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RELAXATION KINETICS OF THE HELIX-COIL TRANSITION OF A SELF-COMPLEMENTARY RIBO-OLIGONUCLEOTIDE: A_7U_7 *

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Relaxation measurements on the kinetics of the double helix to coil transition for the self-complementary ribo-oligonucleotide A_7U_7 are reported over a concentration range of 6.9 μM to 19.6 μM in single strand in 1 M NaCl. The rate constants for helix formation are about 2×10^6 M^{-1} s^{-1} and decrease with increasing temperature yielding an activation enthalpy of −6 kcal/mole. The rate constants for helix dissociation range from 3 to 250 s^{-1} and increase with increasing temperature yielding an activation enthalpy of +45 kcal/mole. The kinetic data reported here for 1 M NaCl is compared with previously published results obtained at lower salt concentrations. These data are discussed in terms of the quantitative effect of ionic strength on the kinetics of helix-coil transitions in oligo- and polynucleotides.

1. Introduction

In this work, the relaxation kinetics of the helix-coil transition of rA_7U_7 has been studied by the temperature-jump method. Two major reasons led to the selection of this molecule for investigation. First, a previous study on the helices formed from self-complementary ribo-oligonucleotides of the form A_nU_n revealed kinetic behavior for A_7U_7 that was inconsistent with the results obtained for A_4U_4, A_5U_5 and A_6U_6 [1]. Secondly, more data was needed in order to evaluate quantitatively the effect of salt concentration on the kinetics of order–disorder transitions in nucleic acids [2,3]. The results reported here should be relevant in this connection since this kinetic study has been carried out at a considerably higher salt concentration than most previous investigations.

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2. Materials and methods

Synthesis, isolation and characterization of the $(Ap)_7(Up)_6U$ was carried out as previously described [4]. All measurements reported here were performed in a buffer system consisting of 1 M NaCl, 0.01 M sodium phosphate and 10^{-4} M sodium EDTA, adjusted to pH 7.

The temperature-jump experiments were carried out on an apparatus that has been previously described by Eigen and de Maeyer [5] and Gralla and Crothers [6]. The relaxation studies were done at three different concentrations; namely, 6.9×10^{-6} M, 10.8×10^{-6} M and 19.6×10^{-6} M in single strand.

For each concentration, temperature jumps were carried out over an average temperature range of 5 to 45°C. For the two lowest concentrations the hypochromicity was monitored at 266 nm while for the highest concentrations the hyperchromicity at 283 nm was monitored.

The concentrations of the oligomer solutions used

in the temperature-jump experiments were spectrophotometrically determined using the 25 and 50°C extinction coefficients (ϵ) per monomer reported by Borer [7].

3. Analysis of the data

3.1. Treatment of T-jump data

Fig. 1(a) shows a typical oscilloscope trace of the change in percentage transmission versus time. In each experiment, one slow and one very fast relaxation effect was observed. The fast relaxation had a time constant smaller than that of the instrument

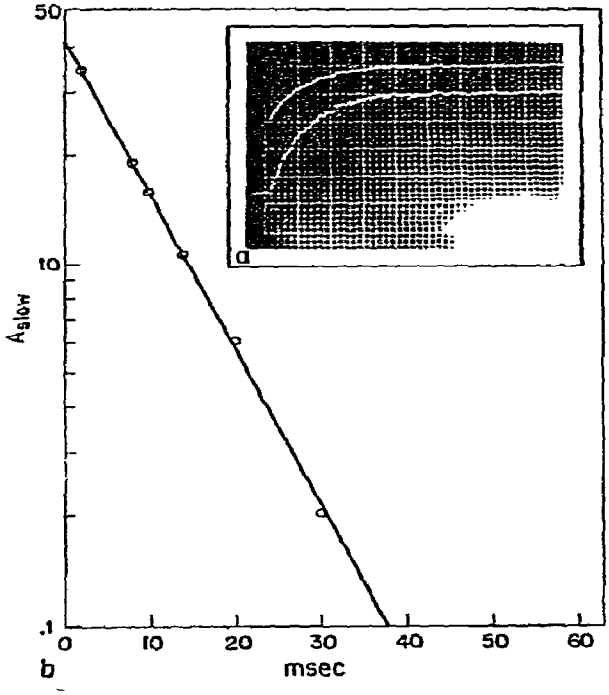

Fig. 1. (a) Observed relaxation signal at 266 nm for the melting of A_7U_7 in 1 M NaCl. Temperature jumped from 35 to 38.7°C. The horizontal sensitivity is 10 ms/cm. The vertical sensitivity is 20 mV/cm for the upper trace and 10 mV/cm for the lower trace. The short signal near the lower left corner represents the baseline before the temperature jump. The absorbance of the solution is 1.4 at 266 nm and 50°C. (b) Logarithm of the slow absorbance change versus time. Extrapolation of this plot to zero time allows partitioning of the total amplitude (92 mV) into 42 mV for the slow effect and 50 mV for the fast effect.

