PROTONATED POLYNUCLEOTIDE STRUCTURES

Thermodynamics of the melting of the acid form of polyadenylic acid

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Thermal denaturation of protonated polynucleotides is accompanied by a change in protonation [1–5]. The thermal transition at a given *p*H can thus be directly related to the *pk'* of the proton-induced structural transition at a given temperature. For poly (A^+).poly (A^+) a strict relationship has been shown to hold over a large range of conditions [4–6]. In fig. 1 the results in the literature and those obtained recently in this laboratory [7] are plotted.

Although the thermodynamics of denaturation of helical polynucleotides has been treated extensively [5,8–13] yielding rather simple expressions, the transitions of protonated polynucleotide structures [5,9,11] showed rather complex behaviour. This is due to the fact that not only the electrostatic contribution of the phosphate-phosphate repulsion has to be considered, but also the ion pair attraction.

In contrast to poly (C^+).poly (C) which can be either deprotonated or protonated upon thermal denaturation depending on the conditions [3] the thermal denaturation of the acid double helical form of poly A is always accompanied by deprotonation. The reaction can be written

$$\text{poly}\,(A^+).\text{poly}\,(A^+) \rightleftarrows 2\,\text{poly}\,(A) + 2\,H^+ \qquad (1)$$

and

$$k' = \frac{[S].[H^+]}{[D]} \qquad (2)$$

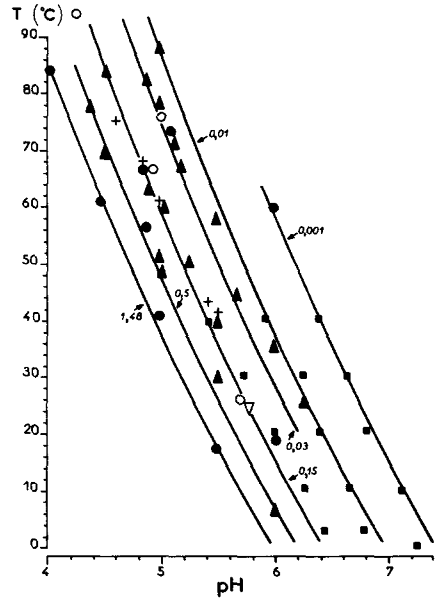

Fig. 1. Plot of T_m against pH_m of the denaturation of the double helical form of poly A at different ionic strengths. The lines are calculated from eq. (10). Data from the literature: X [1]; + [4]; ■ [5]; ▲ [6]; ● [7]; ○ [14]; △ [15].

where [S] is the number of residues in the single stranded form, [D] that of those in double stranded form. At the T_m we have in the case of stoichiometric protonation

$$[A] = [A^+] \qquad (3a)$$

or more generally

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$$[S] = [D] \tag{3b}$$

and

$$pk' = p\mathrm{H_m} \tag{3c}$$

where pH is the pH at which the melting point (T_m) has been determined. Eq. (3a) will not always be true since the two-stranded form can be stable at degrees of protonation as low as $\alpha = 0.25$. (It will generally be $\alpha = 0.5$ at $t > 40°\mathrm{C}$ and $\mu > 0.15$ [5]). In this case one can substitute

$$k' = \frac{(1-\alpha)}{\alpha}[\mathrm{H^+}] \tag{3d}$$

and

$$pk' = p\mathrm{H_m} - \log\frac{(1-\alpha)}{\alpha}\ . \tag{3e}$$

Neglect of the second term will be not too serious, since even at extreme cases ($\alpha = 0.25$) this term will only amount to a value of about 0.4. This neglect will appear in the ΔF_e term (see below) and all results at low temperature and ionic strength, will show slight deviations.

The overall free energy change of the transition can be written as

$$\Delta F' = \Delta F_o + \Delta F_e = \Delta H' - T_m\Delta S' \tag{4}$$

where ΔF_o is the intrinsic free energy change of ionization of the stacked poly (A) at a given temperature in the absence of electrostatic interactions and ΔF_e the change in electrostatic free energy. This latter term will contain the electrostatic free energy change due to phosphate-phosphate repulsion as well as the contribution due to ion pair attraction. The first term can be written as

$$\Delta F_o = -RT\ln k_o = 2.3\,RTpk_o \tag{5}$$

where pk_o is the transition pH in absence of any electrostatic effects, i.e. it should be independent of the ionic strength. Using the treatment of Kotin [8], one can write the second term as

$$\Delta F_e = Ne\psi = RT(c_1 - c_2\log[\mathrm{Na^+}]) \tag{6}$$

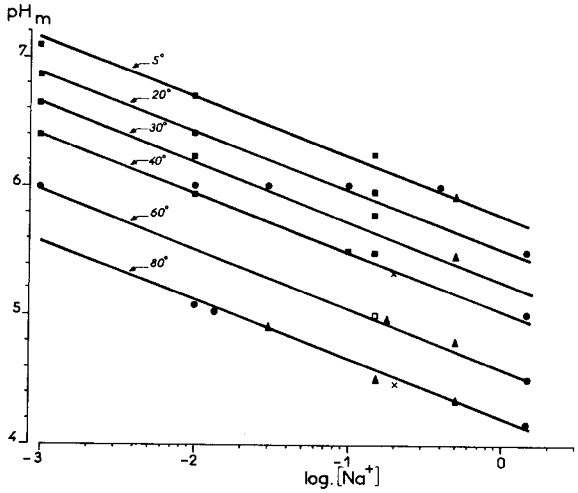

Fig. 2. Plot of $p\mathrm{H_m}$ against ionic strength of the denaturation of the double helical form of poly A at different temperatures. Data from the literature: symbols as in fig. 1.

