Biochemical Pharmacology, Vol. 37, No. 9, pp. 1843–1844, 1988.
Printed in Great Britain.

0006–2952/88 $3.00 + 0.00
Pergamon Press plc

ELASTICITY OF DNA IN NONHELICAL LOOPS

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Introduction

During melting DNA dissociates stepwise by domains of 100-500 bp [1]. In long DNAs the loci of domains is primarily internal so that dissociation leads to the formation of closed loops. Three factors affect the temperature at which such domains dissociate: 1) the energetic costs of interrupting the helix, 2) the costs of unstacking base pairs and 3) the energetic level of the loop. The effects of domain length and chain elasticity on energy level are the least well understood.

The dependence of internal domain stability on length, N, is represented by the loop weighting function, f(N), expressing the statistical weight for ring structures of N dissociated base pairs. This function depends on physical characteristics of dimension, elasticity and elastic uniformity over length of the polydeoxynucleotide chain in the loop [2]. For the purpose of determining f(N) we have constructed a recombinant model, a 4363 base pair (bp) plasmid containing homopoly$(A{\cdot}T)_N$ inserts of length 150 > N > 50. The low stability of these inserts, bounded by considerably more stable (G+C)-rich domains, results in their dissociation as internal loops, manifest as well resolved subtransitions in high resolution melting profiles.

Materials and Methods

The plasmid pBR322 was modified according to Hayashi's procedure, [3] with the addition of homopoly$(dA{\cdot}dT)_N$ inserts of random lengths at position 2069, and grown in E. coli HB101 cells. The length of (A·T) inserts was determined from the 1) altered electrophoretic mobility of the 344 bp Hinf I fragment (2034-2378); 2) relative integrated areas under derivative melting curves of insert and plasmid, and 3) the fit of theoretical curves to observed helix-loop transitions. DNA melting was followed by the loss of hypochromicity by the absorbance difference - approximation method [4] at 260, 270 and 282 nm, and digitized at densities of 100-150 deg^{-1}.

Results and Discussions

Illustrative high resolution melting curves of pBR322$(A{\cdot}T)_{83}$ DNA, linearized at different loci, are shown in Figure 1. Except for the two subtransitions denoted by double headed arrows, the temperatures of subtransitions are conserved within ±0.01°C. Assignments of domains to subtransitions will be described elsewhere; that for $(A{\cdot}T)_{83}$ is well resolved at 71.6°C, and in the enlarged view of the inset figure. Spectral decomposition during melting of pBR322 DNA [4] indicates that melting temperatures of domains vary linearly with the fractional base composition, with a slope of 41.5°C and extrapolated $T_{A,T}$=64.22°C for the generic (A·T) base pair, representing the average stacking stability of AA(TT), AT(TA) and TA(AT) nearest neighbors. This is one degree lower than T_{AA}(65.38°C) for poly dA·dT (Figure 2) and over six degrees above $T_{AT,TA}$ (57.86°C) for poly dA-T·dA-T, reflecting differences in stacking energies for the three different (A·T) neighbors. The value for T_{AA} is significantly lower than that for $...(A{\cdot}T)_{83}...$ (71.61°C), reflecting the greater free energy needed to induce and maintain a disordered loop of 83 residue pairs. Loop energy is determined from the variation of T_{AA}^{N} with the equilibrium contant for loop formation in accordance with the integrated van't Hoff expression. A plot of $1/T_{AA}^{N}$ against 1/(N+1) for 150 > N > 50 appears linear (Figure 3). The extrapolated T_{AA}^{∞} = 65.28°, is almost identical to that for poly dA·dT (65.38°C), which gave a size estimate of > 30 kb, and therefore is a good approximation for the infinite size.

In the consideration of nonideal chains with reduced flexibility and self exclusion, the loop function takes the form $f(N)=(N+D)^{1.7(\pm 0.2)}$ in the equilibrium constant for melting of inserts; where D is an empirical stiffness parameter [5]. From regression of results in Figure 3 we obtain a value for D=97±10bp. This means A and/or T residues are less flexible or otherwise more predisposed

