Investigation of Supramolecular Complexes of Biological Molecules Using a Combination of UV/IR Circular Dichroism and FTIR Spectroscopy.

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A combination of ultraviolet (UV) and infrared (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 UVCD (electronic, or ECD) spectroscopy due to considerable light scattering in such solutions. Based on the analysis of FTIR and UV-CD spectra, the interaction of DNA with nonhistone chromatin protein HMGB1 and linker histone H1 was studied. The data obtained showed that under the conditions of the experiment (15 mM NaCl, protein/DNA ratio r < 1 w/w) the proteins did not reveal any AT or GC specificity in binding to DNA. In the presence of both proteins, mainly interactions in the DNA minor groove were observed, which were attributed to HMGB1 binding. Histone H1 facilitated binding of HMGB1 to DNA by interacting with the negatively charged groups of the sugar–phosphate backbone and binding of aspartic and glutamic amino acid residues of HMGB1. Acting together, HMGB1 and H1 stimulated the formation of supramolecular DNA–protein structures. The structural organization of the ternary complexes depended not only on the properties of the protein–DNA interactions but also on the interactions between HMGB1 and H1 molecules. We have shown that the presence of divalent ions, such as Mn2+ and Ca2+ changes the mode of DNA-protein interaction. Calcium ions stimulate the formation of large but less ordered complexes, while the presence of manganese leads to formation of more ordered DNA-protein complexes.