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Protonated polynucleotide structures. 16. Thermodynamics of the melting of the acid form of polycytidylic acid

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ABSTRACT

A phase diagram (pH, ionic strength, temperature) for the double helical form of poly(C) is presented. The thermodynamic analysis of these data shows that poly(C) behaves essentially as cytidine, if the electrostatic (ionic strength) contributions and the free energy of double helix formation are considered and taken into account.

INTRODUCTION

Polynucleotide structures which form upon protonation are rather rare. Two such homopolymer structures have been studied extensively: poly(A^+).poly(A^+) [1-3] and the semi-protonated poly(C)·poly(C^+)[4-10]. While the first of the complexes shows only one acid transition, evidence has been presented[4,7] that two protonation processes take place in the case of poly(C). It had been shown[7] that this second protonation of poly(C) is responsible for the bell-shaped T_m vs. pH profile (Fig. 1). It also had been argued[7] that above 80° C "the cytosine residues behave essentially as in the monomer state", a statement which had been contested by Wrobel et al.[10]

RESULTS

Phase diagram of the acid form of poly(C)

All published[7,11,12] and unpublished T_m and titration data on poly(C) accumulated in this laboratory in the last years have been summarized in the three dimensional phase diagram in Fig. 1. It includes some 200 T_m measurements and 50 pK determinations. The vertex at about 80°C is at pH 4.5 in 0.01 M [Na^+] and at pH 3.7 in 1.0 M [Na^+]. Above these pH values thermal denaturation yields neutral poly(C), below protonation takes place during denaturation and poly(C^+) is formed.[7]

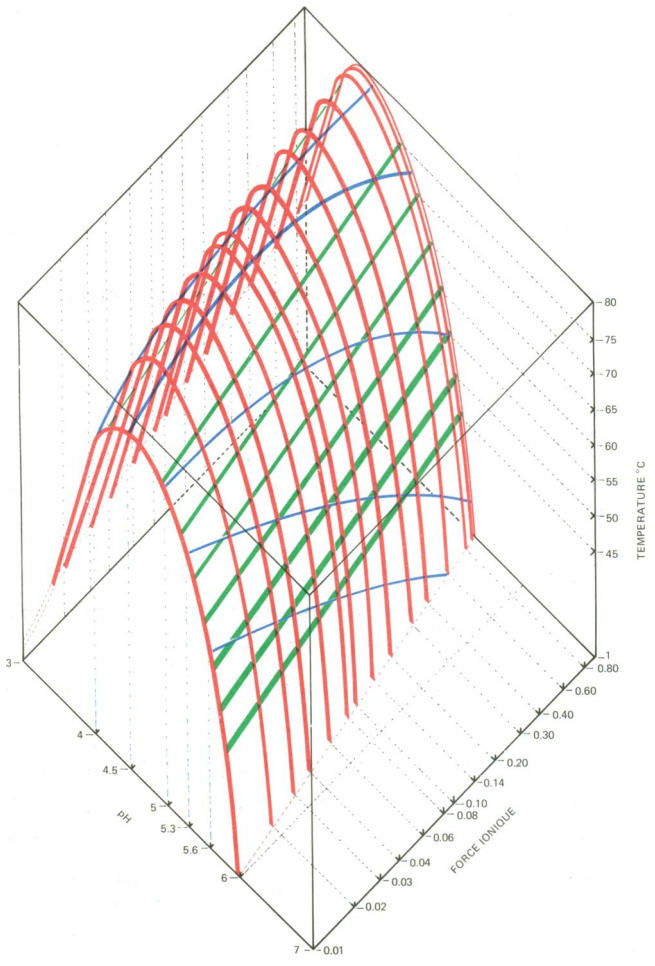

Fig 1: Phase diagram of the acid form of poly(C) as a function of pH, temperature and ionic strength (force ionique). red: isotonic (constant ionic strength), green: isothermic (constant temperature), blue: isoprotonic (constant pH).

