Volume 5 Number 6 June 1978 Nucleic Acids Research

Protonated polynucleotides structures - 23. The acid-base hysteresis of poly(dG).poly(dC)

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Received 24 April 1978

ABSTRACT

The large hysteresis observed during the acid-base titration of poly(dG).poly(dC) was studied by CD and potentiometric scanning curves. Intermediate scanning loops as well as the equilibrium and metastable branches of the hysteresis loop have been determined. The potentiometric titrations showed, however, that the various complexes were not discrete entities, but were linked in "polycomplexes" as had been already suggested. This prevented a thermodynamic study of the system. The acid-base titration was further investigated as a function of ionic strength and temperature. The pK's showed considerably lower ionic strength dependence than observed for polyribonucleotide complexes. The thermal transitions permitted to establish the relative stabilities of the various complexes between pH 2.5 and pH 12.0.

INTRODUCTION

Hysteresis phenomena have been observed in biological macromolecules such as proteins and nucleic acids and interpreted as physical mechanisms for the storage and imprint of environmental information[1]. The acid-base titration of ribosomal RNA[2], DNA[3] and poly(U)•poly(A)•poly(U)[4] led to hysteresis cycles; the last of these systems had been most extensively studied. In previous work we have shown[5,6] that two poly(purine)•poly(pyrimidine) complexes exhibited hysteresis cycles upon acid-base titration: poly(dAG)•poly(dCT) and poly(dG)•poly(dC). The overall process taking place along the acid-base titration of poly(dG)•poly(dC) was apparently simple: upon protonation, the system was going from a stable state (associated complexes) to another stable one (homopolymer mixture, although the total dissociation of the complexes could not be reached). On the backward titration, the homopolymer mixture remained metastable up to pH 7.3 and then returned to the associated complexes; the overall mechanism seemed therefore to be similar to that of poly(U)•poly(A)•poly(U)[4]. The hysteresis of poly(dG)•poly(dC) is complicated by the formation of an acid complex at the beginning of the acid titration[7]. Furthermore, we have shown that most samples of poly(dG)•poly(dC) contained two hetero-complexes, poly(dG)•poly(dC) and poly(dG)•poly(dG)•poly(dC)[8]. The distribution of these

two complexes depended on the past pH conditions. Despite these difficulties, we have attempted to study some features of the poly(dG)•poly(dC) hysteresis cycle and some properties of the different complexes as a function of ionic strength, pH and temperature. We report in this paper results on CD and potentiometric scanning curves, ionic strength dependence of the shape of the hysteresis loop and of the pK values, as well as thermal denaturation of the complexes.

MATERIAL AND METHODS

Poly(dG)•poly(dC) was purchased from Miles laboratories, Elkhart, IN, USA and from Boehringer, Mannheim, GFR. Magnesium determinations were performed with a 290B Perkin Elmer atomic absorption spectrometer and were never found higher than 1 Mg^{++}/ 100 phosphates. The polynucleotide concentrations were determined spectrophotometrically (Perkin Elmer 356), using ε_{254}=7400 for poly(dG)•poly(dC). All solutions were about 0.1 millimolar. CD measurements were performed as previously described[8]. at 22°C.

Potentiometric acid-base titration were performed under nitrogen atmosphere. In order to increase the amount of double stranded poly(dG)•poly(dC) the solutions were first brought to pH 11.5 and back to pH 9.0[8]. The solutions were then dialysed against 0.15M NaCl at pH 9 for 24 hrs under nitrogen atmosphere. Acid-base titration was performed using an ISIS 4000 Tacussel pH meter at 22° on 5 ml polynucleotide solutions by addition of 0.01 M HCL or 0.01 M NaOH, respectively. An equal volume of dialysate was titrated in the same way. At the end of the titration the amount of HCl or NaOH added was around 1 ml; the polynucleotide solutions had used 150 to 200 µl more than the blank. Taking into account pipetting errors, the relative error can be estimated to be about 15%. This gives a maximal protonation of 0.30 ±0.05 H^+/P at pH 3.2.

Thermal denaturations were performed on a Roussel-Jouan Dichrographe II. The CD signal was recorded either at 275 nm or at 255 nm where the changes were maximal. The temperature was measured directly in the cell by a platinum thermistor and recorded on a calibrated MECI (Leeds-Northrup) recorder.

T_m measurements at alkaline pH: The polynucleotides were dissolved in 0.15 M NaCl, 0.001 M Tris pH 8. From pH 10 to pH 12, pH values were determined with a high alkalinity electrode. pH values were greatly affected when the temperature was raised to 90°. On fig. 9 the reported pH values were measured at 20° C. The aim of this experiment was to determine whether poly(dG)•poly(dC) or poly(dG)•poly(dG)•poly(dC) melted at lower temperature at neutral pH. To obtain a very accurate curve of T_m values as a function of pH, it would have

been necessary to work with a buffer the pH of which is less affected at high temperature. The conclusions are, however, not affected by the pH changes during heating.

