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'DNA snapback' peptides

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ABSTRACT. Thermal denaturation studies show that 10-15% of the calf thymus DNA in the heat denatured (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex renatures spontaneously after cooling. The double-strandness of this DNA was verified by its resistance to single-strand *Neurospora* endonuclease and by its elution profile on hydroxyapatite columns. The renatured DNA isolated by the latter technique was found to contain 56% GC compared to the 41% GC content of the whole thymus DNA. Alternating tryptophanyl-glycyl and histidyl-glycyl peptides also catalyze the same renaturation. A linear correlation was found between the thermal stabilization afforded to the DNA by the various peptides and their ability to "catalyze" DNA strand renaturation.

INTRODUCTION

Peptides containing tyrosines, tryptophans, and histidines separated by two or three amino acids, e.g. Tyr-$(Gly)_{2-3}$-Tyr, were all found to stabilize the DNA helix against thermal denaturation.[1] In synthetic polynucleotide studies the tyrosyl peptides were shown to have a strong affinity for guanine and for GC base pairs.[2] A GC specificity has not yet been demonstrated for naturally-occurring DNAs. NMR studies on these complexes show the tyrosyl residues to be intercalated between the polynucleotide bases. The present studies show that a GC-rich fraction of the DNA in these peptide complexes renatures spontaneously after thermal denaturation indicating that the tyrosyl-DNA base bonds are stable at these elevated temperatures and hold the DNA strands in registry for this renaturation.

MATERIALS AND METHODS

Calf thymus DNA was obtained from Worthington. *Neurospora* single-strand specific endonuclease was obtained from Boehringer Mannheim (New York, New York).

Peptides were synthesized by solid phase peptide synthesis on 2% crosslinked chloromethylated polystyrene beads (Schwartz-Mann). The following t-Boc amino acids were used in 2.5 molar excess: tyrosine, benzyl-tyrosine, glycine, tryptophan, im-benzyl-histidine (BACHEM, Marina Del Ray,

California). t-Boc tyrosine and t-Boc benzyl tyrosine derivatives gave similar peptide products. The t-Boc amino acids were deblocked using 6 N HCl-Dioxane, the resins neutralized in triethylamine-chloroform (1:9), and the amino acids coupled to the peptide resins with dicyclohexylcarbodi-imide.[3] The peptides were removed from the resin and deprotected with anhydrous HF. Purification was carried out by TLC on cellulose using a 1-butanol-acetic acid-H_2O(4:1:1, v/v) solvent system. The tryptophan peptides were formylated with 0.1 N HCl-formic acid at the t-Boc deblocking stage and the formyl group removed after HF treatment with 0.1 M piperidine.[4]

% SNAPBACK BY THERMAL DENATURATION

Thermal denaturation studies were all performed in 1 mM cacodylate -0.01 mM EDTA buffer (pH 7.5) at a [1]/[2] peptide to base pairs molar ratio. This ratio gave maximal thermal stabilization and % snapback for all peptides studied. In the "snapback" experiments the complexes were heated for 10 minutes at 100° C and then immediately cooled for 5 minutes in an ice-water bath. Then the % snapback renaturation was determined from the hyperchromicity observed when the samples were "melted" a second time, e.g. Fig. 1d. % SNAPBACK was defined as follows: % SNAPBACK =

$$\frac{\text{Max. } \% \Delta A_{265} \text{ on 2nd Denaturation} - \text{Initial } \% \Delta A_{265} \text{ before 2nd Denaturation}}{\text{Max. } \% \Delta A_{265} \text{ on 2nd Denaturation}} \times 100.$$

All calculations ($\% \Delta A_{265}$) are referred back to the original A_{265} of the native DNA.

