure Chemicals) was made up to 10 m*M* in distilled water. Other details of these measurements can be found elsewhere [20].

Circular dichroism (CD) spectra were recorded at 25°C on a Jobin Yvon Dichrograph Mark IV using 1 cm cuvettes.

2.5 Methods of the measurements of biological activity of polynucleotide complexes

The biological activity, namely the antiviral activity, of the complexes was determined by measuring the level of reduction of the number of plaques of vesicular stomatitis virus (VSV) in chicken embryo cells. The details of this assay have already been described elsewhere [11,12].

3. Results and discussion

3.1 Synthesis of halogenated poly(C) and formation of its complexes with poly(G)

C(5) in cytosine does not take part in the formation of hydrogen bonds of poly(G) · poly(C) or poly(G) · poly(5-halogen-C). It is reasonable to expect that the modification resulting in chlorination or bromination of poly(C) at C(5) has no substantial influence on hydrogen bonding in the poly(G) · poly(C) complex.

Chlorinated and brominated CDP was obtained by the action of free halogen on CDP. The chlorination and bromination take place quantitatively and, because of a short-term reaction, no disturbance of the unstable pyrophosphate group of nucleoside-5′-diphosphates is observed. The characteristics of halogen derivatives of CDP, the

Table 2

The characteristics of halogen-containing polyribocytidylic acids

Polynucleotide	Content of 5-halogen-C (%)	λ_{max} [a] (pH 7.0)	ε [a] $\times 10^{-3}$	Yield (%)
Poly(C)	0	268	6.3	51
Poly(5-Br-C, C)	33	273	–	38
Poly(5-Br-C, C)	67	279	–	35
Poly(5-Br-C)	100	289	5.5	30
Poly(5-Cl-C, C)	10	270	–	40
Poly(5-Cl-C, C)	25	275	–	40
Poly(5-Cl-C)	100	287	5.1	35

[a] λ_{max} and ε have the same meaning as in Table 1.

substrates for the synthesis of halogenated poly(C) used in our work, are given in Table 1. The missing signal in ^{1}H NMR spectra of chlorinated and brominated CDP in D_2O at C(5) gives evidence for a complete replacement of the hydrogen atom in C(5) position of cytosine.

As shown in Table 2, halogenated poly(C) containing different amounts of halogenated cytosine in the pyrimidine chain was synthesized. The yield of brominated or chlorinated poly(C) synthesized with the aid of PNP is decreased with the growing content of 5-halogen-C, and is always lower than the yield of poly(C) synthesized under analogous conditions. Figure 1 shows the curves of the increment of inorganic phosphate during halogenated poly(C) synthesis using PNP. The lower initial rate of the synthesis of polynucleotides containing

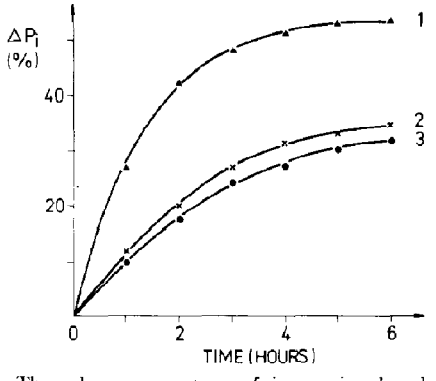

Fig. 1. The release percentage of inorganic phosphate ΔP_i during polycondensation in the presence of polynucleotide phosphorylase of CDP (1), 5-Cl-CDP (2) and 5-Br-CDP (3).

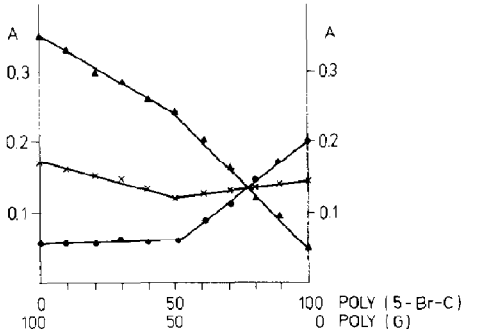

Fig. 2. Mixing curves of equimolar solutions of poly(G) and poly(5-Br-C) (continuous variation experiment) at 260 nm (▲), 280 nm (×) and 290 nm (●): The dependence of optical density A of mixtures heated at 80°C for 10 minutes on molar percentage of poly(G) and poly(5-Br-C).

chlorine or bromine is evident. The order of the substrate activity is 5-Br-CDP < 5-Cl-CDP < CDP.