($\approx < 1$ μs) while the slow effect had relaxation times in the range of milliseconds to seconds. We assume that the slow effect corresponds to the cooperative order–disorder transition of the double helix. The very fast effect could be due to a variety of phenomena, including single-strand unstacking and/or end-effects ("fraying"). A comparison with the results of Pörschke and Eigen [8] on a comparable system, suggests that the very fast effect is most likely due to fraying of the ends. Nevertheless, we will use a simple all-or-none model to interpret the data obtained in this work. This will allow a direct comparison with previously published results on comparable systems which have been analyzed by such a two-state model. However, it should be mentioned that the theoretical calculations of Elson [9] indicate that fast fraying of the ends can have a significant effect upon the overall kinetics.

Analysis of the oscilloscope trace as previously described [6] allowed determination of the fractional absorbance change associated with each of these two effects. These data allow the construction of a differentiated melting curve that corresponds only to the slower cooperative component of the transition (see fig. 2).

Fig. 1(b) shows a plot of the logarithm of the slow absorbance change versus time. The observed linearity indicates that the relaxation effect can be represented by a single relaxation time, τ, which can be calculated from the slope of the line.

The relaxation times obtained in this manner were plotted logarithmically as functions of the reciprocal of the temperature. These data along with the differentiated melting curves determined at each concen-

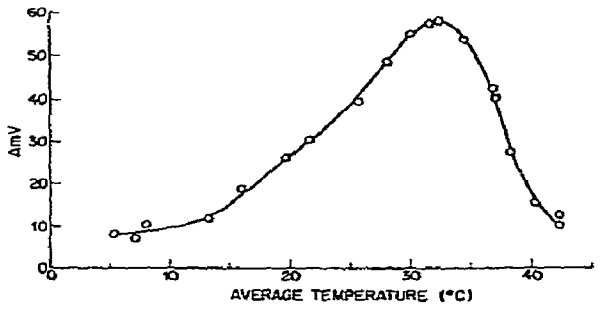

Fig. 2. Differential melting curve determined by temperature-jump for rA_7U_7 at 1M NaCl. The fast effect has been subtracted out and only the slow effect is shown.

tration were used to calculate the relevant kinetic parameters using the procedure described below.

3.2. Calculation of the kinetic parameters

The treatment that follows was originally described by Pörschke and Eigen [8] and has been used here to analyze the T-jump data obtained on rA_7U_7 at 1 M NaCl.

When two strands of a self-complementary molecule (e.g. A) combine in an all-or-none fashion to form a fully bonded helix (e.g. C) one can write general expressions for the reaction, the equilibrium constant, K, and the relaxation time, τ, as

$$A + A \underset{k_D}{\overset{k_R}{\rightleftharpoons}} C, \tag{1}$$

$$k = \frac{k_R}{k_D} = \frac{\alpha}{2(1-\alpha)^2 C_T}, \tag{2}$$

$$1/\tau = 4k_R \bar{C}_A + k_D, \tag{3}$$

where C_T is the total strand concentration, α is the fraction in single strand, k_R and k_D are the rate constants of recombination and dissociation respectively and $\bar{C}_A$ is the concentration of the single stranded form at equilibrium.

Inspection of eq. (2) indicates that at a given α (i.e. a fixed degree of transition) the ratio k_R/k_D (the equilibrium constant) can be expressed as a function of the total nucleotide concentration, C_T. Thus, at the melting temperature where $\alpha \approx 0.5$ one can write $K = k_R/k_D = 1/C_T$. Solving this expression for k_D and substituting in eq. (3) yields an expression for $1/\tau$ in terms of k_R and C_T only. At the melting temperature where $\alpha = 0.5$ this substitution yields $1/\tau = 3.0\, k_R\, C_T$. (Note: at $\alpha = 0.5$ $\bar{C}_A = 0.5\, C_T$.)

If this procedure is followed for a series of α values, the general equations presented in table 1 are obtained. It should be noted that these equations are valid only at the specified degree of reaction.

Inspection of the equations in the last column of table 1 indicate that plots of the reciprocal relaxation time as a function of total concentration, C_T, should yield straight lines. From the slope of these lines one can calculate k_R which in turn allows calculation of k_D using the equations in the second column of table 1.

The origin of the non-integral values of α used above deserves further comment. Gralla and Crothers [6] have shown that at the maximum of a differentiated melting curve $\alpha = 0.59$ and at the half-height $\alpha = 0.19$ and 0.89. Similar calculations reveal that at the two-thirds height of a differentiated melting curve $\alpha = 0.26$ and 0.85 and at the three-quarters height $\alpha = 0.31$ and 0.81. Thus, the non-integral α values simply result from the selection of convenient points for measurement on the differentiated melting curve.

