Evidence from Circular Dichroism and from Melting Behavior for Helix-Inducing Complexation of a Designed β^3-Pentadecapeptide with DNA Duplexes

Preliminary Communication

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Inspired by naturally occurring DNA-binding proteins and their artificial α-peptidic mimics reported to date, a research project was initiated aiming at creating a new class of β-peptides capable of binding to and ultimately regulating the functions of DNA. As an initial foray, a β^3-pentadecapeptide **1**, which bears H-bonding Asn side chains and positively charged Lys side chains, was designed and synthesized on the solid support. DNA-Complexation studies by means of circular dichroism and DNA-melting-temperature measurements revealed the first preliminary indications that support the existence of ordered interactions between β-peptides and DNA.

1. Introduction. – Binding of peptides and proteins to DNA is one of the most fundamental processes in the chemistry of life [1][2]. Particularly well-known and intriguing is the fact that certain classes of natural proteins, such as eukaryotic transcription factors and bacterial repressors, fold parts of themselves into ordered secondary structures upon binding to the major groove of B-DNA duplexes [3][4]. Commonly, α-helix motifs formed as a result of such binding can recognize specific nucleobases in the DNA major groove through H-bonds with Asn amide side chains, whereas sequence-independent phosphate groups in the DNA backbone are often recognized through ionic interactions with cationic Arg, Lys, and/or His side chains. Inspired by the remarkably precise recognition motifs of natural proteins in combination with our previous explorations on β-peptide secondary structures, especially those on 3_{14} helices [5–7], we envisioned that we could construct a new class of β-peptides capable of binding to a DNA duplex in their 3_{14}-helical conformation[3]).

2. Design of the β-Peptide. – To meet our objective, a suitably functionalized β^3-pentadecapeptide **1** (*Fig. 1*) was designed with the following visions in mind:

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[3]) Interestingly, some cationic α- and β-oligoarginines, and α- and β-oligolysines have been observed to accumulate in the cell nucleus [8–11] although their actual binding targets as well as their binding modes are yet to be elucidated.

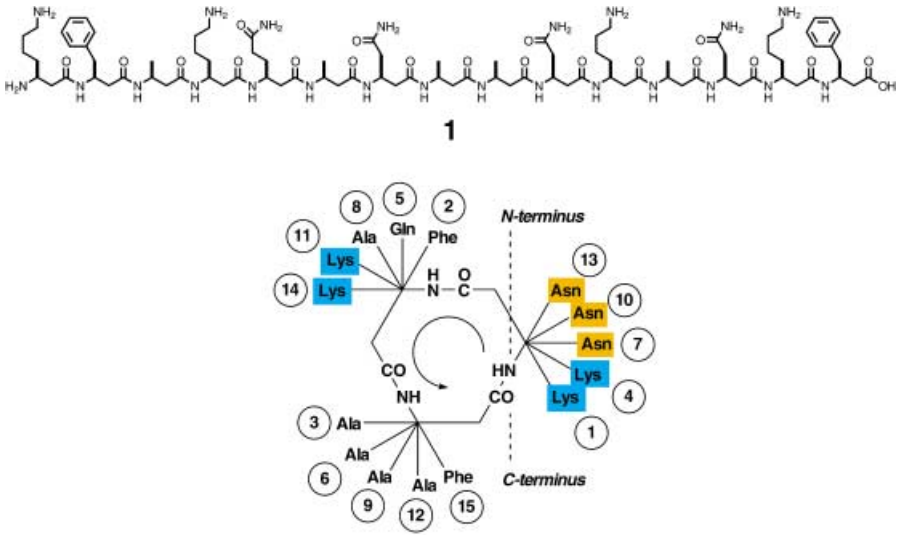

Fig. 1. *Formula and view along the axis of an idealized* 3_{14}*-helical secondary structure of* β^3*-peptide* **1**