The molecular mass of our polyribocytidylates was not lower than 200,000–300,000 g/mol, which is a necessary condition for obtaining highly active preparations of their double-stranded complexes [12,13].

3.2 Physical and physico-chemical properties of complexes of poly(G) with halogenated poly(C)

The molecular mass of the complexes was about 2×10^6 g/mol. Thus the presence of chlorine or bromine in the 5 position of cytosine has no influence on the molecular mass of the polynucleotide complexes.

A hyperchromic effect in the ultraviolet spectra of poly(G) · halogenated poly(C) complexes is observed at 260 nm and a hypochromic effect is found at 280 and 290 nm. Figure 2 demonstrates the mixing curves of poly(G) and brominated poly(C) recorded by measuring absorbances at 260, 280 and 290 nm (the continuous variation experiment [14,15]). The break on these curves at different wavelengths, i.e. the maximum extent of the complex formation, is observed at equimolar ratios of the polynucleotides. The course of the mixing curves recorded at the same wavelengths for chlorinated poly(C) is analogous. These data show that the complexes of poly(G) with chlorinated or brominated poly(C) are double-stranded

like the complex of poly(G) with non-modified poly(C).

The effect of bromination and chlorination at C(5) of cytosine of single-stranded poly(C) on the CD spectrum of this polynucleotide is essentially identical. Figure 3 shows the marked CD spectral changes for poly(C) on increasing the level of its halogenation. The prominent positive CD band at 275 nm is decreased in magnitude and undergoes also a red shift. Fully halogenated poly(C) yields the main positive CD band, the intensity of which is reduced to less than 50% and shifted by more than 20 nm. The formation of a complex between poly(G) and poly(C) or its halogenated analogues is clearly reflected in the CD spectra (Fig. 4). Although the CD spectrum of poly(G) complexed with halogenated poly(C) is substantially different from that of A-RNA, namely poly(G) · poly(C)

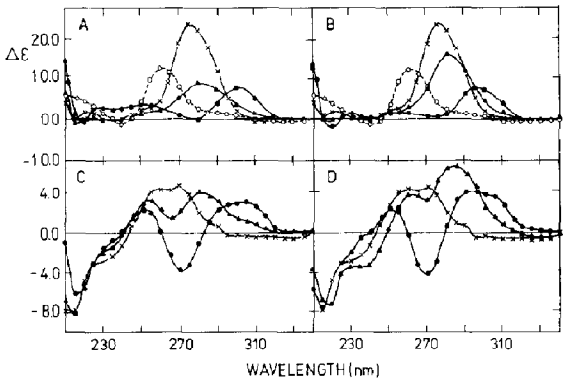

Fig. 3. Circular dichroism spectra of single-stranded poly(C), its halogenated analogues, poly(G) (A, B) and double-stranded complexes of poly(G) with poly(C) and its halogenated analogues (C, D). The spectra were obtained in a medium of 0.1 *M* sodium phosphate, pH 7.0. A: (×——×) poly(C); (▲——▲) poly(5-Br-C, C), the content of halogenated cytosine was 30%; (●——●) poly(5-Br-C), the content of halogenated cytosine was 100%; (○------○) poly(G). B: (×——×) poly(C); (▲——▲) poly(5-Cl-C, C), the content of halogenated cytosine was 20%; (●——●) poly(5-Cl-C), the content of halogenated cytosine was 100%; (○------○) poly(G). C: (×——×) poly(G) · poly(C); (▲——▲) poly(G) · poly(5-Br-C, C), the content of halogenated cytosine in poly(C) was 30%; (●——●) poly(G) · poly(5-Br-C), the content of halogenated cytosine in poly(C) was 100%. D: (×——×) poly(G) · poly(C); (▲——▲) poly(G) · poly(5-Cl-C, C), the content of halogenated cytosine in poly(C) was 20 %; (●——●) poly(G) · poly(5-Cl-C), the content of halogenated cytosine in poly(C) was 100%.