Tm measurements at acid pH: Polymers were dissolved in sodium cacodylate buffer and melting curves performed as described above.

RESULTS AND DISCUSSION

pH and pK values referring to the acid titration are designated pH_a and pK_a, those of the basic titration pH_b and pK_b, respectively.

CD titration curves. Figure 1 shows the CD scanning curves obtained during the acid-base titration of a poly(dG)•poly(dC) sample in 0.15 M Na^+. Five different cycles were performed: all cycles started and terminated at pH 10; their extreme pH's were 5.0, 4.7, 4.2, 3.0 and 2.4, respectively. The scanning curves are presented at three wavelengths, 260, 265 and 295 nm. At all wavelengths, the intermediate scanning curves fall within the greater pH loop. This is characteristic of hysteresis phenomena and was observed at all wavelengths. The main basic pK_b = 7.3 appears clearly at the three different wavelengths in all cycles. Only at 295 nm an intermediate plateau around pH_a 4 can be observed during the acid titration. If we turn to the vectorial presentation which takes into account the whole spectrum[9], a plateau is clearly visible between pH_a 5 and pH_a 4. The two acid steps have pK_a=5.25 and pK_a about 3; the latter pK cannot be given with better precision, because the titration does not appear to be complete at pH 2.4 in 0.15 M NaCl[7]. The reversibility of the titration at pH_b 10 was quite good at 265 and 295 nm, but poor at 260 nm; it was clearly very poor when the whole spectrum was taken into account. We have shown that the irreversibility of poly(dG)•poly(dC) CD spectrum after acid-base titration was due to a change in the distribution of the two heterocomplexes and homopolymers[8]. The possibility of acid degradation of poly(dG) can be disregarded as an additional source of the irreversibility: the CD spectrum of a poly(dG) sample in 0.15 M NaCl showed no significant variation upon return to pH 8.3 after acid exposure at pH 2.5.

In order to observe the hysteresis phenomenon without interference of the variable equilibrium between the double stranded and triple stranded complexes, a wavelength around 290 nm was retained at which only the protonated forms show an important contribution. Figure 2 shows the CD spectrum of the two neutral heterocomplexes and CD spectra of a poly(dG)•poly(dC) sample at pH_a=2.5 and pH_b=6.1. Between 290 and 300 nm the contribution of the neutral complexes is very weak compared to that of the acid species.

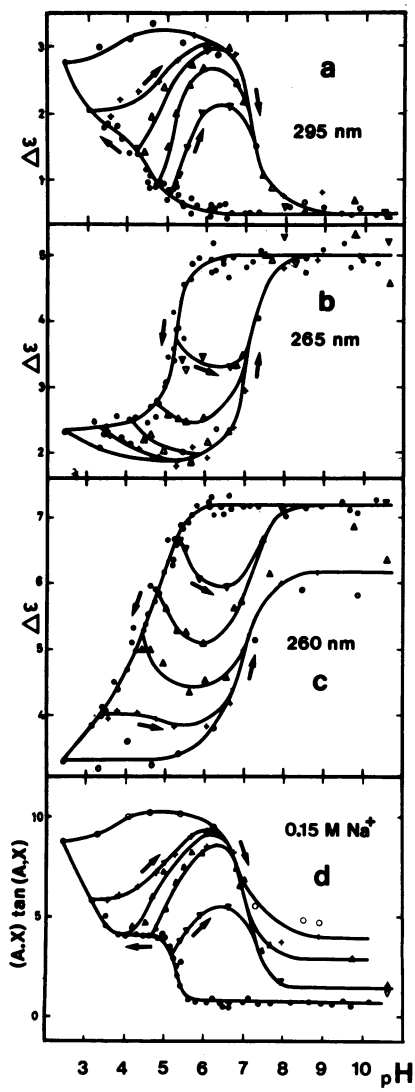

Figure 1: Acid-base titration of a poly(dG)·poly(dC) sample at three different wavelengths: (a) 295 nm, (b)265 nm, (c) 260 nm. (d) is a representation which takes into account the whole spectrum[9]. Five titration loops were performed which stopped at different pH values (see below).
—●— acid titration,
—▽— first alkaline titration from pH 5.0 to neutrality,
—▲— second alkaline titration from pH 4.7 to neutrality,
—△— third alkaline titration from pH 4.2 to neutrality,
—+— fourth alkaline titration from pH 3.0 to neutrality,
—○— fifth alkaline titration from pH 2.4 to neutrality.
(ionic strength 0.15 M NaCl).

In this wavelength range perfectly reproducible and reversible loops were obtained.