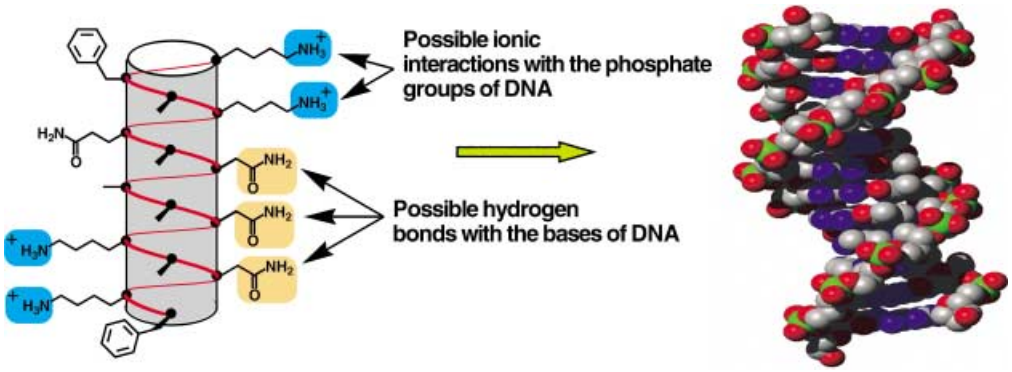

Fig. 2. *Schematic representation of a* 3_{14}*-helical secondary structure of the* β^3*-peptide* **1** *and CPK model of a DNA double helix* (PDB ID: 1D98) [13] *generated by MOLMOL* [12]

a) Generally, the shape of a β-peptide 3_{14} helix can be conceived as a peptide chain wrapped around a hypothetical cylinder in such a way that each pair of amino acid side chains at positions i and $i+3$ are aligned vertically on the side of the cylinder.
b) Consequently, three streaks of side-chain groups run in parallel to the axis of the cylinder at 120° apart from each other. A schematic representation of the helical form of the β-peptide **1** next to a 3-D model of a DNA duplex[4]) is shown in *Fig. 2*.
c) One of these streaks in the peptide **1** was designed to have three Asn side chains (Asn[7], Asn[10], and Asn[13]) to form a H-bonding recognition site.

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[4]) The DNA model was generated with the imaging software MOLMOL [12] from a deposited crystal structure: *Protein Data Bank* (http://www.rcsb.org/pdb) PDB-ID 1D98 [13].

d) Furthermore, four Lys side chains (Lys^1, Lys^4, Lys^{11}, and Lys^{14}) were positioned near the termini of the postulated 3_{14}-helical conformations to serve as ionic-interaction sites[5]).
e) Finally, hydrophobic side chains of Ala and Phe would line up in one of the streaks, endowing amphipathic character on the helix [14].

3. Synthesis of the β-Peptide. – According to the design concepts thus formulated, β-peptide **1** was assembled manually by solid-phase synthesis on *Wang* resin loaded with 0.29 mmol of Fmoc-β^3hPhe-OH, by standard Fmoc strategy [15]. All required building blocks Fmoc-β^3hXaa(PG)-OH were purchased from a commercial source[6]). An aliquot of the crude product was purified by HPLC to yield 7 mg of pure β-pentadecapeptide **1**, which was subsequently characterized by MALDI high-resolution mass spectrometry (*m/z* found: 1861.143 Da $[M+H]^+$, calc. for $C_{89}H_{150}N_{23}O_{20}$: 1861.135 Da).

4. Spectroscopic Studies of β-Peptide–DNA Interactions. – We first examined the CD spectrum of free β-peptide **1** in an aqueous buffer and found almost no negative *Cotton* effect near 215 nm (*Fig. 3,a*), confirming the absence of 3_{14} helix due to the repulsive cationic side chains of lysine residues[7]). We next mixed solutions of DNA duplexes $dA_{20}\cdot dT_{20}$ and $dG_{20}\cdot dC_{20}$[8]) with β-peptide **1** to obtain a 20 μM buffer solution (pH 7.5) for each peptide–DNA complex. As illustrated by the difference CD spectra of the resulting solutions presented in *Fig. 3,a*, the changes in peptide CD profiles caused by the presence of DNA duplexes clearly show that there are certain interactions between the DNA duplex molecules and the β-peptide **1**. More specifically, the distinct pattern of the CD curves thus obtained resembles that of 3_{14}-helical β-peptides [6][16], with normalized minimum Θ values ranging between -15000 and -25000 [deg·cm^2·dmol^{-1}], albeit the minima are slightly shifted toward shorter wavelengths. The effect seems stronger with the CG pair than with the AT pair[9]), although quantitative comparison requires further investigation. We also performed similar experiments with single-strand DNA oligomers dA_{20}, dT_{20}, dC_{20}, and dG_{20}, and their difference CD spectra are presented in *Fig. 3,b*. The apparent deviation of the curves from the control-peptide curve indicates that there are also interactions between β-peptide **1** and single-strand oligonucleotides, but probably in a less-specific fashion.