Thermodynamics of the thermal dissociation of the acid form of poly(C)

The protonation of poly(C) takes place in two steps [4,7] with the double stranded semi-protonated form as intermediate. The following reactions take place between pH 7 and pH 2.5: On the basic side we have:

$$2\ \text{poly(C)} + H^+ \rightleftharpoons \text{poly(C)}\cdot\text{poly}(C^+) \qquad \text{(B1)}$$

$$K_B = \frac{\left[(C^+\cdot C)_d\right]}{[C_s]^2\,[H^+]} \qquad \text{(B2)}$$

and on the acid side we have:

$$\text{poly(C)}\cdot\text{poly}(C^+) + H^+ \rightleftharpoons 2\ \text{poly}(C^+) \qquad \text{(A1)}$$

$$K_A = \frac{\left[C_s^+\right]^2}{\left[(C^+\cdot C)_d\right]\left[H^+\right]} \qquad \text{(A2)}$$

We also assume an intrinsic ionisation without formation of a double stranded structure

$$\text{poly(C)} + H^+ \rightleftharpoons \text{poly}(C^+) \qquad \text{(C1)}$$

$$K_C = \frac{[C_s^+]}{[C_s]\,[H^+]} \qquad \text{(C2)}$$

and an intrinsic double strand formation without protonation

$$2\ \text{poly(C)} \rightleftharpoons \text{poly(C)}\cdot\text{poly(C)} \qquad \text{(D1)}$$

$$K_D = \frac{\left[(C\cdot C)_d\right]}{\left[C_s\right]^2} \qquad \text{(D2)}$$

In the above equations C_s, C_s^+, $(C\cdot C)_d$ and $(C^+\cdot C)_d$ represent the concentrations of neutral and protonated cytidine in single and double stranded conformation, respectively.

For each of the four equilibria one can write the appropriate free energy changes as follows

$$\Delta F_A = -RT\ln K_A = 2.3\ RT\ pK_A = \Delta H_A - T\Delta S_A \quad \text{etc..}$$

It can easily be seen that

$$K_A \cdot K_B = K_C^2 \quad \text{and} \quad pK_A + pK_B = 2\ pK_C \qquad \text{(C3)}$$

The two steps of the protonation of poly(C) can be described by eq. (B1) and (A1) or , alternatively by an appropriate combination of eq. (C1) and (D1). The total apparent free energy change between the single and double stranded form can be written for the acid and basic branch, respectively, as

$$\Delta F'_A = \Delta F_A + \Delta F_e = 2.3RTpK'_A = \Delta F_C + \Delta F_D + \Delta F_e \qquad \text{(A3)}$$

$$\Delta F'_B = \Delta F_B + \Delta F_e = 2.3RTpK'_B = \Delta F_C - \Delta F_D + \Delta F_e \qquad \text{(B3)}$$

where pK'_A and pK'_B are the apparent (experimental) acid and basic transition pH, ΔF_e is the electrostatic contribution to the total free energy change, ΔF_D the free energy of dissociation(positive

