Interaction of tyrosyl, histidyl, and tryptophanyl peptides with DNA: specificity and mechanism of the interaction

Robert L. Novak and James Dohnal

**Department of Chemistry, De Paul University, 1036 West Belden Avenue,
Chicago, Illinois 60614, USA**

Received 18 April 1974

ABSTRACT. In the Tyr-(Gly)₁₋₄-Tyr series maximal thermal stabilization of calf thymus DNA ($\Delta T_m = 10^\circ$) occurred with the Tyr-(Gly)₂-Tyr peptide, where three base pairs could separate the two tyrosyl residues. Tyr-Gly-Tyr-Gly-Tyr stabilized the DNA by 6° . The alternating Trp-Gly-Trp-Gly-Trp and His-Gly-His-Gly-His peptides were equally as effective as the Tyr-Gly-Tyr-Gly-Tyr peptide in stabilizing calf thymus DNA against thermal denaturation. But the alternating Phe-Gly-Phe-Gly-Phe peptide afforded little stabilization, suggesting that a sidechain possessing both a conjugated π -electron system and an electron donor atom is necessary for DNA stabilization. Introduction of electron withdrawing iodo or nitro group into the tyrosyl sidechains almost completely abolished the stabilizing effect. Although the tyrosyl peptides seem to be specific for GC-base pairs, no correlation was found in natural DNA between % GC and % thermal stabilization. Eukaryotic DNAs showed twice the stabilization of prokaryotic DNAs with the same GC content.

INTRODUCTION

A number of critical acidic enzymes interact with nucleic acids, e.g. RNA and DNA polymerases, RNases and DNases, and amino acyl-tRNA synthetases. But the molecular basis for their interaction is mainly unknown, especially if this interaction takes place without the participation of positively charged lysines and arginines. The recent finding that aromatic amino acids bind strongly to native DNAs^{1, 2} suggested to us that acidic proteins may use the same sidechain residues to bind DNA. In order to explore this possibility we had previously synthesized the Tyr-Gly-Tyr-Gly-Tyr peptide, with tyrosines so spaced that they could intercalate between neighboring base pairs in the DNA helix. This peptide did interact with calf thymus DNA raising the T_m 6°C .³ The same study showed the Tyr-Gly-Tyr-Gly-Tyr peptide to have the strongest affinity for poly dGdC and to exclusively precipitate all guanine-containing dinucleotides. From their proton base shifts in the NMR the tyrosines are thought to be intercalated between the bases of the interacting polynucleotides. In this study we have looked further into the nature and specificity of the interaction of aromatic peptides with a number of different DNAs. More specifically we have asked the

following questions:

- (1) What is the effect of the separation between tyrosyl residues on the ability of the peptide to interact with and stabilize the DNA helix?
- (2) Will other aromatic amino acids similarly spaced in the peptide also interact with and stabilize double-stranded DNA?
- (3) What chemical characteristics of the stabilizing amino acid sidechains are responsible for this stabilization, e.g. what type of bonding forces are involved?
- (4) Do the tyrosyl peptides recognize GC-rich regions of the DNA as would be expected from previous model polynucleotides studies?

MATERIALS AND METHODS

Calculations of the maximum amino acid chain separations were performed using LABQUIP (Reading, England) molecular models. Calf thymus DNA was obtained from Worthington Corp. (Freehold, N. J.), Micrococcus luteus DNA from Miles Lab. (Elkhart, Ind.) Salmon sperm DNA and Micrococcus DNA received two phenol purifications before use. All other DNAs were used without any further purification. T2 DNA was purified as previously described.⁴

Thermal Denaturation Studies

Peptides were synthesized by solid phase synthesis and purified as previously described.⁵ All thermal denaturation studies were performed in a Beckman DKB spectrophotometer equipped with an electric thermal heating block. Peptide-DNA complexes were prepared in a 0.01 mM EDTA-1 mM cacodylate buffer (pH 7.5) at a [1]/[2] peptide/DNA base pairs ratio, a ratio found for all peptides to provide maximal stabilization of the DNA.

Chemical Modification of Peptides

The Tyr-Gly-Tyr-Gly-Tyr peptide was nitrated for 60 minutes (37°C) in 50 mM Tris buffer (pH 8.0) with tetranitromethane at a 1:10 (w/w) peptide: modifier ratio.⁶ Iodination was performed with hypiodous acid using 0.6 ml of "hot" ¹²⁵HOI glycine buffer⁷ per 100 µg of Tyr-Gly-Tyr-Gly-Tyr. The modification of Trp-Gly-Trp-Gly-Trp was performed in 50 mM Tris buffer (pH 7.5) using a molar ratio of peptide to 2-hydroxyl-5-nitro-benzyl bromide (Koshland's Rgt) of 1:20. The reaction time was 30 minutes at room temperature. All unreacted modifying agents were removed from the peptides by dialysis through a BIORAD hollow fiber "Osmolyzer".