Some properties of the hysteresis loops: Figure 3 shows a schematic presentation of a hysteresis loop for the transition between a state B and a protonated state A. In the reversible case the protonation takes place via pK_{eq} from branch (1) to branch (3). If the energy barrier between state B and state A is too high, the system remains on branch (2) at a $pH < pK_{eq}$ in a metastable state down to the pK_{BA}. Similarly, during alkaline titration along branch (3) it can remain metastable, continuing on branch (4) up to the transition point pK_{AB}. Hysteresis phenomena can consist of only one metastable branch and the

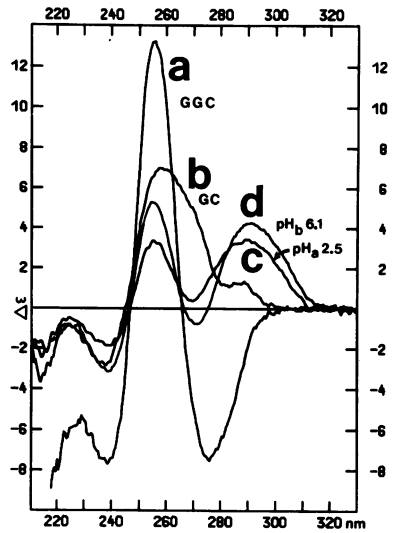

Figure 2: Estimated CD spectra (a) of triple stranded complex poly(dG)•poly(dG)•poly(dC), (b) of double stranded complex poly(dG)•poly(dC) (from ref. 8) and experimental CD spectra of a poly(dG)•poly(dC) sample (45% poly(dG), 55% poly(dC)) (c) at pH_a=2.5, (d) at pH_b=6.1.

equilibrium branch (5); in such cases only one of the states has a large energy barrier to overcome and the hysteresis loop is formed either by surface (a) or (b).

We have first determined the critical pH_c, i.e. the pH above which the acid titration was reversible and below which hysteresis was observed. When poly(dG)•poly(dC) was titrated to pH 6.8 and returned to pH 9, no hysteresis took place. It was necessary to decrease the pH value to 6.35 to observe a small hysteresis cycle upon backward titration. We then examined to what extent the different mixtures present along the whole hysteresis cycle (pH 9⟶ pH 2.5⟶pH 9) were stable or metastable. Heating of a metastable mixture will bring the system to the stable state and maintain it after cooling, while a stable mixture will remain the same after heating and cooling. We where thus able to show that between pH_a 9.0 and pH_a 5.0 the system was stable; from pH_a 5.0 to pH 3.0 the mixture was metastable. Upon back titration, we have observed that between pH_b 2.5 and pH_b 5.5 the system was stable; on the contrary, from pH_b 5.5 to pH 7.0 the system became metastable; in this pH range the mixture contained mainly poly(dC^+)•poly(dC) and poly(dG): heating below pH_b5.5 gave the same spectrum after heating and cooling, while above pH_b 6.0 heating led to a stable mixture of poly(dG)•poly(dG)•poly(dC) and poly(dG)•poly(dC)[8]. It seems therefore that in the poly(dG)•poly(dC) system the two extreme pK's observed (pK_a<2.7 and pK_b=7.3) are the two metastability pK values.

Potentiometric titrations. Figure 4 shows scanning curves obtained upon acid-base titrations of poly(dG)•poly(dC). Acid titration was stopped at pH_a 5.0,

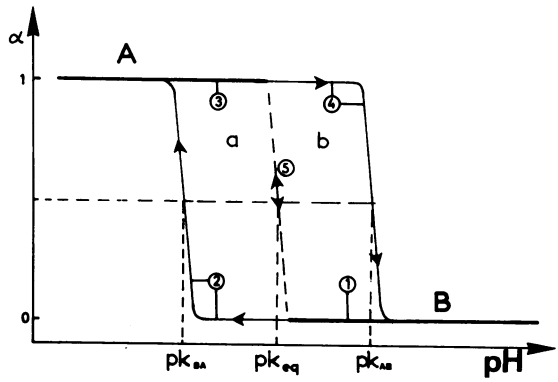

Figure 3 : Schematic presentation of a hysteresis loop. (1) and (3) are stable branches, (2) and (4) are metastable branches. If no hysteresis (metastability) is observed, the system will reversibly titrate at pK_{eq} (branch(5)). For details see text.

3.5 and 3.2, respectively. From pH_a 9.5 to pH_a 6.0 a very slow proton uptake was observed and up to 10 ± 5 % of the total bases were protonated. Between pH_a 6.0 and pH_a 3.2 the proton uptake became steeper. At pH_a 3.2, i.e. at the beginning of the second transition observed in the CD titration curves (fig.1) 35 ± 5% of the bases were protonated. The estimation from the decomposition of CD spectra using the computed spectra of poly(dG)•poly(dG)•poly(dC) and poly(dG)•poly(dC)[8] yielded at pH_a 3.0 11% poly(dC^+)•poly(dC), 49% poly(dG^+)•poly(dC) and some neutral complexes for this sample which contained 45% poly(dC) and 55% poly(dG). This corresponds to a protonation of about 30% of the total bases in agreement with the uptake of protons between pH 6 and pH 3.