The temperatures that correspond to these α values can be determined for each concentration by inspection of the appropriate differential melting curve. The relaxation times associated with these sets of temperatures (which correspond to constant α values) can be obtained from the temperature-jump data. Thus one obtains a series of τ values for each concentration at constant extents of reaction. This allows application of the equations in the last column of table 1 so that k_R and k_D can be determined.

Table 1
Equations for the equilibrium constants and the relaxation times at different degrees of reaction

α	Equilibrium constant, K	Reciprocal relaxation time, $1/\tau$
0.19	$1/0.09\, C_T$	$0.85\, k_R\, C_T$
0.27	$1/0.20\, C_T$	$1.27\, k_R\, C_T$
0.31	$1/0.28\, C_T$	$1.54\, k_R\, C_T$
0.59	$1/1.70\, C_T$	$4.06\, k_R\, C_T$
0.81	$1/7.15\, C_T$	$10.41\, k_R\, C_T$
0.89	$1/14.40\, C_T$	$17.96\, k_R\, C_T$

Table 2
Recombination rate constants as a function of temperature

Temperature, °C	Recombination rate constants k_R, $\ell\ \text{mole}^{-1}\ \text{s}^{-1}$
22.1	2.7×10^6
26.2	2.2×10^6
27.6	2.1×10^6
33.6	1.8×10^6
39.1	1.5×10^6
40.1	1.4×10^6

Table 3
Dissociation rate constants as a function of temperature

Temperature, °C	Dissociation rate constants k_D, s^{-1}
22.1	3.0
26.2	5.8
27.6	7.4
33.6	38.0
39.1	134.0
40.1	250.0

4. Results and discussion

Tables 2 and 3 summarize the values obtained for the recombination and the dissociation rate constants using the data analysis outlined below.

Figs. 3 and 4 present semi-logarithmic plots of k_R and k_D versus the reciprocal of temperature. From the slopes of these plots activation energies have been calculated using the Arrhenius equation

$$\frac{\partial \ln k}{\partial(1/T)} = -\frac{E^{\ddagger}}{R}.$$

The resulting activation energies associated with the recombination and dissociation processes are given in table 4.

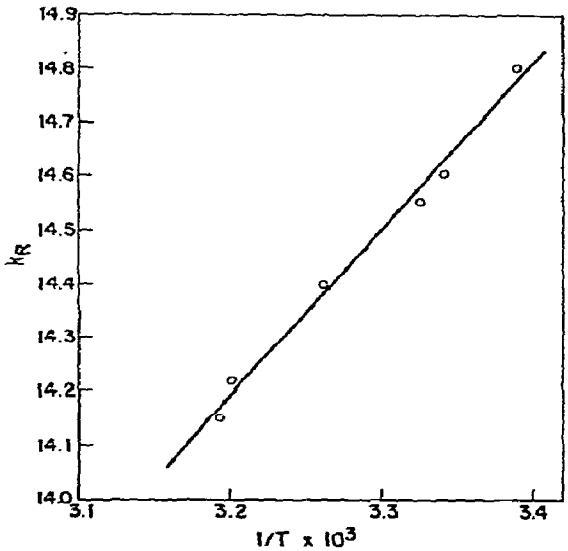

Fig. 3. The logarithm of k_R, the recombination rate constant, versus the reciprocal of temperature for rA_7U_7 at 1 M NaCl.

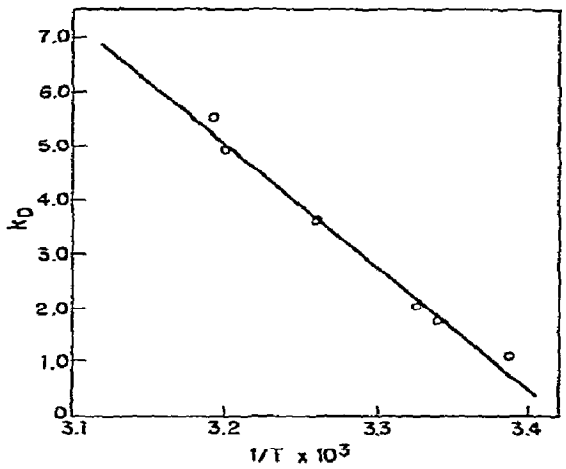

Fig. 4. The logarithm of k_D, the dissociation rate constant, versus the reciprocal of temperature for rA_7U_7 at 1 M NaCl.

Several points deserve emphasis. The dissociation rate constants are strongly temperature dependent and increase with increasing temperature. This trend as well as the sign and magnitude of the activation energy are consistent with previously published data on both $A_n + U_n$ [8] and A_nU_n helices [1].