RESULTS

Tyrosyl peptides were synthesized with varying numbers of intervening glycines in order to determine optimal spacing between tyrosines for the

Figure 1. The effect of tyrosyl spacing on the peptide stabilization of calf thymus DNA.

peptide-DNA interaction. The increase in the thermal stabilization of the DNA helix was taken as a measure of the interaction. The alternating Tyr-Gly-Tyr-Gly-Tyr peptide previously predicted in its "stacked" configuration--tyrosines stacked one over another--to best intercalate between neighboring DNA base pairs (3-4 Å minimal distance between neighboring tyrosines) did not give optimal stabilization (Fig. 1). A comparison of the stabilization of the Tyr-Gly-Tyr-Gly-Tyr and Tyr-(Gly)₃-Tyr peptides suggested that the middle tyrosines do not interact with the DNA. This was confirmed by the ineffectiveness of the Tyr-Gly-Tyr peptide in stabilizing DNA. The peptide with tyrosines interspersed with two glycine residues affords the best stabilization to the DNA. Doubling the Tyr-(Gly)₂-Tyr sequence, i.e., Tyr-(Gly)₂-Tyr-(Gly)₂-Tyr, resulted in no addition stabilization. Increasing the spacing between tyrosines to four glycine almost completely abolished the stabilizing effect. Thus in order for tyrosyl peptides to stabilize native DNA against thermal denaturation the tyrosines have to be spaced three to four amino acids apart.

In order to see if this stabilization of DNA was specific for tyrosine, we synthesized the alternating tryptophanyl, phenylalanyl, and histidyl peptides. The tryptophanyl peptide affords the same stabilization to calf thymus DNA as does the tyrosine peptide, while the phenylalanyl peptide

affords very little stabilization (Table 1). Thus aromaticity is not a

TABLE 1
Stabilization of the DNA Helix by
Tyrosyl, Tryptophanyl & Histidyl Peptides
Increase in T_m (°C)

Tyr-Gly-Tyr-Gly-Tyr	6
Trp-Gly-Trp-Gly-Trp	6
Phe-Gly-Phe-Gly-Phe	2
His-Gly-His-Gly-His	5
Tyr-Gly-Tyr-Gly-Tyr	2
Iodinated Tyr-Gly-Tyr-Gly-Tyr	2
Benzylated 2-hydroxyl-5-nitro-benzyl Trp-Gly-Trp-Gly-Trp	10

sufficient property for stabilization of DNA. Surprisingly, the hetero-aromatic, but conjugated π -electron system in the histidine sidechain affords almost as good stabilization to DNA as do either tyrosine or tryptophan.

In order to explore further the molecular mechanism of these DNA-peptide interactions, we investigated the effect of two chemical modifications of the phenolic sidechain (Table 1). The introduction of either electron-withdrawing nitro or iodo groups completely eliminated the stabilizing effect of the Tyr-Gly-Tyr-Gly-Tyr peptide. Benzylation of tryptophan sidechain with 2-hydroxyl-5-nitro-benzyl bromide increased the stabilization afforded by the Trp-Gly-Trp-Gly-Trp peptide to DNA.

Next we turned our attention to the part of DNA--particular bases or base pairs--recognized by the tyrosyl peptides. Our previous studies on synthetic DNA complexes suggested that the tyrosyl peptides had a specificity for GC base pairs, with the tyrosines probably interacting with the guanine moiety. We explored this possibility by investigating the stabilization and the DNA snapback afforded by the Tyr-Gly-Tyr-Gly-Tyr peptide to DNAs with varying GC-contents (Table 2). There was, however, sur-

TABLE 2
Thermal Stabilization & % Snapback of a Variety
of DNAs with the Tyr-Gly-Tyr-Gly-Tyr

Source of DNA	%GC	Stabilization [†]	% Snapback [‡]
Salmon Sperm	41	9	26
Calf Thymus	42	6	16
Micrococcus luteus	67	4	10
E. coli	52	2	5
Bacteriophage T ₂	35	3	7

[†]Stabilization is given as increases in T_m (°C).