DOUBLE-STRANDNESS OF RENATURED DNA IN THE PEPTIDE COMPLEX

The samples (200 µg of DNA) were applied to 1x5 cm hydroxyapatite (BIO-RAD) columns and denatured and native DNAs eluted with 0.1 M and 0.27 M phosphate buffers, respectively.[5] For nucleotide composition studies the isolated renatured DNA was hydrolyzed with pancreatic DNAase (DNA: enzyme, 20:1 w/w) for 2 hrs. (37° C), and then for 24 hrs (25° C) with venom phosphodiesterase (DNA:enzyme, 2:1 w/w). Both digestions were carried out in a 10 mM $MgCl_2$-10 mM Tris-HCl buffer (pH 7.5). The lyophilized hydrolysates were applied to the 1x50 cm Dowex (50x4) columns in 0.1 M ammonium formate buffer (pH 3.2) and the 5'-nucleotides were eluted with the same buffer.[6]

RESULTS

The cooperativity of the thermal transition found upon "re-melting" the once denatured, quick-cooled (Tyr-Gly-Tyr-Gly-Tyr)-calf thymus DNA

complex first suggested that the tyrosyl peptide "catalyzed" the renaturation of part of the DNA (Fig. 1d). This renaturation occurred within seconds after lowering the temperature of the complex below the T_M. No such cooperative melting was found when the Tyr-Gly-Tyr-Gly-Tyr was introduced after strand separation, nor was as great a hyperchromicity observed. Rather a thermal transition curve similar to that found for denatured calf DNA (Fig. 1c) was obtained.

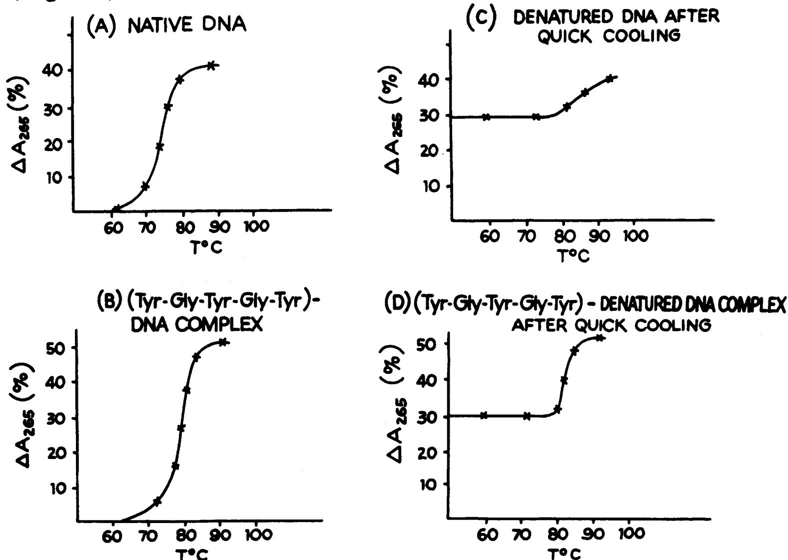


Figure 1. Figures 1a and 1b are the thermal denaturation curves for the first "melting" of calf thymus DNA and of the (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex, respectively. The additional 10% hyperchromicity of the complex is also seen at room temperatures and is attributed to a change in the absorptivity of the peptide upon complexation. After denaturation both samples were cooled for 5 minutes at 0° C and remelted (fig. 1c and 1d).

To confirm the Tyr-Gly-Tyr-Gly-Tyr catalyzed renaturation, we subjected " denatured" DNA from the (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex to digestion with single-strand specific _Neurospora_ endonuclease. As is seen in Table 1, 10-15% of the DNA was undegraded or only partially degraded by this treatment and remained in dialysis bag after this treatment. A further proof of the double-strandness of a portion of the DNA in the denatured, quick-cooled Tyr-Gly-Tyr-Gly-Tyr complex, was the separation of the renatured DNA by hydroxyapatite chromatography (Fig. 2). Again, from its elution profile 10% of the DNA in the complex was found to have renatured after cooling. The nucleotide composition of this DNA showed it to have a 56% GC content compared to a 41% GC content for the entire population of calf thymus DNA molecules (Table 2).