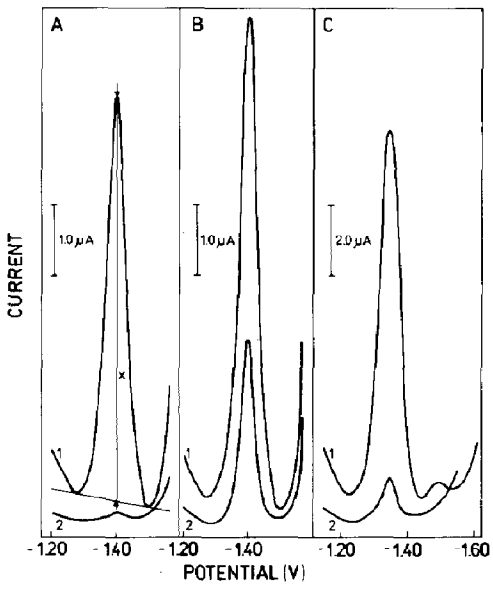

Fig. 4. Differential pulse polarograms of poly(C), its halogenated analogues and their complexes with poly(G) in a medium containing 0.3 *M* CsCl with 0.05 *M* sodium phosphate, pH 6.8. A: poly(C), B: poly(5-Br-C), C: poly(5-Cl-C). Curves 1–single-stranded polynucleotides at a concentration of 2×10^{-4} *M*, curves 2–their complexes with poly(G) at a concentration of 4×10^{-4} *M*.

[16], determination of details of the secondary structure of these homopolynucleotide complexes needs further investigation.

Cytosine and its monomeric derivatives are polarographically reducible [17]. They yield a pH-dependent polarographic reduction current in the potential range around −1.5 V. Bromination and chlorination of cytosine at C(5) does not result in a loss of reducibility of cytosine and its monomeric derivatives. For instance, 5-Br-CDP or 5-Cl-CDP yield similar polarographic reduction currents as the non-modified CDP (not shown). Guanine and its monomeric derivatives are polarographically non-reducible.

Single-stranded poly(C) and its brominated and chlorinated analogues at a concentration of 2×10^{-4} *M* in the medium of 0.3 *M* CsCl plus 0.05 *M* sodium phosphate, pH 6.8, yielded a high DPP peak at a potential close to −1.4 V (Fig. 4, curves 1). While poly(C) and poly(5-Br-C) yielded this peak at −1.40 V, the peak of poly (5-Cl-C) appeared at a potential that was by ca. 60 mV more

positive and almost double that of the non-modified poly(C). Polarographic reduction of cytosine residues is responsible for the peak of poly(C) [18]. Because of the fact that monomeric brominated and chlorinated derivatives of cytosine yield a similar polarographic reduction current as does non-modified cytosine and its monomeric derivatives, it is reasonable to expect that it is electroreduction of halogenated cytosine residues which is responsible for the appearance of the polarographic peaks of single-stranded halogenated poly(C). The nature of the difference in the peak heights and peak potentials of poly(C), poly(5-Br-C) and poly(5-Cl-C) has not been so far investigated in detail. Preliminary results revealed that these differences might be also connected with dissimilarities in molecular mass and adsorbability of the polynucleotide since single-stranded polynucleotides are polarographically reduced only in the adsorbed state and their adsorption is diffusion controlled [19].

It has already been demonstrated [8] that the formation of double-stranded complexes of poly(G) with poly(C) results in a suppression of the polarographic reduction current of poly(C). This is so because the primary reduction sites in cytosine residues are hidden inside the double-helical complex and are involved in hydrogen bonding. These facts represent a steric hindrance for electron transfer from the electrode to cytosine-reducible groups. The small DPP peak yielded by poly(G) · poly(C) sample (Fig. 4A, curve 2), apparently corresponding to a small amount of single-stranded poly(C) appears to be due to the existence of segments in poly(G) during the complex-forming process in which the guanine residues are unable to be hydrogen-bonded with bases in poly(C) [8].

In the case of the complex of poly(G) · poly(C), the height of the small peak of poly(C) (Fig. 4A) corresponded to ca. 3×10^{-6} *M* single-stranded poly(C), i.e. only ca. 1.5% of cytosine residues in poly(C) were not paired with those of guanine in poly(G). When, however, the same DPP analysis was carried out with the complexes of poly(G) with brominated or chlorinated poly(C) (Figs. 4B,C, curves 2), the height of the peak of the halogenated poly(C) corresponded to ca. 37 and 11%, respectively, of halogenated cytosine residues in poly(C) that were not paired with those of guanine in poly(G). Hence, it would appear that the extent of the ordered secondary structure of the complexes of poly(G) with halogenated poly(C) is markedly lower in contrast to poly(G) · poly(C) prepared under identical conditions. It should be also noted that bromination of poly(C) resulted in a less ordered or intact complex with poly(G) than did chlorination of poly(C).

