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Interaction of HMGB-domain proteins from differentiated cells with DNA modified by cisplatin

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Abstract. Using UV absorption spectroscopy and circular dichroism, we studied the interaction of DNA-cisplatin complexes with nonhistone chromosomal proteins HMGB1 and HMGB2. It was shown that the presence of platinum ions affected the ability of HMGB2 to form large supramolecular complexes with DNA. The role of C-terminal domain of the HMGB1/2 proteins is discussed.

1. Introduction

Nowadays, a variety of platinum coordination compounds are used as antitumor agents in medical practice. Among them cisplatin (*cis*-diamminodichloroplatinum(II), *cis*-DDP) is the most successful and widespread drug [1]. Biological activity of cisplatin is based on the ability to bind DNA and change the local structure of the double helix. Formation of a stable complex with platinum drugs leads to a prolonged suppression of gene expression and eventually to a cell death. However in some tissues, the platinum adducts on DNA can be effectively repaired by the cell. It is believed that the structural changes in DNA caused by the formation of platinum adducts are targets for the selective binding of one or more chromatin proteins specific to those tissues where the antitumor activity of the drug is observed [2]. Among such proteins tissue specific HMGB domain proteins are known to play a unique role [3].

The large family of HMGB-proteins is comprised of a great variety of nuclear and cytoplasmic proteins containing a structurally functional motif known as HMGB-domain. HMGB-proteins involved in the formation of DNA-protein complexes responsible for the activation of gene transcription [4-6]. HMGB domain proteins are suspected to be an intermediate substrate in the transport of cisplatin to DNA [7]. Some authors suggest that the binding of HMGB-domain proteins to platinum adducts can block their repair, shielding the adducts on DNA molecule [8].

In this work interaction of *cis*-DDP/DNA complexes with non-histone chromatin proteins HMGB1 and HMGB2 was studied using UV absorption spectroscopy and circular dichroism (CD).

2. Materials and methods

Nuclear proteins HMGB1 (M = 26 500 Da) and HMGB2 (M = 26 000 Da) were isolated from calf thymus by extraction with 5% perchloric acid with subsequent precipitation using 5.5 V pre-cooled acidic acetone at -20 °C overnight as described earlier [9]. The individual proteins were purified by



FPLC. The purity of the proteins was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) [10].

cis-DDP/DNA complexes were obtained from calf thymus DNA (Sigma, USA) by incubation with *cis*-DDP (Sigma, USA) in 15 mM NaCl for 28 hours at 37 °C [11,12]. The formation of the complexes was confirmed by circular dichroism spectroscopy.

DNA/protein complexes were prepared by direct mixing of the equal volumes of DNA and protein solutions in appropriate concentrations in presence of 15 mM NaCl as described elsewhere [13,14]. The final concentration of DNA in the complexes was 20 mg/l. The protein to DNA ratio r (w/w) in the samples varied in the range of 0 – 2.0.

The CD spectra of DNA-protein complexes were registered in 1 cm quartz cells in the range of 200–320 nm using CD6 dichrograph (Jobin Yvon, France). CD of the complexes was registered as the difference in absorbance between left and right circular polarized light ($\Delta A = A_L - A_R$).

3. Results and discussion

One of the characteristic features of the HMGB1 and HMGB2 proteins is their C-terminal domain, sometimes referred as “acidic tail”, which consists of Asp and Glu amino acid residues. This tail includes approximately 30 and 20 Asp/Glu residues in case of HMGB1 and HMGB2 respectively. This domain modulates interaction with DNA and responsible for interactions with other proteins [13,15-18].

