DNA-induced α-Helical Structure in the NH$_2$-terminal Domain of Histone H1*

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It is important to establish the structural properties of linker histones to understand the role they play in chromatin higher order structure and gene regulation. Here, we use CD, NMR, and IR spectroscopy to study the conformation of the amino-terminal domain of histone H1°, free in solution and bound to the DNA. The NH$_2$-terminal domain has little structure in aqueous solution, but it acquires a substantial amount of α-helical structure in the presence of trifluoroethanol (TFE). As in other H1 subtypes, the basic residues of the NH$_2$-terminal domain of histone H1° are clustered in its COOH-terminal half. According to the NMR results, the helical region comprises the basic cluster (Lys$^{11}$–Lys$^{29}$) and extends until Asp$^{23}$. The fractional helicity of this region in 90% TFE is about 50%. His$^{21}$ together with Pro$^{25}$ constitute the joint between the NH$_2$-terminal helix and helix I of the globular domain. Infrared spectroscopy shows that interaction with the DNA induces an amount of α-helical structure equivalent to that observed in TFE. As coulombic interactions are involved in complex formation, it is highly likely in the complexes with DNA that the minimal region with α-helical structure is that containing the basic cluster. In chromatin, the high positive charge density of the inducible NH$_2$-terminal helical element may contribute to the binding stability of the globular domain.

The linker histone H1 has a role in the stabilization of both the nucleosome and chromatin higher order structure. Linker histones contain a globular domain flanked by highly basic amino- and carboxyl-terminal tails (1). The terminal domains have, in general, little structure in solution. The COOH-terminal domain acquires, however, a substantial amount of α-helix in the presence of secondary structure inducers such as TFE$^1$ and NaClO$_2$ (2), suggesting that binding to DNA could stabilize helical segments in the COOH-terminal domain. It has been shown previously by FTIR spectroscopy that a COOH-terminal peptide of histone H1° becomes fully structured upon interaction with the DNA (3). The structures of a turn and of a helix-turn motif belonging to the COOH-terminal domain have been determined by high resolution NMR in the presence of helix stabilizers (4, 5).

It is currently accepted that H1 could have a regulatory role in transcription through the modulation of chromatin higher order structure. In vitro experiments with reconstituted chromatin have shown that H1 can repress promoters containing the RNA start site in the linker DNA, and that some sequence-specific transcription factors can counteract the H1-mediated repression. Preferential binding to scaffold-associated regions and participation in nucleosome positioning have been proposed as other possible mechanisms by which H1 could contribute to transcriptional regulation (6–9). The involvement of H1 in the 300-Å chromatin fiber, which presumably limits the access of the transcriptional machinery, led to the proposal that H1 subtypes may function as generalized repressors. More recently, experiments in vivo have suggested that H1 may regulate transcription at a finer level, participating in complexes that either activate or repress specific genes (6, 7, 10–12). Some gene-specific effects could be attributed only to the globular domain (13), whereas other effects were localized to the tail-like domains (14, 15). The structure of the terminal domains of H1 when bound to the DNA is therefore of great importance to the understanding of H1 function.

The NH$_2$-terminal domain of H1° presents two distinct subregions, which are also found in other H1 subtypes (16). The distal half is devoid of basic residues, whereas the half immediately adjacent to the globular domain is highly basic. The basic cluster is apparently involved in the location and anchoring of the globular domain (17).

We have used CD, high resolution NMR, and IR spectroscopy to study the structure of the NH$_2$-terminal domain of the H1 subtype H1° in aqueous and TFE solution and in the complexes with the DNA. The NH$_2$-terminal domain has little structure in aqueous solution, but in TFE it acquires a high degree of helical structure, predominantly in the region containing the basic cluster. We show that the interaction with the DNA induces a significant amount of α-helical structure in the NH$_2$-terminal domain, similar to that observed in TFE.