Table 3

Physico-chemical analysis of halogenated polyribocytidylic acid and its complexes with poly(G)

Polynucleotide	DPP peak height (μA) [a]	Fluorescence of Tb^{3+} polynucleotide complex [b]
Poly(C)	9.06	0.8
Poly(G)	0.00	100.0
Poly(5-Br-C)	11.43	1.0
Poly(5-Cl-C)	17.14	3.5
Poly(G)·poly(C)	0.14	0.4
Poly(G)·poly(5-Br-C)	4.21	0.6
Poly(G)·poly(5-Cl-C)	1.86	1.1

[a] Single-stranded polynucleotides at a concentration of 2×10^{-4} *M*, double-stranded polynucleotides at a concentration of 4×10^{-4} *M*. Medium: 0.3 *M* CsCl with 0.05 *M* sodium phosphate, pH 6.8.

[b] Relative fluorescence. Fluorescence of poly(G) was arbitrarily set at 100.

Fluorescence of terbium ion–nucleic acid complexes provides a sensitive specific probe for base residues in distorted nucleic acids [20,21]. The interaction of Tb^{3+} with non-hydrogen bonded guanine residues or the guanine residues present in distorted double-stranded regions of nucleic acids in which only the vertical stacking of the base-pairs is altered has been shown to result in a substantial enhancement of the fluorescence of this reaction [20].

The relative intensity of terbium fluorescence is greatly enhanced for poly(G) (ref. [21] and Table 3). On the other hand, a small fluorescence enhancement of terbium was observed for single-stranded poly(C) and its brominated and chlorinated analogues (Table 3). The formation of a double-stranded complex of poly(G) with poly(C) and halogenated analogues resulted in a marked

decrease of the terbium fluorescence enhancement (Table 3). The quenching of fluorescence by the polynucleotide complex formation is likely to be connected with an involvement in base pairing of all the guanine residues. This could be considered as an inconsistency with the results obtained by means of DPP analysis. The latter results indicate 37 and 11% of non-paired cytosine residues in the complex of poly(G) with brominated and chlorinated poly(C), respectively. The explanation may lie in the fact that poly(G) strands have a strong tendency to form four-stranded helices [21] involving tetrads of hydrogen-bonded guanosine residues. If the complex is to be formed in an equimolar mixture of poly(C) and poly(G) relatively extensive secondary structure of poly(G) has to be unwound during the complex-forming reaction. The tendency of single-stranded poly(C) to form a complex with poly(G) is obviously a factor contributing to this unwinding process. Hence, halogenated poly(C) may have the capability to unwind a stable secondary self-structure during the complex-forming process reduced in comparison with non-modified poly(C).

It was shown [22] that bromination of the polyribonucleotide complex poly(G–C) · poly(G–C) can induce in this double stranded nucleic acid a conformational alteration resulting in the formation of a new structure having features of the left-handed Z-conformation observed for DNA. It is evident, however, that our poly(G) · poly(5-Br-C) samples cannot adopt a Z-like conformation. This conformation has been so far observed only in polynucleotides having alternating purine-pyrimidine sequences, i.e. in the polynucleotides in which a dinucleotide is the basic repeating unit. It is very likely that our sample of poly(G) · poly(5-Br-C) (composed of the homopolynucleotides) cannot adopt the Z-conformation. Nevertheless, these facts indicate that in particular bromination of the polynucleotides containing guanine and cytosine residues results in a conformational stress contributing to the transition of the polynucleotide complex to the left-handed Z-form. Thus, although halogenation of poly(C) cannot induce a transition of poly(G) · poly(C) to the Z-conformation it may contribute to the decrease of the stability of its right-handed A-conformation and consequently to the less ordered duplex of this polynucleotide complex. This lowered regularity of the polynucleotide complex could be reflected by the higher number of the conformational distortions, which could be responsible for the high DPP current yielded by the complex of poly(G) with poly(5-Br-C) (Fig. 4B).