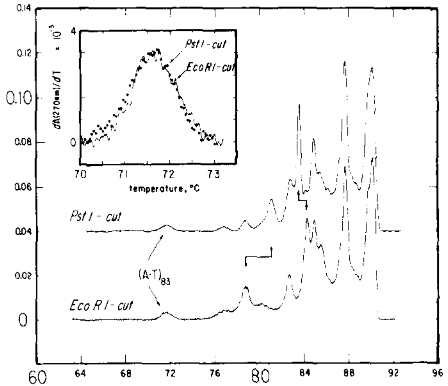

Figure 1

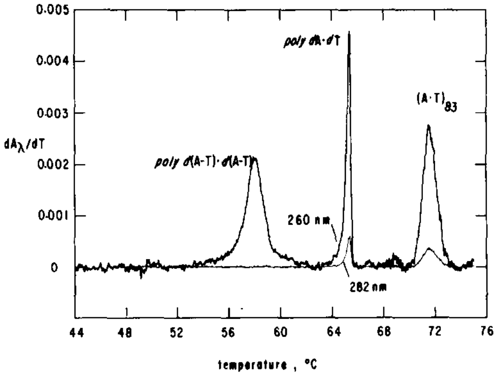

Figure 2

to reclosure than a Gaussian chain. Melting curves of oligo- and poly rA and dA indicate destacking is noncooperative and associated with a transition enthalpy of 9-13 kcal (mol residue)$^{-1}$. Approximately 25% of A residues remain stacked at the temperature of melting of $(A{\cdot}T)_N$ inserts. Stacking in poly dT is nil, therefore the net fraction of stacked residues in $(A{\cdot}T)_N$ loops is ~12%; which is the same as the fractional difference in elasticity between $(A{\cdot}T)_N$ and a Gaussian loop of size N_g that melts at the same temperature, $100(N_g-N)/N_g = 10\%$. The more favorable loop free energy is therefore due to the incipient helical orientation of A residues. The stacking energy of neighboring G and C residues is significantly higher than that of A and T residues, therefore, the stiffness parameter (D) is expected to increase sharply with the (G+C) composition of loop-forming domains.

Polynucleotide elasticity determines the shear force needed to bend the chain by some angle over unit length. The angle is represented by tangents along contours of the separated chains from the point of the helix-loop interface. For small angles the free energy is proportional to the bending force constant and inversely proportional to the persistence length. The smallest loop size examined in this study (55 bp) exceeds the persistence length for the single strand ; and probably explains why the loop free energy increment appears uniform over the size range in Figure 3. The loop energy increment is expected to increase sharply for loops smaller than the persistence length [2].

Indications of the structure of the helix-loop interface can be gleaned from the sensitivity of loop formation on ionic strength. The variation of T^N_{AA} with [Na$^+$], $S_N = dT^N_{AA}/d\log[Na]$, is proportional to the difference in association of Na$^+$ to helix and loop, $\Delta\Psi_N = \Delta[1-(2\xi)^{-1}]$, where the extent of association to the helix is given by $\xi_h = q^2/\epsilon k t b_h$ (and similarly for the loop state), and where b_h is the axial charge separation. Depending on the range of electrostatic effects and size of the loop, the higher charge density in the vicinity of the interface should be evident from $S_N < S_\infty$. Indeed, we find $S_\infty = 19.21°C$ for polydA·dT, but only 16.57°C for $\ldots(A{\cdot}T)_{83}\ldots$, (Figure 4), a surprising difference for such a large domain. The unit transition enthalpy is constant for loops of different size, so that $S_N/S_\infty = \Delta\Psi_N/\Delta\Psi_\infty$. From the salt dependent behavior of loops of different sizes we derive the expression: $\Delta\Psi_N = \Delta\Psi_\infty(1-11.41/N)$. Thus, the differential thermodynamic ion association parameter varies with 1/N , indicating proportionately greater condensation of Na$^+$ to disordered loop domains near the interfaces, and that there is a defined length to the interface region.

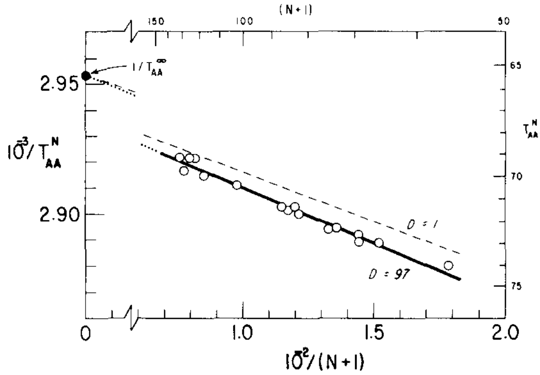

Figure 3

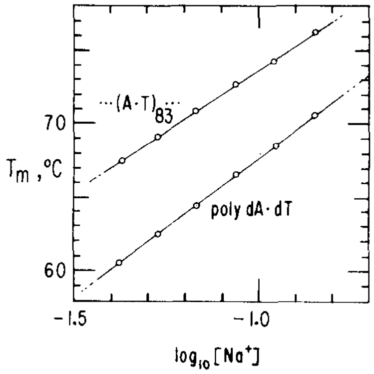

Figure 4

Conclusions

Loops of 150 > N > 50 bp are more stable, less elastic than expected of freely jointed Gaussian chains. The incremental difference in stability is constant over the range examined, and is due to the incipient helical orientation of some residues, predisposing the loop to reclosure. A gradient of counterion association to chains of the loop begins with levels characteristic of the helix at the interface, and drops to levels characteristic of the coil over a distance of > 5 residues. This ion gradient also contributes to the greater stability of loop domains.

Acknowledgement

This work was carried out with funds granted by NIH (GM22827).

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