EXPERIMENTAL PROCEDURES

Peptide Synthesis—The peptides Ac-TENSTSAPAAKPRAKASKKSTDHPKYSDM-NH$_2$ (NH-1) and Ac-PAAKPKRAKASKSTDHPKYSDM-NH$_2$ (NH-2) were synthesized by standard methods (NH-1 by Nsysystem Laboratoire (Strasbourg, France) and NH-2 by DiverDrugs (Barcelona, Spain)). Peptide homogeneity was determined by high performance liquid chromatography on Nucleosil C18 (NH-1) and Kromasil C8 (NH-2) columns. The peptide composition was confirmed by amino acid

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* The abbreviations used are: TFE, trifluoroethanol; NOE, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; FTIR, Fourier transform infrared.

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analysis, and the molecular mass was checked by mass spectrometry. The sequence of NH-1 corresponds to residues 1–20 and that of NH-2 to residues 8–30 of histone H1°. The sequence 1–30 is common to mouse and humans and presents two substitutions in rat, at positions 7 (Ala → Thr) and 18 (Ser → Ala). The peptides were acetylated and amidated during the synthesis process to remove the dipole destabilization effect.

**Circular Dichroism Spectroscopy**—Samples for circular dichroism spectroscopy were 3.7 \( \times \) 10^{-5} M solutions of the peptide in 10 mM NaCl, 5 mM phosphate buffer, pH 3.5. Samples in aqueous and mixed solvent with different ratios (v/v) of trifluoroethanol/H\(_2\)O were prepared. Spectra were obtained on a Jasco J-715 CD spectrometer at 20°C. The results are expressed as mean residue molar ellipticities, [θ]. The helical content was estimated from the ellipticity value at 222 nm, (\([θ]_{222}\)), according to the empirical equation of Chen et al. (18).

\[
\text{% helical content} = \frac{100(\text{[θ]}_{222} - 39,500 \times (1 - 2.57/n))}{\text{Eq. 1}}
\]

where \(n\) is the number of peptide bonds in the helix. The helical length was determined from the NMR data.

**\(^1\)H NMR Spectroscopy**—Samples were routinely prepared as 2.7 mM solutions of the peptide in 90% deuterated TFE, 10 mM NaCl, 5 mM phosphate buffer, pH 3.5. Sodium 3-trimethylsilyl (2,2,3,3-2H\(_4\)) propionate was used as internal reference. Spectra were acquired in a Bruker AMX-600 spectrometer. All two-dimensional spectra were recorded in the phase-sensitive mode using time-proportional phase incrementation (19) with presaturation of the water signal. Correlation spectroscopy (20) and NOESY (21) spectra were obtained using standard phase-cycling sequences. Short mixing times of 150 ms were used in the NOESY experiments to avoid spin diffusion. Spectra were obtained at 25°C. Total correlation spectroscopy spectra were acquired using the standard MLEV16 spin-lock sequence with a mixing time of 80 ms (22). The phase-shift was optimized for every spectrum.

The assignments of the \(^1\)H NMR spectra were performed by standard two-dimensional sequence-specific methods (23, 24).

Quantification of the helix populations on the basis of the up-field shifts of the C\(^2\)H δ-values upon helix formation was carried out according to Jiménez et al. (25). The average helical population per residue was calculated by dividing the average conformational shift, \(\Delta δ = \Sigma (δ_{\text{obs}} - \delta_{\text{H}})\), by the shift corresponding to 100% helix formation. Random coil values, \(δ_{\text{RC}}\), were those given by Wuthrich (24). The ran-

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**Fig. 1.** TFE-dependent conformational transition of the NH-1 and NH-2 peptides measured by CD. Figure shows far ultraviolet CD spectra in the presence of various concentrations of TFE in phosphate buffer 5 mM, pH 3.5 at 5°C. The numbers refer to the TFE concentration in percentage by volume. A, NH-1 peptide; C, NH-2 peptide. Variation of the mean residue molar ellipticity ([θ], degrees cm\(^2\) dmol\(^{-1}\)) at 222 nm with added TFE. The percentage of helical structure, calculated as described under “Experimental Procedures” is also indicated. B, NH-1; D, NH-2.