Having once established that the hyperchromicity exhibited by the "denatured" (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex upon "remelting" (compare Fig. 1c and 1d) reflected the presence of renatured DNA, we used this technique to estimate the extent of renaturation catalyzed by other peptides. As is seen in Figure 3 there is a good correlation between % snapback and the ability of a peptide to stabilize the DNA against thermal denaturation. Tryptophanyl and histidyl peptides are both effective as the analogous tyrosyl peptides in catalyzing DNA strand renaturation. The GC-content of the DNA renatured in the presence of Trp-Gly-Trp-Gly-Trp is the same as that found for Tyr-Gly-Tyr-Gly-Tyr peptide, viz., 56%.

TABLE 1

Resistance of the DNA in the Denatured (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex to single-strand *Neurospora* endonuclease

	A_{260} remaining inside the dialysis bag
Native Calf Thymus DNA	96%
(Tyr-Gly-Tyr-Gly-Tyr)-Denatured DNA complex	3%
Denatured (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex	16%

The A_{260} of 100 μg of each of the respective DNAs was recorded before DNA was treated with 20 μg of *Neurospora* endonuclease (in a 10 mM Tris buffer) (pH 7.5) for 5 hrs at 37° C. The samples were then dialyzed against 3x1000 volumes of 1 M KCl-10 mM Tris (pH 7.5) buffer for 3 hrs each. A_{260} of the material inside was recorded.

TABLE 2

Nucleotide composition of Snapback DNA from the Denatured (Tyr-Gly-Tyr-Gly-Tyr)-DNA complex

	(%)			
	d-GMP	d-CMP	d-AMP	TMP
Whole calf thymus DNA	21	21	29	29
Snapback DNA	28	28	22	22

DISCUSSION

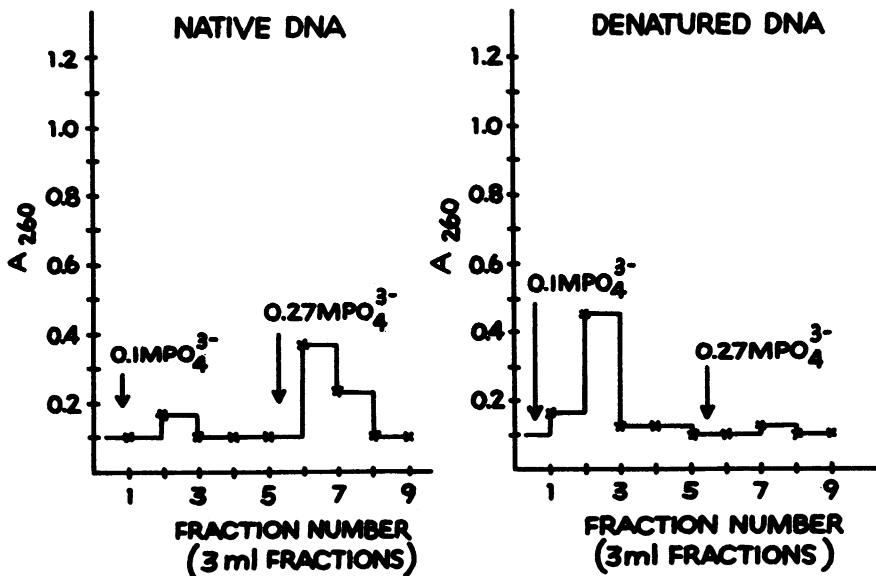
The hyperchromicity of the heated peptide complexes (Fig. 1b) suggests that most of the bases are unstacked at 90° C. Yet the extra 10% hyperchromicity attributed to the complexed tyrosyl residues[2] persists at these elevated temperatures indicating that these tyrosyl-DNA base (guanine) bonds remain intact. If these bonds are weak interactions, such as charge-transfer interactions[1] then the peptides would have to be bound along much of the length of the unwound GC-rich "snapback" DNA to account for its thermal stability. The balance of the DNA that fails to demonstrate "snapback" probably contains too few peptide binding sites to hold the DNA strands in registry. Thus at denaturing temperatures the peptide complex may be pictured as a ladder with the non-covalently bound peptide crosslinkers

representing the rungs between the two unwound DNA strands.