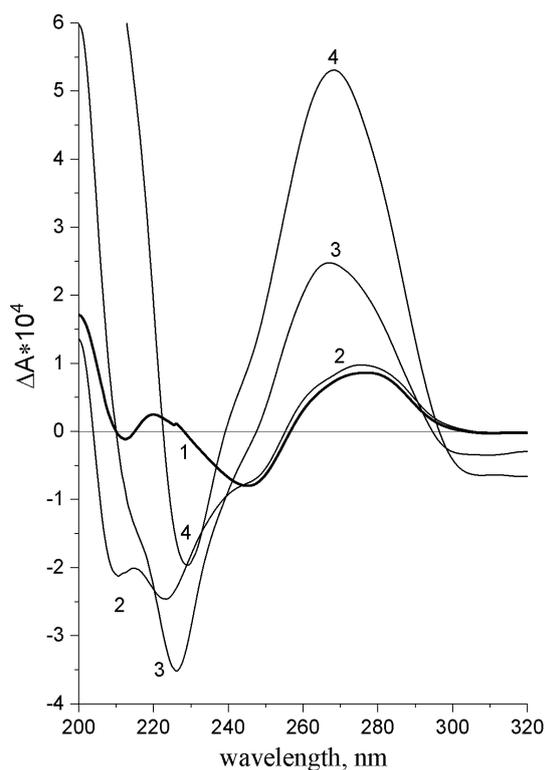


Figure 1. CD spectra of DNA/HMGB2 complexes at different protein/DNA ratio r (w/w): 1 – $r = 0$; 2 – $r = 0.5$; 3 – $r = 0.75$; 4 – $r = 1.0$; 5 – $r = 1.25$.

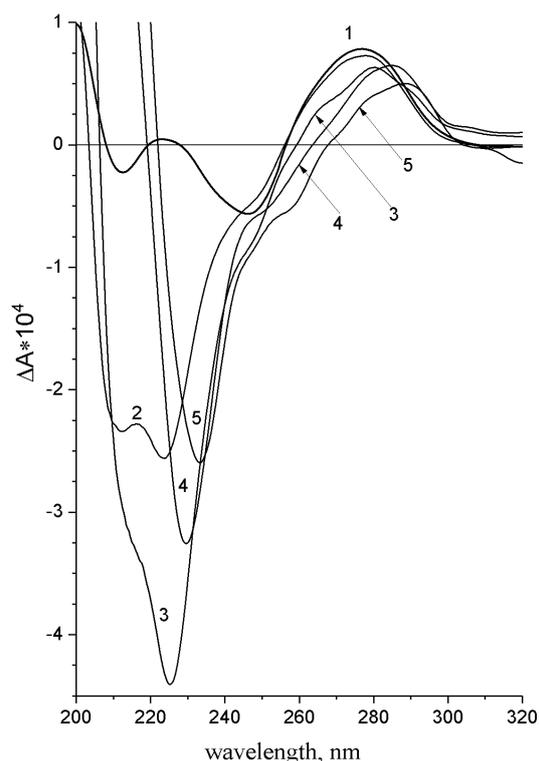


Figure 2. CD spectra of *cis*-DDP-DNA/HMGB2 ([Pt]/[P] 1:15) complexes at different protein/DNA ratio r (w/w): 1 – $r = 0$; 2 – $r = 0.5$; 3 – $r = 0.75$; 4 – $r = 1.0$; 5 – $r = 1.25$.

Although the HMGB1/2 proteins are very similar, there is one important difference between them: HMGB1 can be found both in the nucleus and cytoplasm, while HMGB2 always resides in chromatin. Binding of antitumor drugs, such as *cis*-DDP, have a significant effect on the DNA structure resulting in its capability to interact with nuclear proteins. This might be one of the reasons explaining changes of nuclear/cytoplasmic distribution of such proteins like HMGB1, but not the HMGB2. Here we focus on the effect of *cis*-DDP on DNA/HMGB2 complexes, that still remains under investigated.