To determine whether β-peptide **1** causes any effects upon thermal stability of DNA duplexes, we measured the UV melting curves of $dA_{20}\cdot dT_{20}$ in the absence and the presence of β-peptide **1** ($\lambda = 260$ nm, $10^\circ \le T \le 60^\circ$, 4 μM of $dA_{20}\cdot dT_{20}$, 20 μM of **1** with

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[5]) Charge repulsion between these side chains was actually expected to destabilize the 3_{14}-helical conformation of a free β-peptide in aqueous solutions.

[6]) *Fluka Chemie GmbH* (CH-Buchs); we gratefully acknowledge their discount offers.

[7]) Correlation between the negative *Cotton* effect near 215 nm and the 3_{14} helix was reported from these laboratories [6][16]. For a more-general discussion of 3_{14}-helix formation in H_2O by β-peptides consisting of noncyclic β-amino acids, see [17–19].

[8]) The DNA duplexes were prepared from the corresponding single-strand oligonucleotides dA_{20}, dT_{20}, dG_{20}, and dC_{20}, which were purchased in HPLC-pure form from *Microsynth GmbH* (CH-Balgach).

[9]) Zero-crossing was observed below 205 nm in the CD spectra of both the $dG_{20}\cdot dC_{20}$- and $dA_{20}\cdot dT_{20}$-containing peptide solutions. Remarkably, the CD curve of the former does not level off near zero value at longer wavelength, suggesting alternation of DNA double-helical structure.

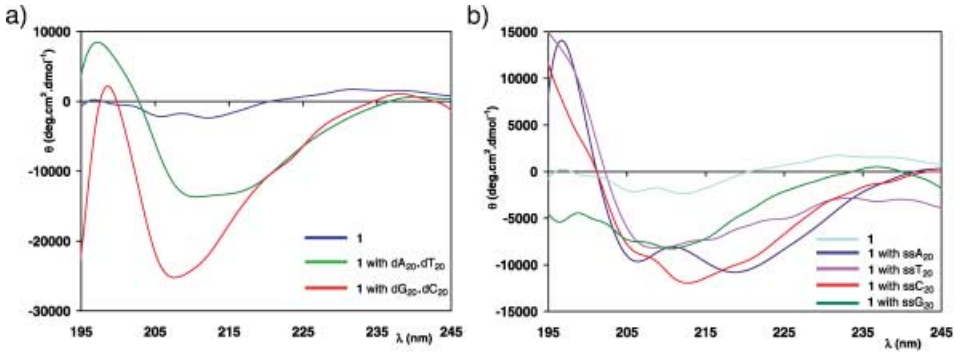

Fig. 3. *CD Spectra of β^3-peptide* **1** *in the presence of ds- and ss-oligonucleotides. a*) In the presence of ds-oligonucleotides $dA_{20} \cdot dT_{20}$ and $dG_{20} \cdot dC_{20}$ the CD spectra of **1** show a pattern with a single minimum between 205 and 210 nm, which can be considered characteristic of a β-peptidic 3_{14} helix. *b*) The change in the CD pattern of **1** in the presence of ss-oligonucleotide probably indicates an interaction between β-peptide **1** and the ss-oligonucleotides. CD Measurements were performed at 10° on a *JASCO J-710* spectropolarimeter in a 1-mm cell. Samples containing 20 μM β-peptide **1**, 20 μM DNA, and 20 mM NaCl in 10 mM phosphate buffer solution (pH 7.5). Spectra of the β-peptide in the presence of DNA were calculated as the difference between the spectrum of the β-peptide–DNA mixture and the spectrum of the free DNA, and then binomially smoothed for clarity.