**Fig. 2.** Selected regions of the two-dimensional NOE spectrum of peptide NH-2. The peptide was 2.7 mM, in 90% TFE, 10% H\(_2\)O, 10 mM NaCl, 5 mM phosphate buffer, pH 3.5, 25°C. Mixing time was 150 ms. Medium range NOE connectivities are boxed. Left, NOE correlations of C\(^2\)H protons with amide or aromatic resonances. Right, NOE correlations of C\(^2\)H protons with other aliphatic protons.
dom coil values used for Ala7, Lys11, and His24 were those given for amino acids followed by Pro (26). A value of -0.39 ppm was used as the shift for 100% helix formation (27). The helical length, n, was determined on the basis of NOE cross-peaks and conformational shifts and confirmed by structure calculations.

Structure Calculations—Calculations of peptide structures were carried out with the program for torsion angle dynamics DYANA (28). Distance constraints were derived from the 150-ms NOESY spectrum acquired in 90% TFE at 25 °C, pH 3.5. The intensities of the observed NOEs were evaluated in a qualitative way and translated into upper-limit distance constraints according to the following criteria; strong NOEs were set to distances lower than 0.3 nm; medium, lower than 0.35 nm, and weak, lower than 0.45 nm. Pseudo atom corrections were set to the sum of the van der Waals radii. φ angles were constrained to the range -180° to 0°.

Infrared Measurements—Peptide samples were measured at 4.6 mg/ml. Peptide-DNA complexes contained 6.7 mg/ml DNA and the appropriate amount of peptide. Samples were measured in 10 mM HEPES, pH 7.0, plus either 10 or 70 mM NaCl. Data treatment and band decomposition of the original amide I' have been described previously (3, 29). The DNA contribution to the spectra of the complexes of the NH-1 and NH-2 peptides with mouse DNA and alternating poly(dA-dT) was subtracted according to Vila et al. (3). In addition, spectra of complexes of different peptide to DNA ratio (0.3 and 0.7, w/w) were recorded. The spectrum of the peptide was independent of the peptide to DNA ratio of the complex inside the statistical error, indicating that the amide I' region was basically free of alterations resulting from DNA spectral changes.

RESULTS

CD Analysis of NH-1 and NH-2—We have studied the peptides Ac-TENSTSAPAAPKRKASKK-NH2 (NH-1), which corresponds to the complete NH2-terminal domain of the histone H1 subtype H1′ (residues 1–20) and the peptide Ac-FKAAPMRKASKKSTDHPKYSDM-NH2 (NH-2), which partially overlaps with NH-1, but without the first 7 residues and which extends 10 residues into the adjacent globular domain (residues 8–29).

The CD spectrum of both peptides in H2O was dominated by the contribution of the random coil. The mean residue molar ellipticity at 222 nm (θ222), taken as criterion of helix formation, was negligible in water. However, the small positive peak at 215 nm, characteristic of the random coil, was not observed, suggesting that a small amount of structure could be present in solution (Fig. 1). Addition of TFE, which stabilizes peptide secondary structure, increased the negative ellipticity at 222 nm. The helical content of the peptides as a function of TFE concentration was estimated by the method of Chen et al. (18). NH-2 had a higher helical propensity than NH-1. In 90% TFE solution, the helical populations were estimated to be 17% for NH-1 and 29% for NH-2 (Fig. 1).

NMR Analysis of NH-1 and NH-2—Fig. 2 shows selected regions of the two-dimensional NOE spectrum of NH-2 in 90% TFE, where NOE correlations corresponding to medium-range interactions are indicated. Fig. 3 and Tables I and II summarize all relevant NOE data for the peptides in 90% TFE. The figure also shows the plot of the conformational shifts of the C=H protons with respect to the random coil values.