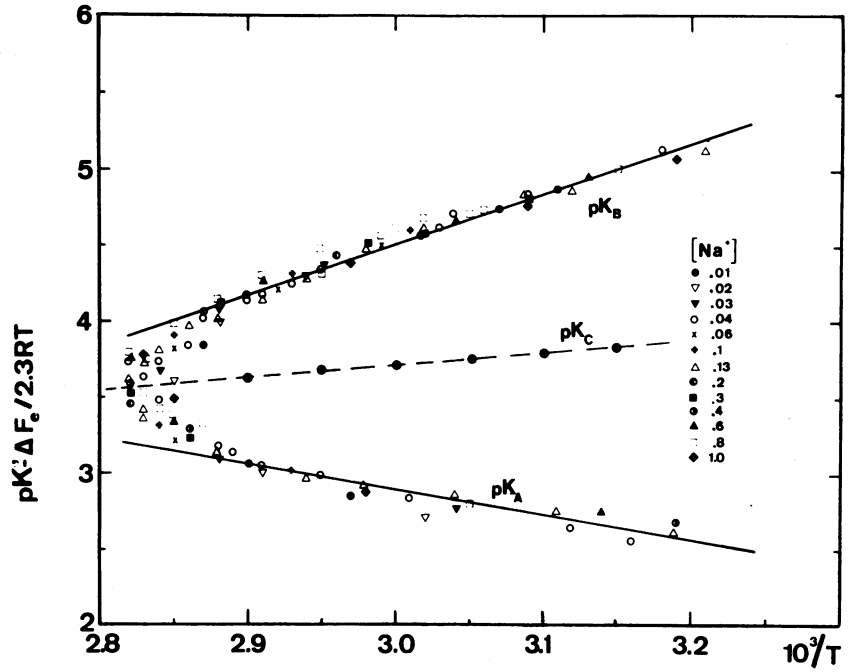

Figure 2: van t'Hoff plot of the reduced pK_A or pK_B (= pK' - ΔF_e/ 2.3·RT) as a function of the inverse of the absolute temperature. ● experimental data for cytidine of Wrobel et al.[10] extrapolated to zero ionic strength.

Table I: Thermodynamic parameters for the protonation of cytidine and cytidine-5'-phosphate compared with those of poly(C)

cytidine			cytidine-5'-phosphate			poly(C)+		
ΔH*	ΔS**	ref.	ΔH*	ΔS**	ref.	ΔH*	ΔS**	eq.
-4.0	5.0	10	-3.64	8.0	10			
-3.75	8.1	15						
-4.83	2.5	14				7.7	36.2	A1
-4.4	5.0	16				-15.5	-25.8	B1
						-3.9	5.2	C1
						11.6	31.0	D1

+ Note that for poly(C) all values except for C1 are per mole base pair.
* in kcal/mole ** in cal/(mole.degree)

on the acid side, negative on the basic side), ΔF_C the intrinsic protonation free energy change. Substraction of ΔF_e gives

$$\Delta F_A = 2.3RTpK'_A - \Delta F_e = 2.3RTpK_A = \Delta F_C + \Delta F_D \quad (A4)$$

$$\Delta F_B = 2.3RTpK'_B - \Delta F_e = 2.3RTpK_B = \Delta F_C - \Delta F_D \quad (B4)$$

ΔF_e can be evaluated from the ionic strength dependence of the experimental pK values according to Kotin[13]

$$\Delta F_e = RT\,(c_1 - c_2 \cdot \log [Na^+]) \quad (D3)$$

where c_2 and $c_1 (= c_2/2)$ are constants; these are found to be c_1=0.525 and c_2=1.05, identical to those obtained for the acid form of poly(A)[3]. A plot of the reduced pK's ($=pK' - \Delta F_e/2.3RT$) against 1/T should give a plot which is independent of the ionic strength. The result in Figure 2 shows the correctness of the choice of variables. From this plot can now be determined the enthalpy and entropy values for the four reactions considered (eq. A1,B1,C1 and D1). The results are summarized in Table I and compared with the literature results on cytidine and 5'CMP. Since one deals with coopérative transitions, the contributions in stacking at the transition point should be small (of the order of scatter in the points in Figure 2).

DISCUSSION

The only complete temperature study (between 10°and 80°C) of pK values of cytidine and its 5'-phosphate and their ionic strength dependence has been published by Wrobel et al.[10]. The agreement between their data (large dots on the broken line in Fig.2) and our computed values of pK_C (broken line in Fig. 2) is striking. From these results it is quite clear that melting and titration of poly(C)·poly(C^+) can be expressed as the sum of three terms: the electrostatic contribution, the dissociation free energy (positive or negative, depending on the branch) and the free energy of protonation of cytidine. In the absence of double strand formation and ionic strength effects (i.e. above the vertex in Figure 1 at temperatures higher than 80°C) poly(C) behaves like cytidine according to equation (C1).

