

Structural organization of DNA–protein complexes of chromatin studied by vibrational and electronic circular dichroism

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Abstract. Structure and functioning of chromatin is determined by interactions of DNA with numerous nuclear proteins. The most abundant and yet not completely understood non-histone chromosomal proteins are those belonging to a High Mobility Group (HMG) namely HMGB1. The interplay of this protein on DNA with linker histone H1 and other proteins determines both structure and functioning of the chromatin. A combination of UV and IR absorption and circular dichroism (CD) spectroscopy was applied to investigate the structure and formation of large supramolecular DNA–protein complexes. This combination of techniques was used to overcome limitations of UV-CD (ECD) spectroscopy due to considerable light scattering in such solutions. Based on the analysis of FTIR and UV circular dichroism spectra and AFM imaging the interaction of DNA with high-mobility group non-histone chromatin protein HMGB1 and linker histone H1 was studied.

Keywords: DNA, chromatin, HMGB1, circular dichroism, VCD

1. Introduction

Structural organization of DNA in chromatin has been extensively studied for many years. When the structure of nucleosome was solved, the investigations of the higher levels of the structural organization became one of the most important steps in understanding the chromatin functioning [17,18]. A great variety of DNA-binding proteins interact with DNA forming intricate DNA–protein complexes. DNA-bound protein factors normally cooperate to assemble higher-order nucleoprotein structures in which multiple protein–DNA and protein–protein contacts increase the specificity and stability of the final complexes [4]. There is the whole class of ‘architectural’ proteins in chromatin that perform these structural functions. Among such proteins are HMGB-domain proteins. Their common features are the DNA-binding domains, often called HMG-Box domains [11,13] whose structure is highly conservative. Some of the proteins in this family contain only one HMGB-domain but a great number of others possess two or more of these domains. The members of the family are able to distinguish and preferably bind to DNA four-way junctions, binding sites of the anticancer drug cisplatin and different bends

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and crossovers in general [14]. Our previous studies revealed the ability of HMGB1 protein to induce formation of ordered multi-molecular complexes upon binding to DNA [2,9]. However, their structural investigation using UV-CD, the spectroscopic technique that can be routinely applied to DNA and protein studies, is limited due to the emerging light scattering in the solutions resulting from the big size of the complexes. The aim of the present study is to demonstrate the possibility to study such DNA–protein complexes spectroscopically combining UV-CD with IR circular dichroism.

2. Materials and methods

To obtain the DNA–protein complexes, histone H1 (MW = 21,000) and non-histone protein HMGB1 (MW = 26,500) were used. Both proteins were isolated from calf thymus as described earlier [2]. Calf thymus DNA (Sigma) was sonicated as described elsewhere [6]. All aqueous solutions were prepared using double-distilled water. Heavy water (99.9% D₂O) for the IR experiments was purchased from Sigma. The protein content in the system was described in terms of the protein to DNA weight to weight ratio (r , w/w). In all complexes the H1 to HMGB1 w/w ratio was maintained at 1:1. UV absorbance and ECD spectra of the complexes were recorded with a Jasco-715 spectropolarimeter (Jasco Corp., Japan) in 1 mm cylindrical quartz cells; concentration of DNA in the samples was 0.3 mg/ml. All VCD and IR spectra were measured simultaneously in the range of 1800–750 cm⁻¹ in D₂O with the VCD instrument described earlier [15]. Concentration of DNA in the samples for VCD-measurements was 35 mg/ml. The detailed description of both UV and IR spectroscopic approaches is given elsewhere [6]. The AFM images were obtained on mica in a tapping mode with NanoWizard microscope (JPK Instruments, Germany).

3. Results and discussion

One of the most useful tools for investigating the structure of biological macro-molecules is ultraviolet circular dichroism (CD). However, the applicability of the method appeared to be limited for the investigation of DNA–protein assemblies. In most cases, large DNA–polymer complexes cause considerable light scattering, which affects the shape of the spectral bands resulting in “ ψ -type” CD spectra. In such a situation, direct structural analysis of the CD data is impossible. To overcome this limitation we combine UV-CD with another structure-sensitive spectroscopic approach: infrared or vibrational, CD (VCD). Being a chiroptical technique, like its counterpart in the UV region, VCD is also very sensitive to structural changes in the macromolecules, but in addition is considerably more informative for structural analysis compared to ECD. For example, unlike ECD, VCD spectra reveal not only DNA conformations, i.e., A, B or Z form, but also the mutual orientation of the different chemical groups within the DNA as well as their participation in interactions with other molecules or ions.