The NMR analysis in 90% TFE confirmed the higher helical propensity of NH-2, as shown by more abundant nonsequential (i, i + 3) and (i, i + 4) connectivities in NH-2 than in NH-1. In NH-1, a stretch of αN(i, i + 3) connectivities spanned the basic cluster between Lys11 and Lys20. NOE connectivities αN(i, i + 3) between Pro12 and Lys14, αH(i, i + 3) between Pro12 and Lys16, αβ(i, i + 3) between Pro12 and Ala15, and αN(i, i + 4) between Lys11 and Arg14 were also present. The calculation of the amount of helical structure based on the C=H chemical shifts gave a value of 17%. The negative values (−0.08) of C=Hαδ for the sequence Ala7−Ala9, together with an αN(i, i + 3) connectivity between Ala7 and Ala9 suggest that the peptide could have a slight helical propensity between Ala7 and the beginning of the basic cluster at Lys11. However, as shown below, the structures calculated on the basis of distance constraints derived from observed NOE cross-correlations had helical structure only in the second half of NH-1. Accordingly, we chose Lys11 and Lys19 as the limits of the helical region of NH-1.

The higher helical propensity of NH-2 over NH-1 suggested that the helical element in NH-1 could extend beyond the limit of the NH2-terminal domain, as defined by trypsin cutting at Lys20. Indeed, in NH-2 stretches of αN(i, i + 3), αβ(i, i + 3), and αN(i, i + 4) NOE connectivities spanning from Lys11 to His24 were observed. The abundance of αN(i, i + 4) correlations between Lys11 to His24 unequivocally showed the presence of α-helical structure in this region. The C=Hαδ between Lys19 and His24 were less negative than would be expected from the abundance of medium range cross-correlations in this region. This might be the result of the ring current shift effect pro-
duced by the histidine ring system, and precluded accurate calculation of the amount of helical structure of this peptide based on the C\textsuperscript{13}H chemical shifts.

Structure Calculations—The structure calculations were performed on the basis of the NOE cross-correlations observed in 90\% TFE. A set of 42 distance constraints, composed of 25 sequential and 17 medium range constraints, was used to calculate the three-dimensional structure of NH-1. In NH-2, 53 distance constraints, 26 sequential and 27 medium range, were used. A number of 50 structures were generated for each peptide by using the distance geometry program DYANA (28). The best 20 converged structures were chosen for each peptide. The global RMS deviation of the backbone atoms for this set of structures, excluding the first and last residues, was 0.32 ± 0.09 nm for NH-1 and 0.23 ± 0.07 nm for NH-2. The maximum NOE violations were 0.05 and 0.02 nm for NH-1 and NH-2, respectively. A superposition of the backbones of the best 20 selected structures of each peptide is shown in Fig. 4.

In NH-1, the region spanning from Lys 11 to Lys 19 adopts a well defined α-helical structure. In NH-2, the α-helical conformation spans from Lys 11 to Asp 23, with His 24 as Ccap. His 24 presents an αN(i, i + 4) cross-correlation with Lys 20, but its torsion ψ (−31.7 ± 1.9) indicates that the α carbon departs slightly from the helix cylinder, whereas torsion ψ is proper of an extended configuration (ψ = +95.3 ± 0.1).

Fig. 5A shows a view down the helix axis of the helical region of one of the calculated structures of NH-2. B, clustering of the basic residues on opposite faces of the helix.

| Residue 1 | Residue 2 | Intensity |
|-----------|-----------|-----------|
| ββ′Asn\textsuperscript{3} | NHThr\textsuperscript{5} | Weak |
| ββ′Asn\textsuperscript{3} | NHSer\textsuperscript{6} | Weak |
| αAla\textsuperscript{8} | βPro\textsuperscript{12} | Weak |
| αAla\textsuperscript{8} | βPro\textsuperscript{12} | Weak |
| αLys\textsuperscript{11} | δδArg\textsuperscript{14} | Weak |
| εδLys\textsuperscript{16} | NHSer\textsuperscript{18} | Weak |