In contrast, the recombination rate constants, k_R, decrease with increasing temperature and are only slightly temperature dependent. This indicates that k_R cannot be the rate constant of a simple elementary reaction but rather may reflect a combination of a pre-equilibrium and a rate-determining step. This behavior has been observed previously by Craig et al. [1] for the kinetics of recombination associated with A_4U_4, A_5U_5, and A_6U_6. However, they found the opposite behavior for A_7U_7; that is, they report that k_R increased slightly with increasing temperature thereby indicating a small positive activation energy. This stands in contrast to the results reported here which indicate that the kinetic behavior of A_7U_7 is completely consistent with the results obtained by

Table 4
Activation enthalpies for the recombination and dissociation processes

Reaction	$E^{\ddagger}$ (kcal $mole^{-1}$)
recombination	−6
dissociation	+45

Craig et al. [1] for A_4U_4, A_5U_5, and A_6U_6.

Several possible explanations can be offered to explain the observed differences between these two studies on A_7U_7. Craig et al. suggest that the "unusual" behavior which they observe for A_7U_7 can be explained if it is assumed that intramolecular hairpin helices form from A_7U_7 at temperatures near and below the melting temperature of the bimolecular helices. As they point out, such a situation would cause their equilibrium melting curve to be broadened and shifted upwards which in turn would lead to an overestimate of α, the extent of reaction, at any temperature. This overestimate of α would in turn result in an underestimate of k_R which would be most severe at the lower temperatures.

In contrast, in the present work α is determined from the differentiated melting curve which refers only to the slow (millisecond) component of the transition. Since hairpin structures melt with a time constant in the microsecond range [10], the recombination rate constants reported here should be less sensitive to errors introduced by the existence of any hairpin structure. However, it should be noted that the formation of any monomolecular structure would still have a small indirect effect on the differentiated melting curve since hairpin formation is coupled to the double strand to single strand equilibrium. Furthermore, if some hairpin helices do exist, the total concentration, C_T, would be slightly overestimated.

An alternative explanation relates to the fact that this investigation was carried out at four times the salt concentration and three to eight times the strand concentration used by Craig et al. Both of these differences in solution conditions favor formation of bimolecular helices relative to hairpin structures [11]. Furthermore, these same solution conditions cause the bimolecular helices of this study to melt at considerably higher temperatures than the 23.5°C found by Craig et al. [1]. Thus, interference from hairpin structures at these higher temperatures seems unlikely.

4.1. Salt dependence

The effect of ionic strength on the rate of association between complementary polynucleotide strands has been studied by a number of workers [2,12–14]. For relatively low ionic strengths (10^{-2}–10^{-1} M), the apparent second order rate constant k_R has been found to vary directly as the third or fourth power of the ionic strength [12].

In contrast, very few data are available on the salt dependence of k_R for short helices formed from complementary oligonucleotides.

For the self-complementary hexamer A_2GCU_2. Pörschke et al. [15] report that k_R increases with approximately the square root of the ionic strength over the range of 0.05 to 1.05 M sodium ion.

These same investigators found that for the self-complementary decamer, A_4GCU_4, k_R increased with the power of 0.7 of the ionic strength over a sodium ion concentration range of 0.05 to 1.05 M.

In an earlier study, Pörschke [16] found that for the reaction between $A(pA)_{13}$ and $U(pU)_{13}$ k_R varied as the square of the salt concentration over a sodium ion concentration range of 0.05 to 0.17 M.

The present study of rA_7U_7 was done at 1 M sodium ion concentration as compared with the 0.25 M sodium ion concentration used by Craig et al. As expected based upon screening by the sodium ions of the repulsion between the polyelectrolyte backbones, the k_R values reported here are significantly higher than those found by Craig et al. [1]. In fact k_R reported here for rA_7U_7 at 22°C in 1 M Na^+ is three times larger than that reported by Craig et al. for rA_7U_7 at 22°C in 0.25 M Na^+.

These data allow one to calculate that k_R for rA_7U_7 increases approximately with the power of 0.8 of the ionic strength over a sodium ion range of 0.25 to 1.0 M. This is quite similar to the ionic strength dependence of k_R found by Pörschke et al. [15] for A_4GCU_4 but is lower than that reported by Pörschke [16] for $A(pA)_{13} + U(pU)_{13}$.

These results are of interest in connection with a theoretical model recently developed by Manning [2] in which polyelectrolyte theory is used to quantitatively predict the dependence of k_R on ionic strength.

However since such few data are presently available on the salt dependence of k_R, it is quite possible that such quantitative comparisons are not yet justified. Suffice it to say that our data clearly indicate that k_R increases with increasing ionic strength. Further studies are required before quantitative comparisons can be made so that existing theories which deal with the ionic strength dependence of renaturation kinetics can be tested.

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