To demonstrate the applicability of this approach for investigating supramolecular DNA–protein systems, we prepared complexes between DNA and two chromosomal proteins: histone H1 and non-histone protein HMGB1, which are some of the most abundant chromatin proteins. Both proteins play an important role in the structural organization of chromatin at the post nucleosomal level. Both proteins also initiate the formation of supramolecular structures when complexed with DNA and each is able to induce ψ -type CD spectra. Acting together, they form supramolecular complexes at even smaller protein to DNA ratios.

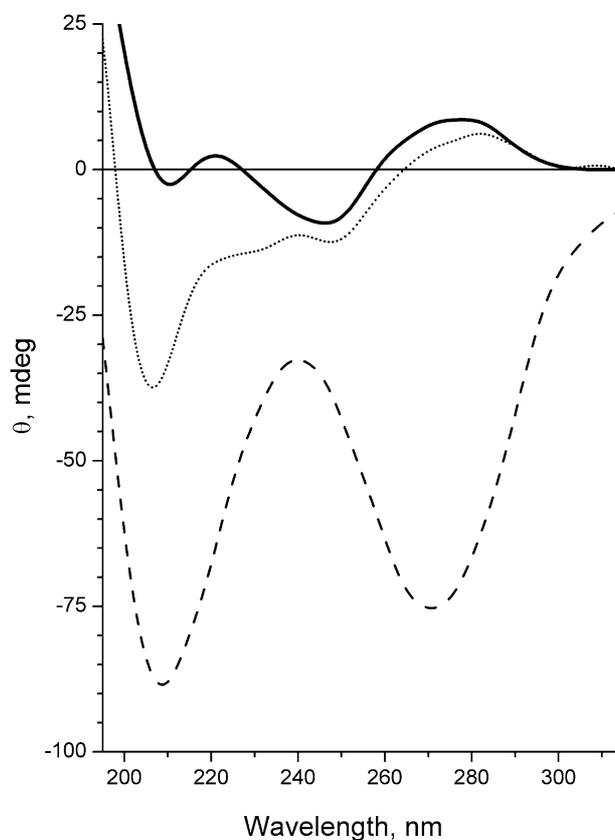


Fig. 1. CD spectra of DNA (solid line) and its complexes with non-histone protein HMGB1 and histone H1 at different protein to DNA ratios (r , w/w): $r = 0.10$ (dotted line) and $r = 0.25$ (dashed line). Concentration of DNA in all samples was 0.3 mg/ml, optical path length was 1.0 mm. The molar ratio of HMGB1 to H1 in the samples was maintained 1:1.

The typical UV-CD spectra of DNA and its complexes with proteins HMGB1 and H1 are given in Fig. 1. At low protein to DNA ratios the spectra of the complexes do not reveal any detectable changes in the secondary structure of the proteins, while decrease of DNA band in vicinity of 275 nm reveals DNA–protein interactions in the major groove of the DNA double helix. Considerable light scattering of the samples with $r = 0.25$ leads to the formation of the ψ -type CD spectra. Such CD spectra indicate that large multi-molecular complexes are likely present in the solution. However, these spectra contain no information about the secondary structure of the macromolecules within the complexes. Nevertheless, IR/VCD spectra of the complexes with the same ($r = 0.25$) and even higher ($r = 0.75$) protein to DNA ratios, taken at higher DNA concentrations, essential for reliable VCD signal, give well-resolved spectral pattern (Fig. 2). Based on the previously published results [1,3,5,6,10,12,16] it is possible to assign the major spectral features as summarized in the Table 1. Thus, IR/VCD spectra of large supramolecular complexes can be analyzed at higher protein to DNA ratios, unreachable for conventional UV-CD spectroscopy.