There are three strong pieces of evidence that indicate that the Tyr-Gly peptides bind preferentially to GC-base pairs. Tyr-Gly-Tyr-Gly-Tyr preferentially precipitates out guanine-containing dinucleotides. Tyr-Gly-Tyr-Gly-Tyr bound to the Merrifield resin specifically adsorbs poly dG·dC, but not poly dG,. poly dC or any of the adenine containing duplexes.† The "snapback" DNA has an enriched GC content. However, that these peptides seem to recognize something more than GC base pairs is suggested in the next paper by the lack of correlation between % snapback and GC content of the DNA.[1]

Finally, the "snapback" activity of these Tyr-Gly, Trp-Gly, and His-Gly, sequences might be used by enzymes, e.g. RNA polymerase. Their occurrence in an enzyme, such as RNA polymerase, might occasion a re-evaluation of the hypothesis that DNA "renaturation" during RNA synthesis by the RNA polymerase is dependent only on the relative affinities of the participating polynucleotide strands.

†Novak, R. and Dohnal, J. (unpublished data).



Figures 2a and 2b. The elution profiles of native and denatured calf thymus DNA are shown for comparison (Fig. 2a and 2b, respectively). Presence of Tyr-Gly-Tyr-Gly-Tyr had no effect on the elution patterns.

SPONTANEOUSLY RENATURING CALF THYMUS DNA
FROM THE DENATURED Tyr-Gly-Tyr-Gly-Tyr COMPLEX

$0.1MPO_4^{3-}$
$0.27MPO_4^{3-}$
A_{260}
1.1
1.0
0.8
0.6
0.4
0.2
1 3 5 7 9 11
FRACTION NUMBER
(3ml FRACTIONS)

Figure 2c. The elution profile of snapback DNA isolated from the denatured complex.

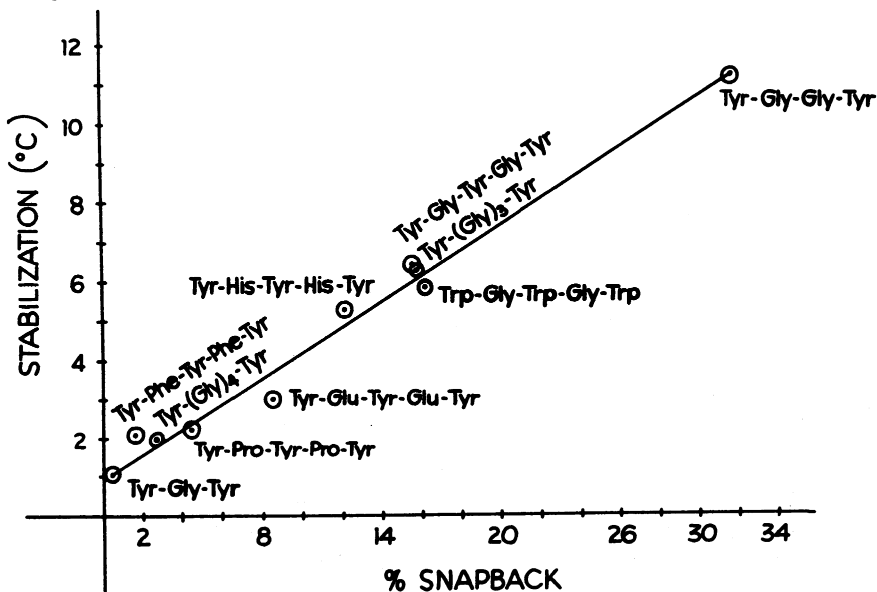


Figure 3. Correlation between calf thymus DNA stabilization and snapback activity. Stabilization is given in terms of increased T_M.

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