| Residue 1 | Residue 2 | Intensity |
|-----------|-----------|-----------|
| αLys\textsuperscript{19} | γH\textsubscript{3}Thr\textsuperscript{22} | Medium |
| αSer\textsuperscript{21} | δHis\textsuperscript{24} | Weak |
| αThr\textsuperscript{22} | δHis\textsuperscript{24} | Weak |
| βThr\textsuperscript{22} | δHis\textsuperscript{24} | Weak |
| αThr\textsuperscript{22} | εHis\textsuperscript{24} | Weak |
| βThr\textsuperscript{22} | εHis\textsuperscript{24} | Weak |
| αPro\textsuperscript{23} | ββ′Asp\textsuperscript{29} | Medium |
| NHLys\textsuperscript{26} | ββ′Asp\textsuperscript{29} | Weak |
| αTyr\textsuperscript{27} | γMet\textsuperscript{30} | Weak |
| αTyr\textsuperscript{27} | γMet\textsuperscript{30} | Weak |

Table I

Summary of side-chain to side-chain and side-chain to main-chain NOE connectivities other than those expected for regular helices (αN(i, i + 3)) found for the NH-1 peptide in 90\% TFE

Table II

Summary of side-chain to side-chain and side-chain to main-chain NOE connectivities other than those expected for regular helices (αN(i, i + 3)) found for the NH-2 peptide in 90\% TFE

Fig. 5. Distribution of the basic residues in the helical region of NH-2. A, end view down the helix axis of one of the calculated structures of NH-2. B, clustering of the basic residues on opposite faces of the helix.
Infrared Spectroscopy Analysis of the NH-1 and NH-2 Peptides in Solution—We have used FTIR spectroscopy to study the conformation of NH-1 and NH-2, both in solution and in the complexes with DNA. IR spectroscopy is particularly well suited to the study of the complexes of DNA with basic peptides because it is not affected by turbidity. The number and initial position of the amide I component bands were obtained by Fourier deconvolution as described (29). Values corresponding to band position and percentage area are given in Tables III and IV.

In aqueous (D$_2$O) solution, the amide I of both NH-1 and NH-2 was dominated by the random coil band at 1641 cm$^{-1}$ (representing 42% of the total amide I intensity in NH-1 and 44% in NH-2) (30). The other main component (29% of the total amide I intensity in NH-1 and 24% in NH-2) at 1661 cm$^{-1}$ was assigned to turns. Minor components at 1674 cm$^{-1}$ were also attributed to turns. Small amounts of α-helix at 1651 cm$^{-1}$ were also present (6% in NH-1 and 8% in NH-2).

The helical population increased considerably in TFE solution, both in NH-1 and NH-2. In NH-1, the α-helix amounted to 22% of the total amide I intensity, whereas, in NH-2, it amounted to 30%. These values are compatible with those estimated by CD. The random coil component decreased reciprocally with the increase of α-helix. In TFE, the turn components increased moderately in NH-1, whereas they remained approximately constant in NH-2. The relative intensities of the different turn components varied in the two peptides.

Infrared Spectroscopy Analysis of the NH-1 and NH-2 Peptides Bound to the DNA—We have studied the structure of NH-1 and NH-2 bound to mouse DNA and alternating poly(dA-dT)poly(dA-dT) (Fig. 6). In the peptide-DNA complexes, the α-helix (1651 cm$^{-1}$) amounted to 18–20% of the total amide I intensity in NH-1 and 25–26% in NH-2. As the percentage of α-helix in aqueous solution was only 6–8%, these results indicate that interaction with the DNA stabilizes the helical structure of the peptides. The amounts of helical structure observed in the complexes with DNA are similar to those observed in TFE solution, by both IR and CD. The contribution of the random coil band at 1641 cm$^{-1}$ in the complexes was 17–20%, which is similar to that observed in TFE solution. Turn components were observed at 1662, 1672, and 1682 cm$^{-1}$. They represented between 32 and 40%, depending on the peptide. The complexes of both peptides presented a component at 1630–32 cm$^{-1}$, which amounted to 16–17% of the total amide I intensity. Vibrations at this wavenumber are usually attributed to β-sheets (31), although they have also been assigned to extended structures (30) and even to α-helix vibrations (32).