In both phosphate ($1150\text{--}900\text{ cm}^{-1}$) and carbonyl ($1750\text{--}1500\text{ cm}^{-1}$) regions interaction of DNA with the proteins at moderate r values leads to decreasing intensity in IR absorbance, while at higher ratios intensity of the corresponding bands increases (Fig. 2). As it was shown earlier [6,8] this type of behav-

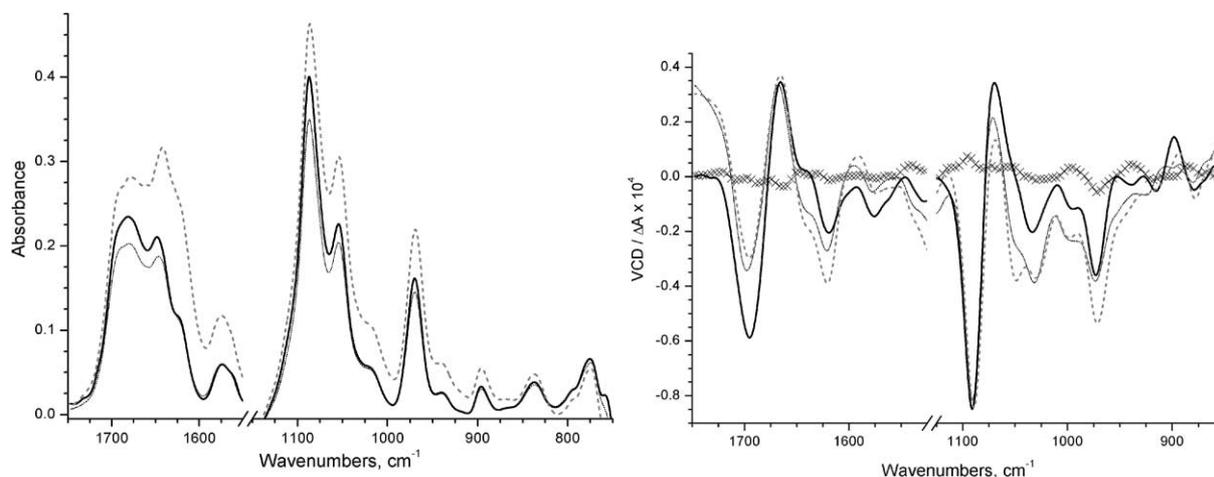


Fig. 2. IR absorbance (left panel) and VCD (right panel) spectra of DNA (solid line) and its complexes with non-histone protein HMGB1 and histone H1 at different protein to DNA ratios (r , w/w): $r = 0.25$ (dotted line) and $r = 0.75$ (dashed line). The estimated level of noise for VCD signal of DNA at a particular wavenumber is given as separate trace (crosses). Concentration of DNA in all samples was 35 mg/ml, optical path length was 0.050 mm. The molar ratio of HMGB1 to H1 in the samples was maintained 1:1.

Table 1

The assignment of the major vibrations in the DNA–protein complex

Band position, cm^{-1}		Proposed assignment
Absorption	VCD	
1693	1698(-)/1688(+)	Stretching vibrations of $\text{C}_2=\text{O}$ in thymine
1678	1662(+)	Stretching vibrations of $\text{C}=\text{O}$ groups in guanine and cytosine in presence of proteins
1644	–	$\text{C}_2=\text{O}$ stretching of cytosine and $\text{C}_4=\text{O}$ of thymine, $\text{C}_6=\text{O}$ of guanine, $\text{C}=\text{C}$ of cytosine
1623	1638(+)	Adenine, thymine ring vibrations
1572	–	Superposition of purine ring vibrations, including $\text{C}-\text{ND}_2$ (1572 cm^{-1}) and
1560		$\text{C}=\text{N}$ (1560 cm^{-1})
1086	1091(-)/1074(+)	Symmetrical vibrations of $\text{O}=\text{P}=\text{O}$ bonds
1053	1056(+)	Stretching vibrations of $\text{C}-\text{O}$ bonds in sugar ring
1021	1034(-)/1008(+)	Deoxyribose vibration
970	972(-)	Sugar ring vibrations
938, 895, 836	936(-)/896(+)	Sugar ring vibrations. DNA B-form markers

ior can be attributed to the initial stabilization of the double helix followed by numerous intermolecular interactions. Comparison of the spectra in the both regions at the different r values reveals significant differences indicating that interactions of the proteins take place with the phosphate backbone and with the DNA bases, which is slightly different from the cases of DNA–HMGB1 and DNA–H1 complexes [7,8]. Changes in the spectra of the DNA bases at 1693 cm^{-1} (thymine $\text{C}_2=\text{O}_2$) and 1643 cm^{-1} (cytosine $\text{C}=\text{O}$) show dominant interactions between DNA and HMGB1, which occur in the minor groove. Weaker interactions of the histone H1 in the major groove can also be detected as slight changes in intensities at 1678 cm^{-1} (guanine $\text{C}=\text{O}$) as well as those at 1590 , 1572 and 1560 cm^{-1} (guanine $\text{C}=\text{N}$