The results of physical and physico-chemical studies of the complexes of poly(G) with brominated or chlorinated poly(C) indicate that the extent of the ordered double-stranded structure of these complexes is somewhat lower than that of poly(G) · poly(C). It was suggested [3–8] that the regular and intact double-helical structure of the polyribonucleotide complex is essential for interferon inducing and antiviral activity of this nucleic acid. It is therefore possible to anticipate that the double-stranded complexes of poly(G) with brominated or chlorinated poly(C) will not exhibit a better biological activity than the complex of poly(G) with non-modified poly(C). Moreover, the complex containing chlorinated poly(C) was more intact than the complex containing brominated poly(C). Therefore, the latter complex was expected to exhibit a lower interferon inducing or antiviral activity than the complex with

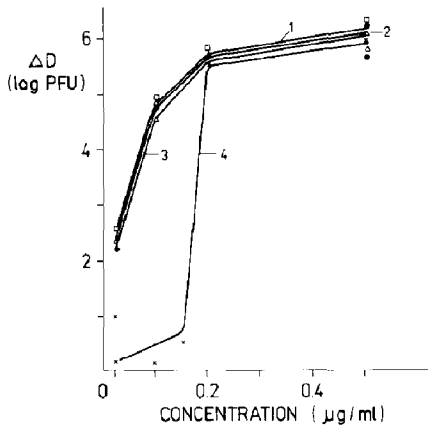

Fig. 5. Relative antiviral activity of the complexes of poly(G)·poly(5-Cl-C, C) differing in the content of 5-Cl-cytosine in the cell culture of chicken embryos: The dependence of the concentration decrease of vesicular stomatitis virus ΔD expressed in plaque-forming units (PFU) on the polynucleotide complex concentration in μg/ml. The content of 5-Cl-cytosine in poly(C) was 0% (curve 1), 10% (curve 2), 25% (curve 3), and 100% (curve 4).

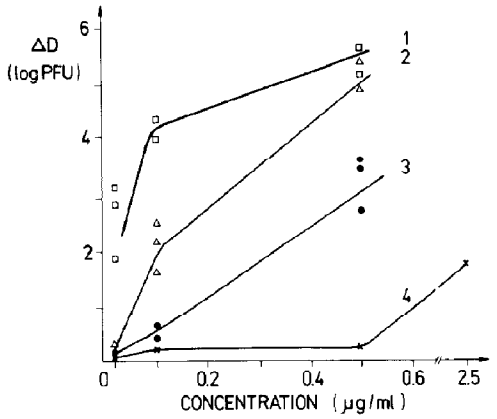

Fig. 6. Relative antiviral activity of the complexes of poly(G)·poly(5-Br-C, C) differing in the content of 5-Br-cytosine in the cell culture of chicken embryos: The dependence of the concentration decrease of vesicular stomatitis virus ΔD expressed in plaque-forming units (PFU) on the polynucleotide complex concentration in μg/ml. The content of 5-Br-cytosine in poly(C) was 0% (curve 1), 33% (curve 2), 67% (curve 3), and 100% (curve 4).

chlorinated poly(C). The verification of the latter assumption was subject to further investigation.

3.3 Antiviral activity of the complexes of poly(G) with halogenated poly(C)

Figures 5 and 6 show the results of the investigation of antiviral activity of the complexes of poly(G) with halogenated poly(C) samples used in physical and physico-chemical studies. A decrease of antiviral activity of the polynucleotide complex in the cell culture of chicken embryos with the growing content of 5-halogen-cytosine in poly(C) was observed. However, the complexes containing chlorinated poly(C) were somewhat more active than the complexes containing brominated poly (C). A 10% content of 5-Cl-cytosine in poly(C) had only little influence on the activity of the complex (Fig. 5, curve 2). The complexes of poly(G) with completely brominated poly(C) were inactive (Fig. 6, curve 4), while an activity was exhibited by the complex of poly(G) with completely chlorinated poly(C) under analogous conditions (Fig. 5, curve 4).