Upon back titration three important features were observed:

- The proton release was delayed compared to the proton uptake, giving rise to hysteresis loops.
- Back titration did not show any particular transition at the poly(dC^+)•poly(dC) pK=7.3 as was observed on the CD titrations. This could be due to the fact that a minimum amount of bases had to be deprotonated before the poly(dC^+)•poly(dC) was cooperatively dissociated; apparently single stranded poly(dC) remained partially protonated.
- The proton release was not terminated at pH 9, particularly in the case of the third cycle: 10± 5% of the total amount of bases was still protonated at this pH.

This unusual behaviour of poly(dG)•poly(dC) upon potentiometric titration could be due to the existence of "polycomplexes", some parts of which could be protected against protonation and deprotonation. This led, however, to

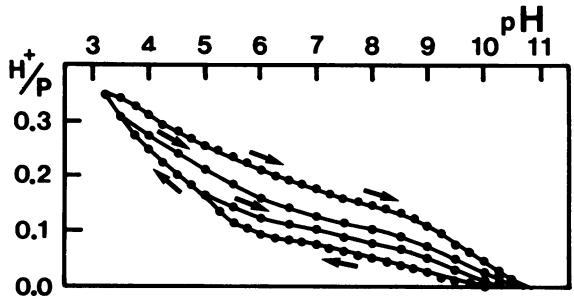

Figure 4: Potentiometric titration curves of poly(dG)•poly(dC in 0.15 M NaCl.

the unfortunate conclusion that no numeric data can be obtained from these results and that it is impossible to undertake a thermodynamic study of this hysteresis system.

Influence of ionic strength on the acid-base titration of poly(dG)•poly(dC)

Figure 5 shows the acid-base titration of poly(dG)•poly(dC) in four different ionic strength conditions: 0.03, 0.06, 0.6 and 1.0 M NaCl. In 0.03 and 0.06 M NaCl the acid titration showed two distinct steps, which both went to completion. In 0.15 M NaCl the plateau was clearly distinguishable only if the whole spectrum was analyzed (fig. 1) and the lower transition was not terminated at pH 2.4. The back transition showed two steps for all these three ionic strengths.

At higher salt concentrations, 0.6 and 1.0 M NaCl, only one acid transition was observed and it was not possible to decrease the pH below 3, because the polymers precipitated before the second step began. Here again the back titration showed two steps.

The apparent pK_a and pK_b values obtained from these titration experiments are presented in figure 6, together with those of poly(dC^+)•poly(dC). When the pH had not been decreased below pH 5, this polymer complex exhibited only a very small hysteresis loop of 0.5 pH units and very cooperative transitions allowing a precise determination of the pK values. The comparison of the pK_b as a function of ionic strength of poly(dG)•poly(dC) with that of poly(dC) shows quite clearly that the basic branch of the hysteresis cycle involves the deprotonation of the acid selfstructure of poly(dC^+)•poly(dC). The slopes are slightly different, probably due to the presence of poly(dG) which enters in the formation of poly(dG)•poly(dC) while poly(dC^+).poly(dC) is deprotonated. A similar phenomenon has been observed in the poly(U)•poly(A)•poly(U) system,[4] where the pK of the transition poly(A^+)•poly(A^+) ⟶ 2 poly(A) is slightly different from the pK of the transition 1/2[poly(A^+)•poly(A^+)] + 2 poly(U)

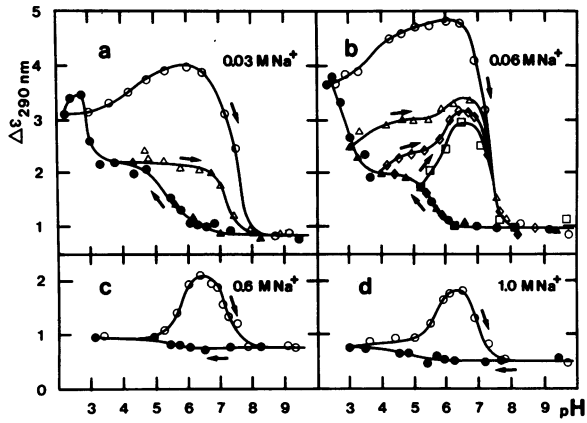

Figure 5: Acid- base titration of the poly(dG)•poly(dC) system at different ionic strengths. (a) 0.03 NaCl: ▲● acid titration, Δ first base titration pH 4.3 → pH 8.8, ○ second base titration pH 2.2 → pH 9.7; (b) 0.06 M NaCl: ■◆▲● acid titration, □ first base titration pH 5.2→ pH 9.7, ◇ second base titration pH 4.0 → pH 8.7, Δ third base titration pH 3.0→pH 9.3, ○ fourth base titration pH 2.3→pH 9.8. (c) 0.6 M NaCl: ● acid titration, ○ base titration. (d) 1.0 M NaCl: ● acid titration, ○ base titration.