The characteristic CD spectra of DNA/HMGB2 and *cis*-DDP-DNA/HMGB2 systems are presented in figures 1 and 2, demonstrating noticeable difference in the complexes. Binding of the HMGB2 to DNA results in formation of characteristic ψ -type CD spectrum of DNA (PSI: Polymer and Salt Induced) at $r > 0.75$ (figure 1), typical for the formation of large supramolecular DNA-protein particles [18,19]. Similar changes in the CD spectrum were previously observed for the HMGB1 protein lacking its acidic tail [9,10,13,14,20]. Thus, shorter C-terminal domain allows more effective binding of the HMGB domains to DNA, increasing the complex stability by numerous interactions between the closely positioned HMGB- and the C-terminal domains of the adjacent HMGB2 molecules. It is interesting to mention, that in case of HMGB1 we do not observe formation of the large DNA protein complexes in this range of r values (figure 3). As it was demonstrated earlier [15,20] the length of the HMGB1 acidic tail is just enough to bind the adjacent HMGB-domain within the same protein molecule. The latter provides two consequences: (1) one of the HMGB-domains of the HMGB1 molecule is not able to interact with DNA being blocked by the tail, and (2) the tail itself is not able to make intermolecular contacts. In the case of HMGB2, the shorter tail it is not long enough for the effective intramolecular interaction, allowing tight binding of the HMGB-domains on DNA and stimulating intermolecular interactions of the acidic tails.

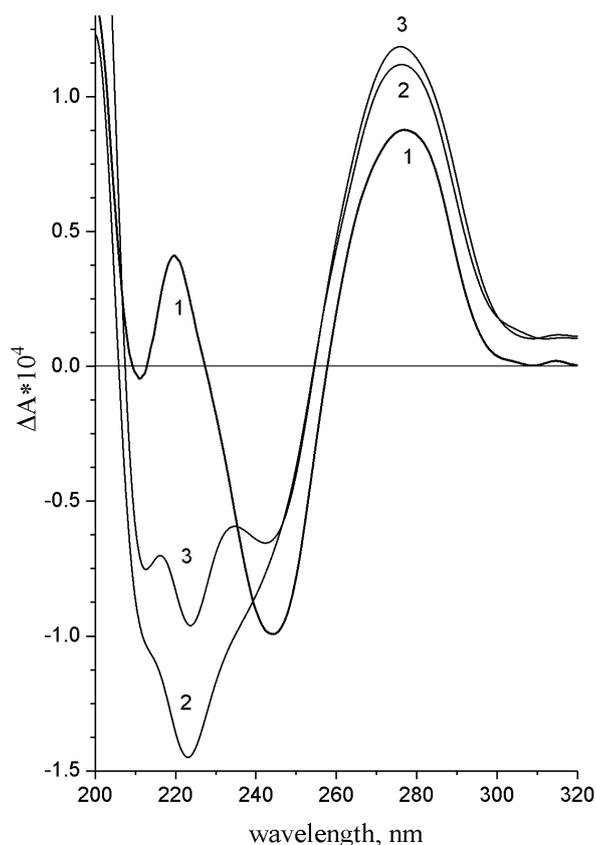


Figure 3. CD spectra of *cis*-DDP-DNA/HMGB1 complexes at $r=1$ ($[Pt]/[P]$ 1:15). 1 – DNA; 2 – DNA/HMGB1; 3 – *cis*-DDP-DNA/HMGB1.

The situation is different in presence of the platinum adducts on DNA, where no ψ -type CD spectra were observed (figure 2). Preferential binding to the adducts results in more distant distribution of the protein on DNA, making impossible direct interaction of two HMGB2 molecules bound to the nearest platinum adducts. In this case the length of the tail makes little or no effect on protein binding, which is clearly seen if one compare CD spectra of the complexes of platinated DNA with HMGB1 and HMGB2 proteins (figure 3). Thus, the platinum adducts prevent the formation of the ordered supramolecular DNA-protein structures modulating the distribution of the protein molecules on DNA.

4. Conclusions

We suggest that the antitumor activity of the drugs based on platinum coordination compounds, among the other factors, might also be related to the stability and structural organization of the complexes that form tissue-specific HMGB-domain proteins when interact with platinum adducts on DNA. As we have demonstrated above the most significant effect of DNA platination on the chromatin structure can be observed when the cells contain HMGB-domain proteins with shorter C-terminal domains. Indeed, according to the results of recent experiments, cisplatin is the most effective in treatment of the tumors in the tissues, which have tissue-specific HMGB-proteins with shorter or no C-terminal domain, such as HMGB4 [3,5,21].

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