Our observation that the complex of poly(G) with fully brominated poly(C) almost completely lost its antiviral activity even though its significant part (ca. 60%) remained in the ordered conformation deserves further explanation. We propose that the extensive disturbance of the regular secondary structure induced by the complete bromination of poly(C) does not apparently comply with the conformational demands made on polyribonucleotide complexes with a significant antiviral activity. A rough estimate of the extent of distortions in the conformation of poly(G) · poly(C) which results in a complete loss of its antiviral activity is ca. 40%.

4. Conclusions

Chlorination and bromination at C(5) of cytosine in poly(C) do not improve antiviral activity of double-stranded poly(G) · poly(C) studied in cell culture. Moreover, physical and physico-chemical investigations reveal that the regular double-helical conformation of the poly(G) · poly(C) complex is disturbed as a result of the halogenation of poly(C). Hence, the results of this work support the view that the regular and non-disturbed secondary structure of polyribonucleotide complexes is an important factor determining their biological activity. Differential pulse polarographic analysis of polynucleotide complexes appears to be a very sensitive and simple assay for testing the quality of these RNAs from the point of view of their interferon inducing or antiviral activity.

References

1 E. De Clercq, D. Hermann and W. Guschlbauer, Biochim. Biophys. Acta 741 (1983) 358.
2 A.S. Novokhatsky, F.I. Ershov, A.L. Timkovsky, S.E. Bresler, E.M. Kogan and N.S. Tikhomirova-Sidorova, Acta Virol. 19 (1975) 121.
3 E. De Clercq, P.F. Torrence, B.D. Stollar, J. Hobbs, T. Fukui, N. Kakiuchi and M. Ikehara, Eur. J. Biochem. 88 (1978) 341.
4 J.J. Greene, J.L. Alderfer, I. Tazawa, S. Tazawa, P.O.P. Ts'o, J.A. O'Malley and W.A. Carter, Biochemistry 17 (1978) 4214.
5 E. De Clercq, G.-F. Huang, B. Bhooshan, G. Ledley and P.F. Torrence, Nucleic Acids Res. 7 (1979) 2003.
6 E. De Clercq, B.D. Stollar, J. Hobbs, T. Fukui, N. Kakiuchi and M. Ikehara, Eur. J. Biochem. 107 (1980) 279.

7 N. Kakiuchi, C. Marck, N. Rousseau, M. Leng, E. De Clercq and W. Guschlbauer, J. Biol. Chem. 257 (1982) 1924.
8 V. Brabec and A.L. Timkovsky, Gen. Physiol. Biophys. 2 (1983) 487.
9 K. Reuss, K.-H. Scheit and O. Saiko, Nucleic Acid Res. 3 (1976) 2861.
10 P.F. Torrence, E. De Clercq, J.A. Waters and B. Witkop, Mol. Pharmacol. 13 (1974) 4400.
11 L.M. Vilner, G.A. Platonova, E.M. Kogan, N.S. Sidorova and A.L. Timkovsky, Vopr. Virusol. 30 (1985) 337 (in Russian).
12 L.M. Vilner, E.M. Kogan, N.G. Naumovich, N.S. Sidorova, E.A. Glazunov and A.L. Timkovsky, Vopr. Virusol. 33 (1988) 331 (in Russian).
13 A.L. Timkovsky, O.A. Aksenov, S.E. Bresler, A.A. Smorodintzev, N.S. Tikhomirova-Sidorova and E.M. Kogan, Vopr. Virusol. 18 (1973) 350 (in Russian).
14 G. Felsenfeld, D.R. Davies and A. Rich, J. Am. Chem. Soc. 79 (1957) 2023.
15 G. Felsenfeld and A. Rich, Biochim. Biophys. Acta 26 (1957) 457.
16 D. Thiele and W. Guschlbauer, Biopolymers 10 (1971) 143.
17 B. Janik and E. Palecek, Arch. Biochem. Biophys. 105 (1964) 225.
18 E. Palecek, J. Electroanal. Chem. 22 (1969) 347.
19 V. Brabec, J. Electroanal. Chem. 50 (1974) 235.
20 Z. Balcarová and V. Brabec, Biophys. Chem. 33 (1989) 55.
21 M.D. Topal and J.R. Fresco, Biochemistry 19 (1980) 5531.
22 C.C. Hardin, D.A. Zarling, J.D. Puglisi, M.O. Trulson, P.W. Davis and I. Tinoco, Jr., Biochemistry 26 (1987) 5191.