——→ poly(U)•poly(A)•poly(U). It should be noted that the variation of the apparent pK with ionic strength is considerably lower than in the case of polyribonucleotide complexes.

Table I summarizes the slopes observed for some synthetic and natural polynucleotides. The variation of the pK values as a function of ionic strength has been studied to a lesser extent than the T_m variations. Polyelectrolyte theory has generally been applied using a cylindrical DNA model[10,11]. The negative charges on its surface give rise to an electric potential varying with the nature and amount of counter ions present in solution. Daune[12] has shown that the proton concentration on the surface of DNA can be twenty times larger than in the surrounding solution at an ionic strength of 0.1 M NaCl. Aronssohn and Travers[13] have used Kotin's[10] approach to study the ionic strength dependence of the pK values of poly(C) and poly(I)•poly(C). Unfortunately, none of these theories can account for the differences in ionic strength dependence of the pKs of polyribonucleotides compared with that of the deoxyseries (Table I).

Thermal denaturation studies:

In order to characterize the different complexes and to determine their respective domains of existence, melting curves in alkaline, neutral and acid pH were performed.

Melting curves in alkaline medium: Although two complexes, poly(dG).poly(dC) and poly(dG)•poly(dG)•poly(dC) are present at neutral pH[8], only one transition

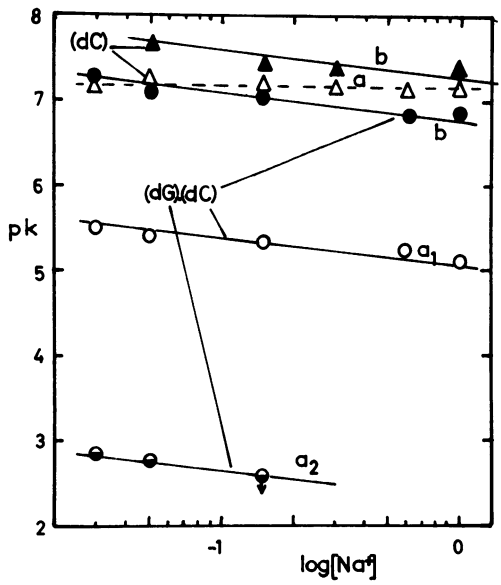

Figure 6: Apparent pK values of the poly(dG)•poly(dC) system and of poly(dC) as a function of ionic strength.
○ a_1: pK values of the first acid transition of the poly(dG)•poly(dC) system;
◒ a_2: pK values of the second acid transition of the poly(dG)•poly(dC) system;
● b: pK values of the second alkaline transition of the poly(dG)•poly(dC) system;
△ a: pK values of the acid transition of poly(dC);
▲ b: pK values of the alkaline transition of poly(dC).

was observed in 0.06 M NaCl between 20°and 95°. We have therefore performed thermal denaturation experiments in the range of the alkaline transitions of the two complexes (pH 10 to 12)[8], to determine which of them melted at pH 8. The sample used was from Boehringer and contained an excess of poly(dG). No preliminary alkali treatment was done. The analysis of the CD spectrum at pH 9 showed that the sample contained 52% poly(dG)•poly(dG)•poly(dC) and 48% poly(dG)•poly(dC)[8]. Such samples undergo two alkaline transitions[8] at pK=11.35 and pK=11.95 (in 0.15 M NaCl), respectively, corresponding to the reactions

poly(dG)•poly(dG).poly(dC) ⟶ poly(dG) + poly(dG).poly(dC) (1)

poly(dG)•poly(dC) ⟶ poly(dG) + poly(dC) (2).

Thermal denaturations were followed at 275 nm and 255 nm. 275 nm corresponds to the first negative peak of the triple stranded complex[8], while 255 nm is the positive maximum of the spectra of both complexes (see fig. 2).

Between pH 11.95 and pH 11.35 a single transition was observed which corresponds to reaction (2) (fig. 9). Between pH 11.35 and pH 10.8 two transitions were found (figs. 7 & 9). Analysis of the spectra recorded at the plateaus indicated that above pH 10.8, the first transition was due to the dissociation of the triple stranded complex (reaction (1)), followed by the melting of the double stranded complex (reaction (2)). At pH 10.8 only one melting step was observed, both at 275 nm and 255 nm. Below pH 10.8, two transitions were again found. The shapes of the melting curves at 275 nm were opposite in shape to those found above pH 10.8 (fig. 7). In this case, the

TABLE I: Slopes of apparent pK's *versus* logarithm of ionic strength for different reactions taking place during acid or acid-base titration of some polyribo- and polydeoxyribonucleotides. This slope is related to Kotin's[10] constant c_2 (2.3 times slope = c_2).