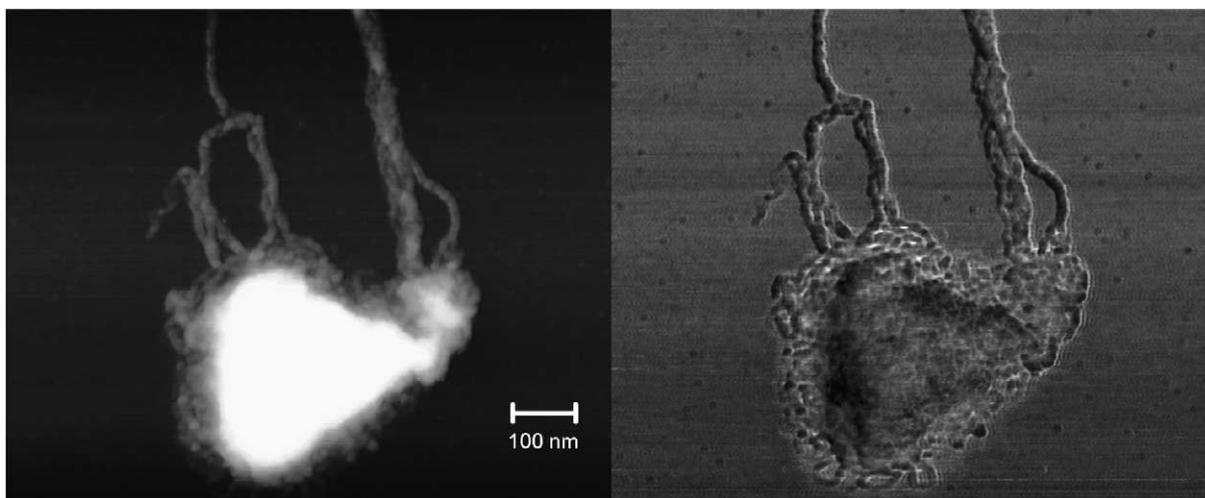


Fig. 3. AFM height trace (left panel) and phase trace (right panel) images of DNA–HMGB1–H1 complexes taken in a tapping mode on mica. Protein to DNA ratio (r , w/w) $r = 1$, HMGB1:H1 molar ratio is 1:1. The scanned area is approximately $1 \mu\text{m} \times 1 \mu\text{m}$.

and C–ND₂). Protein binding is also accompanied by considerable DNA unwinding and bending, resulting in distortions in the mutual orientation of the bases, and thereby causes changes in corresponding VCD spectra at $\sim 1700 \text{ cm}^{-1}$, $1666(+)$ cm^{-1} and $1636(+)/1620(-)$ cm^{-1} . Decreasing coupling of phosphate groups at $1088(-)/1068(+)$ cm^{-1} and increasing VCD of the sugars at 1034 and 972 cm^{-1} indicate intensive binding of the histone H1 with phosphate groups and changes in geometry of the sugar–phosphate backbone.

Hence, we may conclude that acting together histone H1 and the non-histone chromatin protein HMGB1 do not demonstrate a competitive protein binding. Although binding of HMGB1 likely prevents binding of the histone molecules to the same site, partial neutralization of the negative charges of the phosphate groups of DNA by the histone facilitates HMGB1–DNA interactions and the formation of supramolecular complexes. To find out more about these large structures we applied atomic force microscopy, which shows (Fig. 3) that DNA molecules form some ordered fibrillar structures. This process is facilitated by the proteins, which hold together DNA molecules. The individual fibrils in turn interact with each other producing large shapeless aggregates, which easily precipitate in solutions.

Thus, the combination of UV and IR CD techniques allowed us to study large multi-molecular DNA–protein complexes, overcoming limitations due to the light scattering of the solutions.

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