the same buffer concentration as CD measurements). Upon increasing the sample temperature from 10° to 60°, both UV absorbance curves with and without the peptide followed the essentially identical trajectory, the melting temperature of which fell into the range of 36.7–36.9° (*Fig. 4*,*a* and *b*; [20]: T_m for free $dA_{20} \cdot dT_{20}$: 38.0°). However, when the temperature returned from 60° to 10°, the corresponding UV spectra showed that the dissociated complementary single-strand DNA did not rewind to form the original duplex in the presence of β-peptide **1** (*Fig. 4*,*b*), contrary to the completely reversible thermal hysteresis of free DNA melting in the absence of the peptide (*Fig. 4*,*a*). One reasonable interpretation is that the peptide interacts with DNA single strands and hence interferes with the rewinding process, giving rise to an altered DNA structure at the end of the melting cycle. Remarkably, the resulting new structural entity shows quintessentially reversible thermal hysteresis with distinct hyperchromicity around 36° (*Fig. 4*,*c* and *d*), indicating the existence of a certain form of stable DNA double-helical structure.

5. Conclusions. – Combining simple and straightforward spectroscopic methods with our accumulated insight into β-peptide structures, we demonstrated that the designed β-peptide **1** can interact with both DNA duplexes and single strands. To the best of our knowledge, this is the first published report on the structurally ordered interaction[3]) of a β-peptide with DNA molecules. Further investigations into thermodynamics and kinetics of the interaction, as well as structure-affinity relationships and sequence specificity between β-peptides and DNA, are in progress.

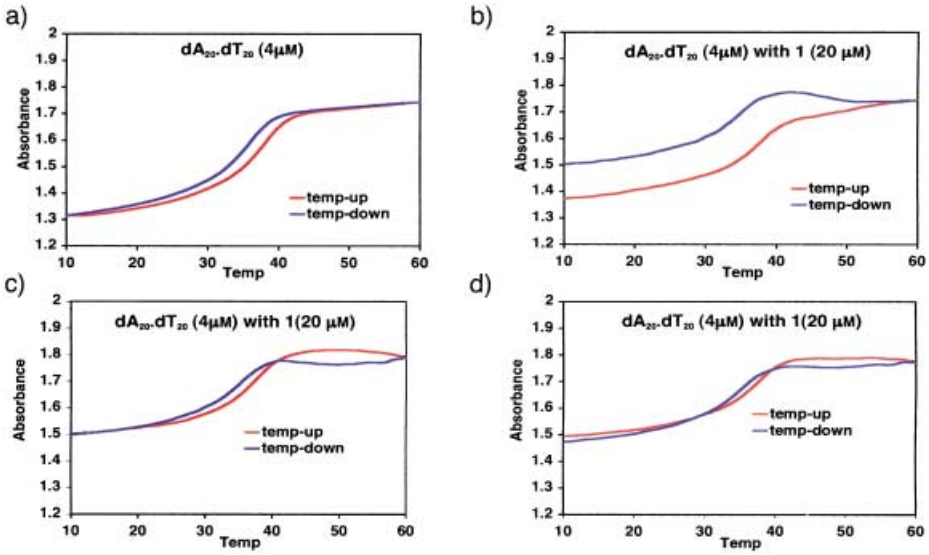

Fig. 4. *Melting curves of $dA_{20}\cdot dT_{20}$ in the presence of β-peptide* **1**. Free $dA_{20}\cdot dT_{20}$ (control) showed thermally-reversible sigmoidal melting curves (*a*), whereas $dA_{20}\cdot dT_{20}$ with peptide **1** behaved similarly to the control only during heating, but eventually started to deviate from the ideal reversible hysteresis upon cooling of the mixture (*b*). After the first melting measurement, the melting properties of the mixture shifted to a new thermal hysteresis (*c*), which remained reversible over repetitive heating-cooling cycles (*d*). Melting curves were measured at 260 nm on a *Varian Cary 100* UV/VIS spectrophotometer in a 1-cm cell with the temperature increasing from 10° to 60° at 1°/min and decreasing from 60° to 10° at the same rate. Samples were prepared with the same concentration as the CD measurement except for DNA concentration (4 μM).

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