Polymer system	Reaction	Slope	Ref.
poly(rC)	$2\,(rC) \rightleftharpoons (rC^+)\cdot(rC)$	0.46	13,14
poly(rG)	$(rG)_n \rightleftharpoons (rG^+)$	0.60	16
poly(rA)	$2\,(rA) \rightleftharpoons (rA^+)\cdot(rA^+)$	0.46	17
poly(rG)·poly(rC)	$2\,[(rG)\cdot(rC)] \longrightarrow (rC^+)\cdot(rG)\cdot(rC) + (rG)$	0.75	16
poly(rI)·poly(rC)	$2\,[(rI)\cdot(rC)] \longrightarrow (rC^+)\cdot(rI)\cdot(rC) + (rI)$	0.75	13,15
	$(rC^+)\cdot(rI)\cdot(rC) + (rI) \longrightarrow 2\,[(rC^+)\cdot(rI)]$	0.75	15
poly(dC)	$2\,(dC) \longrightarrow (dC^+)\cdot(dC)$	0.0	fig.6
	$(dC^+)\cdot(dC) \longrightarrow 2\,(dC)$	0.2	fig.6
poly(dG)·poly(dC)	pK_{a1}	0.25	fig.6
	pK_{a2}	0.25	fig.6
	pK_b	0.25	fig.6
poly(dI)·poly(dC)	$2\,[(dI)\cdot(dC)] \longrightarrow 2\,(dI) + (dC^+)\cdot(dC)$	0.55	a
	$2\,(dI) + (dC^+)\cdot(dC) \longrightarrow 2\,[(dI)\cdot(dC)]$	0.30	a
DNA *E. coli*	strand separation by protonation	0.65	a

a: unpublished results from our laboratory.

increase of the negative CD signal at 275 nm indicated that the first transition was due to an increase of the concentration of triple stranded complex. Spectra recorded at the plateaus of the melting curves are shown in figure 8 and distributions of concentrations of the various complexes in Table II.

These results summarized in figure 9, lead to the following conclusions: curve (1) corresponds to the dissociation of triple stranded complex, either partial, leading to the double stranded complex (branch (1a)), or complete, leading to a mixture of the homopolymers (branch (1b)), depending on the pH. The reaction along curve (2) is more complex: along branch (2a) double stranded complex dissociates into free homopolymers, while along branch (2b) disproportionation occurs according to

$$2\,[\text{poly(dG)}\cdot\text{poly(dC)}] \longrightarrow \text{poly(dC)} + \text{poly(dG)}\cdot\text{poly(dG)}\cdot\text{poly(dC)} \quad (3).$$

In this pH range large changes of the pH take place between 20° and 90°; this, however, does not affect the main result of this study: around neutrality, the triple stranded complex is more stable than the double stranded complex.

The complexity of the thermally induced disproportionation of poly(dG)·

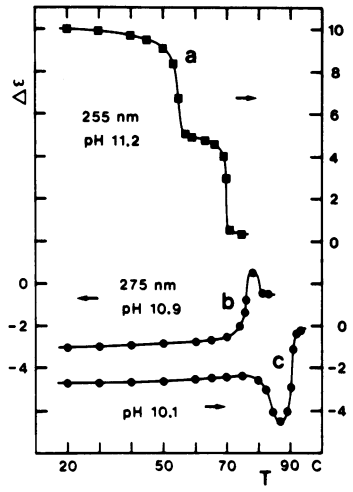

Figure 7: Melting curves of poly(G) poly(dC) at different alkaline pH a 255 and 275 nm. pH values were measured at 20°C.
(a) Melting curve at pH 11.2 at 255 nm. T_{m1}=55°, T_{m2}=70°;
(b) melting curve at pH 10.9 at 275 nm. T_{m1}=74°, T_{m2}=80°;
(c) melting curve at pH 10.1 at 275 nm. T_{m1}=85°, T_{m2}= 90°.

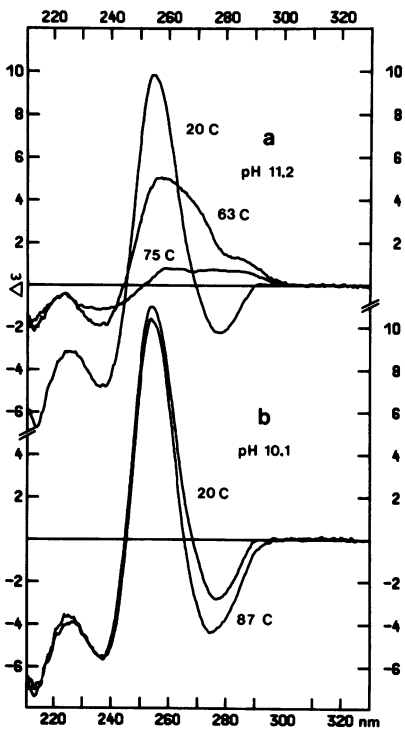

Figure 8: CD spectra of a poly(dG)·poly(dC) sample containing 52% poly(dG)·poly(dG)·poly(dC) at alkaline pH at different temperatures. ▲ △:T_m, ■□:pK.
(a) pH 11.2 at 20°, 63° and 75°.
(b) pH 10.1 at 20° and 87°.