[‡]Percent snapback was calculated and experiments carried out as previously described.⁵

prisingly little correlation between these two activities and the GC content of the DNAs studied. The Tyr-Gly-Tyr-Gly-Tyr peptide seems to stabilize the eukaryotic DNAs better than the prokaryotic DNAs with the same GC content.

DISCUSSION

The lack of DNA stabilization by the Tyr-Gly-Tyr peptide and the equal effectiveness of the Tyr-Gly-Tyr-Gly-Tyr and Tyr-(Gly)₃-Tyr peptides indicates that the tyrosines sidechains have to be separated by more than 8 Å for the interaction with DNA to take place. This almost rules out the possibility that the tyrosines are intercalated between neighboring base pairs unless the peptide is wrapped around one of the DNA strands. The latter is unlikely since the tyrosines in the Tyr-(Gly)₂-Tyr peptide, which provides maximal DNA stabilization, are separated by a maximal distance of 11 Å, approximately the radius of the DNA helix. If the tyrosyl residues interact with bases on both chains, which might be expected from their stabilization of the DNA helix, their 11 Å separation would probably preclude the latter encircling arrangement. It seems more likely that the neutral tyrosyl peptides with their limited solubility in water prefer to be located in the interior grooves of the DNA helix. With a maximal distance of 11 Å between the tyrosyl sidechains in Tyr-(Gly)₂-Tyr, three base pairs could separate the tyrosyl residues in the DNA complex.

Using this model for the tyrosyl peptide-DNA complexes we might now explain their selective stabilization of eukaryotic DNAs. Previous homopolymer studies have shown that Tyr-Gly-Tyr-Gly-Tyr peptide selectively stabilized poly dGdC. Other studies show that polystyrene bound Tyr-Gly-Tyr-Gly-Tyr selectively binds poly dGdC.* In the previous paper⁵ the portion of the calf thymus DNA stabilized by this peptide is highly enriched in % GC. Yet in our study on a number of different DNAs, there was no correlation between % GC and thermal stabilization of DNA. One possible explanation for this is that DNA stabilization reflects the number of available GC pairs separated by another 3-4 intervening base pairs. If this turns out to be the case, the thermal stabilization and snapback studies in Table 2 would reflect the frequency of occurrence of these guanine-containing sequences, and an analysis of these DNAs should show these sequences to be more abundant in the eukaryotic DNAs than in the prokaryotic DNAs.

The comparative peptide study in Table 1 indicates that the prerequisites for the peptide interaction with DNA is that the interacting sidechain

*Novak, R & Dohnal, J. (unpublished data).

residues possess a conjugated π -electron system and an electron donor atom. Previous NMR studies on the (Tyr-Gly-Tyr-Gly-Tyr)-poly A complex show "stacking" interaction between the purine and tyrosine π -electron systems.³ The same studies show an increased hyperchromicity of the tyrosine residues in the DNA complex. The attachment of electron withdrawing groups to the tyrosyl sidechains completely inhibits DNA stabilization (Table 1). All of these observations are consistent with hypothesis that the tyrosyl, tryptophanyl, and histidyl peptide sidechains form charge transfer complexes with the GC base pairs in synthetic and naturally occurring DNA's.^{8, 9} Further studies are in progress to investigate this hypothesis.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the U.S. Public Health Service Grant GM17335, of the American Cancer Society Grant 72-8 (Chicago Chapter), and of the Research Corporation.

REFERENCES

1. Helene, C., Montenay-Garestier, T., Dimicoli, J. (1971a) *Biochem. Biophys. Acta* 254, 349.
2. Helene, C., Dimicoli, J., Brun, F. (1971b) *Biochemistry* 10, 3802.
3. Novak, R., and Dohnal, J. (1973) *Nature, New Biology* 243, 155.
4. Thomas, C. A., and Abelson, J. (1966) *Procedures in Nucleic Acid Chemistry*, Cantoni, G. L. and Davies, D. R., Ed., pp. 553-561, Harper & Row, New York.
5. Novak, R., and Dohnal, J. (1974) *Nucleic Acids Research* (Preceding Paper).
6. Riordan, J. F., and Vallee, B. L. (1972) *Methods in Enzymology*, 25B, 515-521.
7. Rohott, D. A., and Pressman, D. (1972) *Methods in Enzymology*, 25B, 438-449.
8. Slifkin, M. A. (1971) *Charge Transfer Interaction of Biomolecules*, Academic Press, New York.
9. Slifkin, M. A. (1973) in *Physico-chemical Properties of Nucleic Acids*, Duchesne, J. Ed., pp. 67-98, Academic Press, New York.