Effect of Mn$^{2+}$ and Ca$^{2+}$ Ions on Formation of Structurally Ordered Nanoscale Complexes of DNA with Nuclear Proteins HMGB1 and H1

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Abstract. The structural organization of DNA in complex with linker histone H1 and non-histone chromosomal protein HMGB1 in presence of calcium and manganese ions have been studied using FTIR and UV circular dichroism spectroscopy. We have demonstrated that the presence of calcium ions leads to the formation of highly ordered DNA-H1-HMGB1 structures, while manganese ions decrease the order in the earlier reported nanoscale complexes.

1. Introduction

Interactions between protein molecules play important role in various biological systems. One of the most interesting examples of such complex multicomponent systems is chromatin. Structural organization of the chromatin strongly depends on DNA-protein and protein-protein interactions within the linker DNA in the regions between nucleosomes. The most abundant nuclear proteins are linker histone H1 and non-histone chromosomal proteins HMGB1 [1-3]. Both of these proteins interact with DNA and play critical role in the structural organization of the chromatin. The non-histone protein HMGB1 is a member of a large superfamily of High Mobility Group (HMG) chromosomal proteins. The characteristic feature of the proteins attributed to the HMGB sub-family is the presence of structural-functional domains known as HMG-Box [3-6]. Having an L-shaped tertiary structure these domains are responsible for the interactions of the HMGB proteins with DNA. HMGB-domain proteins demonstrate high affinity to pre-bent DNA regions, including super helical and cruciform DNA, Holliday junctions and cisplatin adducts [7-9]. The particular functions of the HMGB proteins remain a matter of debates, however it is believed, that structural organization of chromatin depends on their functioning. The HMGB1 protein is considered to be an “architectural” factor of transcription [4, 5], and participates in transcription [10-12], recombination [13, 14] and replication [15]. However, the exact positioning on DNA and the pattern of its interactions with other nuclear proteins remains unclear. HMGB-domain demonstrates ability to form highly ordered complexes with DNA [16-19]. Linker histone H1 in turn is one of the best studied chromosomal proteins [1-3, 20, 21]. Interacting with DNA on entrance/exits of a nucleosome it plays a crucial role in formation of 30-nm chromatin fibre. Earlier we have demonstrated that HMGB1 co-operate with histone H1 also forming structurally ordered complexes with linker DNA [22]. Here we investigate the effect of calcium and manganese ions on the formation and stability of the DNA-HMGB1-H1 complexes reported earlier using UV circular dichroism (CD) and Fourier-transformed infrared absorption spectroscopy (FTIR) [22].
2. Results and Discussion

Nuclear proteins histone H1 (M=21 000 Da) and HMGB1 (M=26 500 Da) were isolated from calf thymus by extraction with 5% perchloric acid with subsequent precipitation using 3.5 V for H1 and 5.5 V for HMGB1 pre-cooled acidic acetone at −20 °C overnight as described earlier [17]. The purity of the protein was tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis [23]. The protein concentration was determined by UV absorbance using extinction coefficients $\varepsilon_{280} (\text{HMGB1}) = 33000 \text{ M}^{-1}\text{cm}^{-1}$ and $\varepsilon_{230} (\text{H1}) = 41000 \text{ M}^{-1}\text{cm}^{-1}$ [24]. Calf thymus DNA sodium salt (Sigma) was used without further purification. The concentration and nativity of DNA were determined spectrophotometrically after hydrolysis by 6% perchloric acid [25]. DNA-protein complexes were prepared by direct mixing of equal volumes of DNA and proteins H1 and HMGB1 solutions of appropriate concentrations, to obtain desired protein to DNA ratio r (w/w) in the complex maintaining equimolar HMGB1/H1 ratio. The total protein to DNA ratio r varied from 0 to 0.3. The CD spectra of DNA-protein complexes were registered in 1 cm quartz cells in the range of 200–320 nm using Mark V dichrograph (Jobin Yvon, France). CD of the complexes was registered as the difference in absorbance between left and right circular polarized light ($\Delta A = A_L - A_R$) and further converted into ellipticity.

![Figure 1. CD spectra of complexes of DNA with HMGB1 and H1 proteins in presence of 0.8 mM MnCl₂ at different protein/DNA ratios r.](image)

For FTIR measurements samples were placed in a demountable cell composed of two BaF₂ windows separated by a 50 μm Teflon spacer. The mid-IR absorption spectra were registered using FTIR spectrometer equipped with MCT-detector. The spectra obtained are the average of 128 accumulations at the 2 cm⁻¹ resolution. Data preprocessing was performed using OPUS (Bruker) software provided with the spectrometer. For FTIR measurements isotopic substitution of H₂O by D₂O was performed [26-28].
Figure 2. FTIR spectra (left panel) of complexes of DNA with HMGB1/H1 proteins without (blue) and in presence of MnCl$_2$ (red) and their difference spectrum (right panel).