The dissociation enthalpy ΔH_D = 11.6 kcal/(mole base pair) compares favorably with the value determined by Pohl[17] for poly(dG-C). In both cases three hydrogen bonds per base pair are broken. Similarly, the entropy values are comparable. These values are considerably larger than those obtained for

other polynucleotide complexes[18-20,23-25] containing only two hydrogen bonds per base pair. In contrast, Podder[21] determined

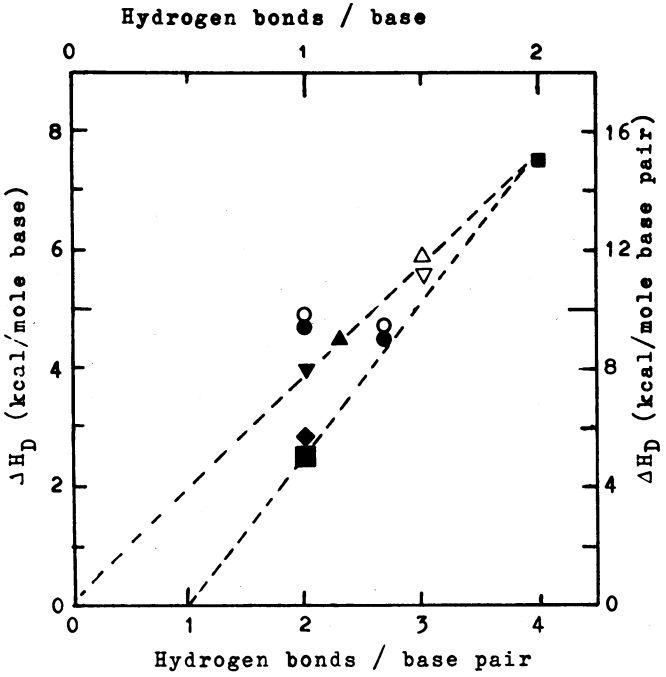

Figure 3: Dissociation enthalpy ΔH_D of various polynucleotide complexes as a function of hydrogen bonds formed.
○● : poly(A).poly(U) and poly(A).poly(U).poly(U) (ref. 18,19);
◆ : poly(I).poly(C) (ref. 20); ▽ : poly(dG-C).poly(dG-C)(17);
▼ : poly(dA-T).poly(dA-T) (ref. 23); ▲ : T_2-DNA (ref. 25);
■ : poly(U).poly(U) (ref. 24); △ : poly(C).poly(C^+) this work;
■ : GMP-gels (ref. 22).
closed symbols: calorimetric data; open symbols: T_m data.
This compilation is not exhaustive.

for the interaction of GpGpGpC with CpCpCpG a ΔH_D = 5.4 kcal/(mole base pair), without taking into account end effects and nucleation. A value of 7 to 8 kcal/(mole base) was found by Chantot[22] for the gel formation of guanylic acids when high polymerisation was observed. In this case two hydrogen bonds are formed for each base in a tetrameric structure which has a total of eight hydrogen bonds. In Figure 3 an attempt is made

to correlate these observations. It appears that the dissociation enthalpy ΔH_D depends on the number of hydrogen bonds formed in the polynucleotide structure. Extrapolations indicate the low stability of structures with only one hydrogen bond per base pair (which have not been observed).

The rather high dissociation enthalpy of poly(C).poly(C^+) suggests that three hydrogen bonds are formed and that the base pairing scheme (with parallel strands and a shared proton forming the third hydrogen bond) generally accepted[4-8] may be correct. Recent viscosity data[9] had been interpreted as a bent-back hairpin structure for poly(C).poly(C^+) which, however, could only form two hydrogen bonds (anti-parallel strands). Kinetic experiments and precise x-ray fibre diffraction data will be needed to definitively clear this point.

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