poly(dC) samples was further illustrated by the following experiment: two aliquots of native poly(dG)·poly(dC) were adjusted to pH 10.5 and heated to 100° for 30 min. One of the aliquots was chilled in an ice-water bath and the other was allowed to cool slowly for 6 hrs. The quickly cooled sample exhibited a slight trough around 270 nm in its CD spectrum, characteristic of the pre-

TABLE II: Distribution of polymercomplexes and homopolymers at different pH values and temperatures (in percent).

complex	pH 10.1		pH 11.2	
	20°C	87°C	20°C	63°C
(C)	2	6	2	11
(G)•(C)	48	6	54	82
(G)•(G)•(C)	50	78	44	0
(G)	0	10	0	7

sence of triple stranded complex. Computation of the concentrations of the different complexes present by least square fit[8] revealed the absence of triple stranded complex in the slowly cooled sample. One week later, the CD spectra of both samples were unchanged. These results are similar to those of Wells et.al.[18] on poly(dAG)•poly(dCT); these authors had observed that quick cooling increased the buoyant density of the polymer compared to slow cooling. The higher density obtained after quick cooling was interpreted as being due to the formation of a triple stranded complex poly(dTC$^+$)•poly(dAG)•poly(dTC).

Melting curves during acid-base titration: T_m values were obtained during acid titration (T_{ma}) and during base titration after acid exposure to a given pH (T_{mb}). The results obtained at two ionic strengths (0.06 and 0.15 M NaCl) are summarized in fig. 10.

Study of T_{ma}: The values obtained for the T_{ma}'s fall on two curves, the slopes of which are of opposite sign; they cross at about pH 5.1. These curves delineate four domains: A, B, C and D.

Thermal transitions along curve (1): T_{ma} values of poly(dG)•poly(dC) measured

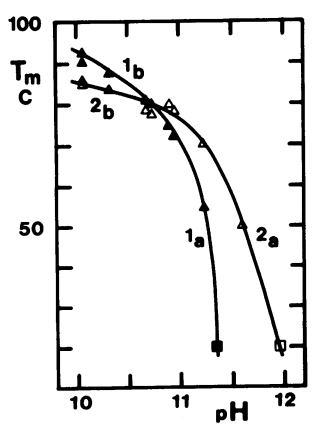

Figure 9: Melting points of a poly(dG)•poly(dC) sample containing 52% poly(dG)•poly(dG)•poly(dC) as a function of pH.
(1) melting of poly(dG)•poly(dG)•poly(dC)
(1a) (G)•(G)•(C) ⟶ (G)•(C) + (G),
(1b) (G)•(G)•(C) ⟶ 2(G) + (C)
(2) melting of poly(dG)•poly(dC)
(2a) (G)•(C) ⟶ (G) + (C)
(2b) 2 (G)•(C) ⟶ (G)•(G)•(C) + (C)

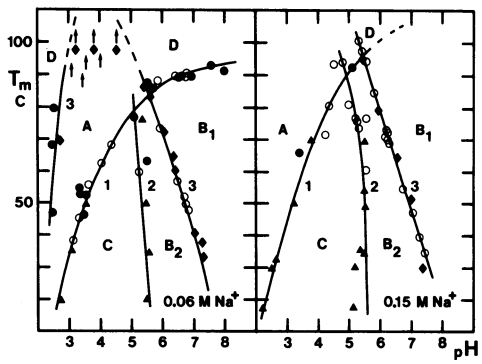

Figure 10: Melting temperatures of the poly(dG)•poly(dC) system as a function of pH at (a) 0.06 M NaCl; (b) 0.15 M NaCl.

● T_m's during acid titration,
○ T_m's during alkaline titration,
▲ pK's at different temperatures,
◆ T_m's of poly(dC),
↑ incomplete melting curves.

between pH 8.5 and 7.0 in 0.06 M NaCl are in good agreement with the values obtained by other authors[18,19]. Below pH 5.1 the transitions obtained in 0.06 and 0.15 M NaCl corresponded also to the melting of poly(dG)•poly(dC); it has been shown that this complex existed down to pH 3[7]. The difference spectra characteristic of the transition that defines curve (1) appeared to be homothetic in the two pH ranges, taking into account the difference between the CD spectra of poly(dC^+)•poly(dC) at pH 6 and at pH 3.5.

Thermal transitions along curve (2): The difficulty of an exact determination of T_m's along curve (2) due to its very steep slope led us to determine pK values at different temperatures (fig. 11). The pK values found at 35°, 50° and 76° C define curve (2) (0.06 M NaCl), which delimits with curve (1) the domain C in the low pH range where poly(dG)•poly(dC), poly(dG).poly(dG). poly(dC) and the acid form are present[7].