The type of DNA does not appear to affect significantly the properties of the complexes, as the percentages and positions of the different components of the amide I were similar with poly(dA-dT)poly(dA-dT) and mouse DNA.

**DISCUSSION**

The NH$_2$- and COOH-terminal domains of histone H1 have little structure in aqueous solution. However, the COOH-terminal domains of several H1 subtypes acquire variable amounts of α-helical structure in the presence of TFE and other secondary structure stabilizers (2). The presence of a inducible helix-turn motif in the COOH-terminal domain of histone H1$^\circ$ has been demonstrated by NMR and FTIR spectroscopy (3, 5). The structure of the NH$_2$-terminal domain had not been addressed before, either in solution or in the complexes with the DNA. Here, we show that the interaction with the DNA induces a significant amount of α-helical structure in the NH$_2$-terminal domain of H1$^\circ$.

The first half of the NH$_2$-terminal domain of H1$^\circ$ is devoid of basic residues, and it is thus not expected to interact strongly with the DNA. In contrast, the second half of the domain is highly basic, with 1 Arg and 5 Lys residues. The proximity of this region to the globular domain suggests that it may contribute to the binding stability of the globular domain in chromatin. The clustering of the basic residues of the NH$_2$-terminal domain in the vicinity of the globular domain, leaving the rest of the domain free of basic residues, is a common feature of H1 subtypes (16).

We have studied two peptides: one comprising the 20 residues of the NH$_2$-terminal domain (NH-1), as defined by trypsin cutting at Lys$^{20}$, and the other of 23 residues, spanning 10 residues into the globular domain, and lacking the first 7 residues of the protein (NH-2). NH-1 and NH-2 have little structure in aqueous solution. The IR spectrum of both peptides is dominated by the random coil and to a lesser extent by turn conformations, probably in rapid equilibrium with the unfolded state. Small amounts of α-helix are also present. TFE induces a significant increase in the amount of α-helical structure in both peptides. NH-2 has a much higher helical propensity than NH-1, with 30% helical structure compared with the 18% of NH-1. When the amount of α-helix for the entire NH-2 peptide is corrected for the length of the helical region (13 residues), a fractional helicity of 57% is obtained. This relatively high helical propensity explains the abundance of medium range NOE cross-correlations in this region.

The NMR analysis shows that the α-helix is induced in the positively charged second half of the NH$_2$-terminal domain. In NH-1, the helical region spans from Lys$^1$ to Arg$^{19}$. In NH-2, the helical element also begins at Lys$^{11}$, but it spans until Asp$^{23}$, with His$^{24}$ as Ccap, surpassing the limit of the globular domain as defined by trypsin cutting at Lys$^{20}$. The NH$_2$-terminal helix element is thus close to helix I of the globular domain, which starts at Lys$^{26}$. The extension of the structural limit of the NH$_2$-terminal domain beyond the proteolytic limit, together with the proximity of the NH$_2$-terminal helical element.

| Assignment | Band position | Band area | D$_2$O | 90% TFE | NH1/mouse DNA | NH1/poly(dA-dT) poly(dA-dT) |
|------------|---------------|-----------|--------|---------|---------------|-----------------------------|
| β-Structure | 1632 | 0 | 0 | 0 | 17 | 17 |
| Random     | 1641–1642 | 42 | 17 | 18 | 20 | 18 |
| α-Helix    | 1651–1653 | 6 | 22 | 18 | 17 | 17 |
| Turns      | 1651–1663 | 29 | 25 | 16 | 15 | 15 |
| Turns      | 1672–1674 | 7 | 19 | 1 | 1 | 1 |
| Turns      | 1682–1685 | 2 | 13 | 8 | 7 | 7 |
to helix I, supports the view that the NH$_2$-terminal basic cluster and the globular domain of histone H1 may act in concert in chromatin structure (17). The proximity of the NH$_2$-terminal basic cluster to the DNA at the entry/exit point of the nucleosome is supported by the cross-linking of His$_{25}$ of histone H5, an avian H1 subtype closely related to H1° (34), to the terminal regions of chromatosomal DNA (35). Fig. 7B shows a model structure with the NH$_2$-terminal domain of H1° connected to the globular domain of H5 (36).