We have studied the effect of manganese and calcium ions on interaction of DNA with non-histone chromatin proteins HMGB1 and H1 using spectroscopic approaches. One of the characteristic features earlier revealed for the complexes between DNA and linker chromatin proteins was the formation of large supramolecular complexes manifested themselves in form of $\psi$-like type CD spectra [17-19]. To evaluate the effect of the divalent ions on such complexes we have registered their CD spectra in presence of manganese (Figure 1). Presence of manganese in the DNA-protein system leads to considerable condensation of DNA molecule. Noteworthy, the condensation occurs at protein to DNA ratios one order of magnitude lower, compared to similar DNA-protein complexes [29]. However, the above CD spectra demonstrate considerable contribution of light scattering in the spectral pattern, which is likely due to the increasing size of the forming complexes. The formation of supramolecular particles makes the CD spectra less informative from the structural point of view. To reveal some additional structural details we obtained IR absorption spectra of the complexes.

Measurements of IR spectra in aqueous solutions require to use rather high sample concentrations, which in presence of nuclear proteins may rise some solubility issues. To avoid such problems we prepared samples at relatively low protein to DNA ratios ($r < 0.3$). Figure 2 represents spectra of DNA/proteins complex (blue line), DNA/proteins/Mn$^{2+}$ (red line) and their difference spectrum (black line, right panel). The comparison of these spectra revealed several vibrations, which change their spectral properties in presence of manganese ions. The assignments were performed according to the earlier published data (see [22] and references therein). The properties of the spectrum of the DNA–protein complex in absence of manganese were described in greater details elsewhere [22, 29]. Absorption of the proteins in the amide I/II regions can be considered constant for both complexes due to the negligible spectral changes of these bands compared to changes in the spectra of DNA achieved by significant excess of DNA over proteins in the complexes. The addition of the manganese ions to the DNA-protein complex increases absorption at 1678 cm$^{-1}$ corresponding to the C=O stretch vibrations of guanine and cytosine. These carbonyl groups participate in base pairing, and changes of their vibrational modes provide strong evidence that some distortions in geometry of the double helix occur. Presence of manganese ions also resulted in significant decrease of the band at 1087 cm$^{-1}$, corresponding to the symmetric vibration in phosphate group. Also a distinct shoulder appears in the vicinity of 1108 cm$^{-1}$, attributed to the in-plane bending vibrations in Asp and Glu side chains. These changes together indicate that the interaction (and hence screening) of the negatively charged phosphate groups with Mn$^{2+}$ is accompanied by emerging interactions of Asp and Glu side chains with the DNA bases. The latter is likely caused the changes in DNA geometry, mentioned above. These data confirm that the extensive light scattering observed in UV region might be due to the ability of manganese ions to facilitate DNA compaction by HMGB1 and H1.
Figure 3. Circular dichroism spectra of DNA complexes with HMGB1 and histone H1 \((r = 0.125)\) at different concentrations of CaCl\(_2\).

Earlier it was demonstrated, that at protein to DNA ratios \(r < 0.2\) HMGB1 and H1 proteins interact with the DNA independently. The increasing of the protein content in the system \((r > 0.2)\) induces interaction of positively charged H1 with the negatively charged HMGB1's C-terminal domain [29]. Manganese ions significantly affected the interaction of DNA with the proteins. Mn\(^{2+}\) facilitates DNA compaction by HMGB1 and H1. In ternary DNA-protein complex HMGB1 interacts with the bases and histone H1 mostly with DNA phosphate groups screening their negative charge. This leads to the amplification of the interactions between the protein molecules resulting in the increasing number of associations of the complexes. The significant condensation of DNA at low protein content is not very typical for the DNA-protein complexes and suggests some structural reorganization of the complexes after the addition of the manganese ions, similar to those observed earlier for HMGB1-(A+B)/DNA complexes, which were also characterized by the high level of DNA compaction [16-18]. The characteristic feature of the HMGB1-(A+B)/DNA complexes was strong intermolecular interaction promoted by the absence of the negatively charged C-terminal domain in the HMGB1-(A+B) recombinant protein. It was also suggested that increasing ionic strength could contribute to inactivation of the C-terminal domain in the native HMGB1, promoting stronger DNA-protein interactions. Histone H1 also demonstrated the ability to screen negative charge of the C-terminal region of HMGB1, and as a result, to induce DNA condensation in the complex. Most likely the presence of manganese ions also contributed to the electrostatic screening of the HMGB1 tail charge, promoting condensation of DNA at earlier stages of interaction. If so, then another divalent cation can demonstrate similar effect. To check this assumption we have registered CD spectra of the DNA-protein complexes in presence of Ca\(^{2+}\) ions (Figure 3), which are in contrast to manganese bind only to the negatively charged phosphate groups. Calcium ions significantly increased the affinity of the
proteins to DNA resulting in 30-50 times higher circular dichroism of DNA in the complex, compared to its unbound state, that is even higher than in the above case of the manganese ions.

Most likely, Ca\(^{2+}\) ions inactivate electrostatic repulsion of the negatively charged groups of DNA and the proteins. Manganese ions in contrast bind not only to the negatively charged phosphate groups of DNA and HMGB1 but also to the DNA nitrogen bases. This interplay leads to weakening of the DNA-protein interaction and disrupting the DNA-protein complexes. At the same time we have observed strengthening DNA-condensation processes and increasing level of interactions between H1 and HMGB1 proteins. Thus, the presence of calcium ions most likely leads to the formation of more condensed structures while manganese ions decrease the order in DNA-H1-HMGB1 complexes.

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