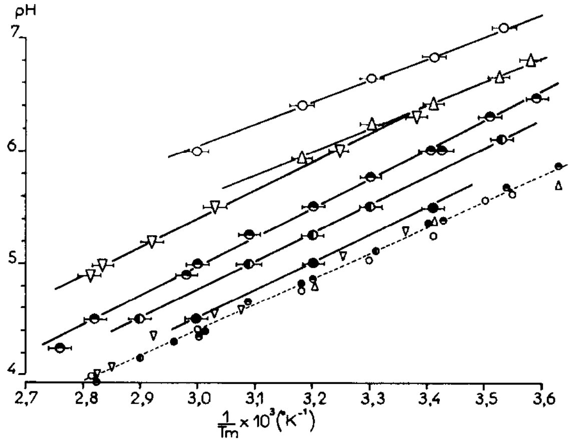

Fig. 3. Van 't Hoff plot of the data in fig. 1 at different ionic strengths (in [$\mathrm{Na^+}$]) : ○–○ : 0.001M, △–△ : 0.01M, ▽–▽ : 0.03M, ⊖–⊖ : 0.15M, ◐–◐: 0.5M, ●–● : 1.48M. Small symbols: same ionic strength as large symbols: intrinsic dissociation constant pk_o versus $1/T_m$ (independent of ionic strength).

Table 1
Thermodynamic parameters of the transition poly (A^+).poly (A^+) $\rightleftarrows$ 2 poly (A) at 20°C (assumed $\alpha = 0.5$).

μ	$\Delta H'$ (kcal/mole)	$\Delta S'$ (e.u.)	$\Delta F'$ (kcal/mole)	ΔF_e (kcal/mole)	ΔF_o (kcal/mole)	pk_o	ΔS_e (e.u.)
0.001	9.0	− 0.5	9.2	2.16	7.0	5.25	−11.5
0.01	9.6	3.5	8.6	1.54	7.1	5.3	− 7.5
0.03	11.15	9.0	8.5	1.26	7.2	5.4	− 2.0
0.15	11.15	10.9	8.0	0.81	7.2	5.4	− 0.1
0.5	11.15	12.0	7.7	0.49	7.2	5.4	+ 1.0
1.48	11.15	13.2	7.3	0.20	7.1	5.3	+ 2.2

$\Delta H_o = 10.3 \pm 0.3$ kcal/mole.
$\Delta S_o = 11.0 \pm 0.5$ e.u.

where c_1 and c_2 are constants. Kotin [8] computed values of $c_1 = 0.95$ and $c_2 = 1.9$ from polyelectrolyte theory for a cylinder of 20 Å diameter and an average linear charge density of 3.4 e/Å and found $c_1 = 0.3$ and $c_2 = 0.6$ for DNA. Clauwaert and Stockx [9] determined $c_1 = 0.73$ and $c_2 = 1.7$ for poly(A). poly(U).

In order to determine these constants one can write eq. (4)

$$\Delta F_e = \Delta F' - \Delta F_o \tag{7}$$

$$RT\,(c_1 - c_2 \log\,[\mathrm{Na^+}]) = 2.3\,RT\,pk' - 2.3\,RT\,pk_o \tag{8}$$

or

$$2.3\,\Delta pk = c_1 - c_2 \log\,[\mathrm{Na^+}]. \tag{9}$$

From a plot of pk_m against ionic strength (fig. 2) these constants can now be determined using Kotin's assumption that $c_1/c_2 = 0.5$. One thus obtains $c_1 = 0.525$ and $c_2 = 1.05$. All thermodynamic parameters of the overall free energy change can now be determined from a Van t'Hoff plot (fig. 3).

The results obtained are summarized in table 1 and indicate the strong influence of ionic strength and the concomitant electrostatic contribution which disappears at high ionic strength. The values for ΔF_e compare reasonably well with those computed by Holcomb and Timasheff [5] from potentiometric titration curves. After deduction of ΔF_e a value of ΔF_o is obtained which is independent of the ionic strength. An intrinsic dissociation constant $pk_o = 5.3 \pm 0.1$ at 20°C can be computed from this. This dissociation constant which is temperature-dependent but independent of ionic strength (fig. 3) corresponds to the protonation of partially stacked and thus structured poly A and is therefore not equal to the pk of the monomer AMP.

The charge-independent (intrinsic) enthalpy change can be computed from ΔF_o and is found $\Delta H_o = 10.3 \pm 0.2$ kcal/mole with a corresponding intrinsic entropy contribution $\Delta S_o = 11.0 \pm 0.5$ e.u.; deducing the latter from the corresponding values of $\Delta S'$ one obtains the values for the electrostatic entropy term. They range from $\Delta S_e = -11.5$ e.u. (in 0.001 M $\mathrm{Na^+}$) to +2.2 e.u. (in 1.48 M $\mathrm{Na^+}$) (table 1).

Using the values of ΔH_o and ΔS_o obtained the T_m values at any pH and ionic strength can be computed.

$$T_m = \frac{\Delta H_o}{2.3\,R\,pk + \Delta S_o - R(c_1 - c_2\,log\,[\mathrm{Na^+}])}\,. \tag{10}$$

The slightly curved lines in fig. 1 show excellent agreement with the data in the literature and indicate that the thermodynamic values and Kotin's constants are essentially correct.

At low ionic strength the pk's of the transition are high and the intrinsic entropy contribution is balanced by the electrostatic entropy term (table 1). This will stabilize the two forms relatively to each other. Protonation will be necessary in order to shift this equilibrium to the double stranded form and small changes in the degree of protonation will favor this by ion pair formation [5]. On the other hand, at high ionic strength the phosphate charges are shielded and ion pair formation shall not be essential for the stabilization of the double helix. An unprotonated double helix at high ionic strength and low temperature may thus exist in principle.

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