The question is whether the interaction with the DNA induces a significant increase in the helicity of the NH$_2$-terminal peptides. IR spectroscopy shows that it is indeed the case. The percentages of $\alpha$-helix of NH-1 and NH-2 in the complexes are 18–20% and 27–29%, respectively. These percentages are close to those observed in TFE solution and agree well with the different helical propensities of the two peptides. They reflect an important stabilization of the helical structure when compared with the low percentages present in water (6–7%) and demonstrate that the DNA displaces the coil (disordered) helix equilibrium toward helix formation. As a low resolution technique, IR spectroscopy cannot, in general, specify which

### Table IV

| Assignment | Band position (cm$^{-1}$) | D$_2$O | 90% TFE | NH2/mouse DNA | NH2/poly(dA-dT)$\cdot$poly(dA-dT) |
|------------|--------------------------|--------|---------|---------------|----------------------------------|
| $\beta$-Structure | 1629–1630 | 0 | 9 | 14 | 18 |
| Random | 1640–1644 | 44 | 25 | 19 | 16 |
| $\alpha$-Helix | 1651–1655 | 8 | 32 | 29 | 27 |
| Turns | 1663 | 24 | 8 | 20 | 17 |
| Turns | 1672–1674 | 6 | 16 | 10 | 9 |
| Turns | 1681–1684 | 3 | 2 | 6 | 5 |

**Fig. 6.** Amide I’ decomposition of the spectra of the complexes of NH-1 and NH-2 peptides with the DNA. A, difference spectrum of the complex of NH-1 with mouse DNA. B, difference spectrum of the complex of NH-1 with poly(dA-dT)$\cdot$poly(dA-dT). C, difference spectrum of the complex of NH-2 with mouse DNA. D, difference spectrum of the complex of NH-2 with poly(dA-dT)$\cdot$poly(dA-dT). The reconstitution of the spectra from the component bands is indicated by dashed lines. The dashed and continuous lines are virtually superimposed, because of the goodness of the fit. The Fourier deconvolution of the absorption spectra is included. Peptide/DNA ratio = 0.7 (w/w).

**Fig. 7.** Connection of the NH$_2$-terminal and globular domains. A, view of one of the calculated structures of NH-2 showing the NH$_2$-terminal helix (Lys$_{11}$$\rightarrow$Asp$_{23}$) and the first turn of helix I of the globular domain (Lys$_{38}$$\rightarrow$Met$_{50}$); B, model structure of the NH$_2$-terminal domain of H1° connected to the crystal structure of the globular domain of H5.
particular residues are in helical conformation, but, because coulombic interactions between basic residues and DNA phosphates promote helical compaction in model and natural peptides (3, 37, 38), it is likely that the minimal region with helical structure in NH-2 is that containing the basic cluster, between Lys11 and Lys20, although, as in TFE, the helical structure could extend to Asp23.

Significant as they are, the percentages of α-helix in the complexes are, nevertheless, indicative of the presence of alternative modes of binding. Indeed, if the helical elements as defined by NMR in TFE solution were always in a helical conformation in the complexes, the amount of α-helix should be ~45% for NH-1 and about 56% for NH-2, instead of ~20 and 30%, respectively. These other modes of binding could be at the origin of the component band at 1630 cm⁻¹, and contribute to the components attributed to turns. A possible explanation of the observed values of fractional helicity would be that DNA can only partially mimic the nucleosome environment of the NH₂-terminal domain.

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