Study of T_{mb}: Melting points obtained upon alkaline titration of acid exposed samples lie at any pH value also on curves (1) and (2). A new transition appears, however, the T_m's of which define a third curve (3); this curve (3) delimits the domains B_1 and B_2 in fig. 10, within the domain B. The amplitude of the melting transitions for the T_{mb}'s of curves (1) and (2) are smaller the lower the pH of the acid exposure was. The values obtained for the various T_{mb}'s at different pH values on curve (3) confirm that this transition corresponds to the melting of poly(dC^+)•poly(dC), since the T_m values obtained for the homopolymercomplex fall on the same curve. For samples brought to various acid pH values and retitrated to pH 6 the amplitudes of the transition on curve (3) were proportionally larger the lower the pH of acid exposure was. This confirms that the amount of poly(dC^+)•poly(dC) was increasing along the acid titration due to the dissociation of the complexes. It should be noted that the values obtained for the T_{mb}'s did not depend on the pH of acid expo-

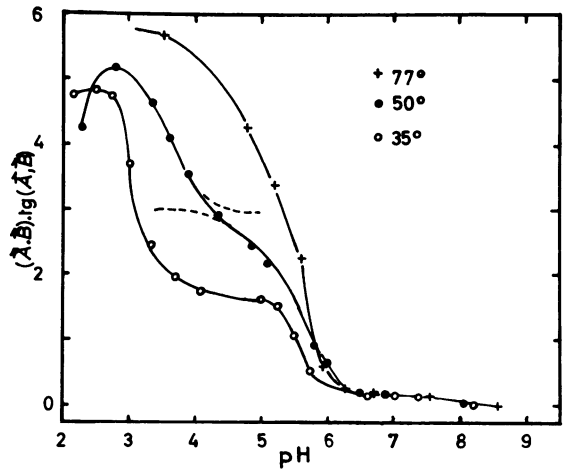

Figure 11: Acid titration of the poly(dG)•poly(dC) system in 0.06 M NaCl at (o) 35°, (•) 50° and (+) 77°. Same vectorial presentation as in fig. 1d.

sure, but on the pH at which the thermal denaturation was performed.

We can summarize the melting results by giving the complexes present in each domain:

B_1: poly(dG)•poly(dG)•poly(dC), poly(dG)•poly(dC), poly(dC);

B_2: poly(dG)•poly(dG)•poly(dC), poly(dG)•poly(dC), poly(dC^+)•poly(dC);

C : poly(dG)•poly(dG)•poly(dC), poly(dG)•poly(dC), poly(dC^+)•poly(dC), acid form;

A : poly(dG), poly(dC^+)•poly(dC);

D : poly(dG), poly(dC) or poly(dC^+) precipitated.

Radding et al.[19] had performed melting experiments on poly(dG)•poly(dC) and had obtained results in agreement with our interpretations: at 0.015 M Na^+ a single transition was observed at pH 6.5 with a T_m=82°; at 0.0025 M Na^+ a second, small transition was found at 82°, while the bulk of the melting was observed at 67°. At both ionic strengths, the main cooperative transition was preceeded by the melting of a minor component at 65° and 48°, respectively. Our results suggest that the second small transition observed in 0.0025 M Na^+ at 82° corresponded probably to the melting of poly(dG)•poly(dG)•poly(dC). The T_m of the lower melting component falls on the curve corresponding to the T_m's of poly(dC^+)•poly(dC) (see fig. 6).

CONCLUSION

The study of poly(dG)•poly(dC) has shown that the acid-base titration of this complex gave rise to a large hysteresis loop and that three different

phenomena took place along this loop:

1) A hysteresis phenomenon similar to that observed for the acid-base titration of poly(U)•poly(A)•poly(U)[4].

2) The formation of an acid complex which did not disturb the overall hysteresis phenomenon, since upon neutralization this complex dissociated into free homopolymers.

3) A partially irreversible (or reversible only with very slow kinetics) transition poly(dG)•poly(dG)•poly(dC) ⟷ poly(dG)•poly(dC) + poly(dG)[8]. This reaction perturbed the hysteresis loop and led to the impossibility to undertake a quantitative thermodynamic study. Another problem which prevented such a study consisted in the formation of "polycomplexes" where the different structures are not individualized.

Finally, this study showed the interest of accurate numerical interpretation of CD spectra. The CD spectra of the three heterocomplexes have been computed; the spectrum best estimated was that of poly(dG)•poly(dC) which is in agreement with the one determined by Gray and Bollum[21]. We also have used this computed double stranded poly(dG)•poly(dC) spectrum as input data for the computation of nearest neighbour frequencies of quasi random sequence DNA's[22]: the frequencies of the GpG/CpC first neighbour configuration was always resolved within a 1% error; this provides a further indication that the CD spectrum of poly(dG)•poly(dC) we have computed is correct.

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