CIRCULAR DICHOISM – THEORY AND APPLICATIONS OF THE INTERACTION OF DEOXYRIBONUCLEIC ACID WITH MANGANESE IONS

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Abstract

Understanding the mechanism of recognition and interaction between nucleic acids and molecules affecting their function is important not only for biological or biochemical studies but also for the design of molecules with therapeutical or biotechnical qualities. Numerous biological processes involving deoxyribonucleic acid (DNA) are successfully used in biotechnology. Spectroscopic techniques with polarised light, mainly linear dichroism (LD) and circular dichroism (CD) and fluorescence techniques are developed and applied together with kinetic and thermodynamic techniques to describe and explain specific binding mechanisms of molecules of biological importance. It was demonstrated that manganese is involved in interactions with phosphate oxygens and with the bases of DNA.

Keywords: circular dichroism, DNA-Mn$^{2+}$ interaction

Introduction

Because of the helical structure of double stranded DNA, circular dichroism is very useful in following structural alterations of the basic DNA conformation, the so-called B form. The chiral nature of the helix gives rise to a strong CD signal which varies under certain conditions, e.g. state of hydration, temperature etc. Basic information is given in modern biochemistry textbooks (Balasubramanian, 1976; Darnell, 1986; Gârban, 2004). Chiral transitions metal-ion complexes are studied with respect to affinity, geometry, mechanism of DNA binding and structural changes.

The structural transition can be characterized by the specific ellipticity ($\Psi$) associated with maxima or minima in the CD spectrum. The molar ellipticity can be used to calculate the difference in extinction coefficient for left versus right circularly polarized light.
DNA conformation varies in direct response to the types and positions of cations. Helical parameters such as groove width, helical twist and propeller twist are sensitive to the position of cations. Changes in cation type and concentration can induce large scale conformational transitions in DNA. The structure and dynamics of the grooves are of great importance for recognition of DNA by proteins and small molecules as well as for packaging of DNA into nucleosomes and viral particles.

Nucleic acids are optical active substances. The circular dichroism (CD) phenomenon - differential absorption of circularly polarized light - is evidenced by the Cotton effect. Circular dichroic effects occur as the wavelength of the incident light approaches that of the absorption band. Cotton effects in DNA are determined by the optical active deoxyribose, by the purine and pyrimidine bases whose cromophores become optical active thanks to the binding at the deoxyribose, as well as by the double helix structure of the macromolecular edifice of DNA (Crabbé, 1965; Yang, 1971; Gray, 1996; Rodger, 1997; Avacovici, 2003).

Molecular interactions are the basis for information storage and transfer of genetic code, substrate binding to proteins, cell-cell recognition processes, signal transduction etc.

**Experimental**

The interaction between DNA with Mn$^{2+}$ ions was studied on DNA from thymus at a concentration of $10^{-4}$M in a solution of sodium perchlorate (NaClO$_4$ .10$^{-2}$M) and MnCl$_2$ .4H$_2$O, at the concentrations $10^{-4}$M. Out of the initial solutions of DNA and chlorides, there were further on prepared solutions with two increasing molar ratios, defined by the fraction M$^{2+}$ / DNA-P (related to the number of phosphate groups from the DNA macromolecule). The ratios were: 0.5/1; 1.0/1; 2.5/1; 5.0/1. The solution of sodium perchlorate does not affect either the conformational state of DNA or the interaction with divalent metals.

Dichroic spectra were obtained with a Roussel-Jouan dicrograph composed of: a monochromator, a polarizer and a crystal of monoammonia phosphate under an electric field.

**Results and Discussions**

Metal ions interact with many groups in different sites of nucleic acids influencing the canonical DNA structure and essential in the
formation of noncanonical forms such as triplexes, quadruplexes etc. The influences of cations on nucleic acid structure are multifaceted and subtle. Cations can simultaneously stabilize and stack.

In double or triple helical structures the preferred sites for nucleobase/ion interaction are the N\textsubscript{7} and O\textsubscript{6} positions of guanine and the N\textsubscript{7} of adenine. Simultaneous coordination to base and phosphate groups is also observed. Cations may interact with other sites on nucleobases, e.g. N\textsubscript{3} of cytosine, N\textsubscript{1} of guanine, that are not accessible under the Watson–Crick base pairing. The strength of interaction is determined by the interaction of the ion with the molecular electric field of the nucleobase.

The use of quantum-chemical methods to study the interactions of metal ions with DNA bases and base pairs was until recently strongly limited by the availability of sufficiently powerful computer software in order to take into consideration the electron correlation effects, molecular relaxation of base pairs upon ion binding and the basis set superposition error.

Dichroism spectroscopic methods rely extensively on the know-how from basic photophysical and quantum chemical studies, such as the exact directions and sizes, absorption overlaps, transition moments in a given molecule and about how these properties are affected by substitution, solvents and interaction with neighbour cromophores (Xu You-Cheng, 1997; Israelachvili, 1991; Gray, 1996).

The DNA conformation appears to vary in direct response to the types and positions of cations. Helical parameters such as groove width, base pair roll, helical twist, propeller twist appear to be sensitive to positions of cations (Johnson, 1996; Gârban, 2004).

Manganese was found to be an essential element for a number of DNA-binding proteins, which possess structural rather than enzymatic activity. Mn\textsuperscript{2+} ions are able to change the enzymatic activity of some nuclear proteins.

Conformational transition towards the C-form of DNA was observed in solution at the molar ratio Mn\textsuperscript{2+}/DNA-phosphates between 0.1 and 1.5. The exact ratio depended on the ionic strength and increased with increasing NaCl concentration. Although manganese interacted with the phosphates and nucleobases of DNA at higher metal concentrations, it is unlikely that direct chelation occurred. A model for the interaction between manganese ions and DNA mediated by water is suggested destabilizing the double helix and partially breaking the hydrogen bonds.
between the base pairs. At high Mn\(^{2+}\) concentrations DNA aggregation was observed (Pullman, 1980).

As Mn\(^{2+}\) ion concentration increases, chelation between phosphate oxygens and the nucleobases can occur. Such a binding mode is unfavourable for the stability of the base pairs thereby inducing more GC openings and opportunities for Mn\(^{2+}\) binding to C=O of cytosine.

Important modifications of the Cotton effect are induced by the Mn\(^{2+}\) cation on the positive domain (Gârban, 1998). In our investigations the specific ellipticity was expressed in relationship with the wavelength \([\Psi]\) = f(\(\lambda\)) as shown in fig.1.

![Fig. 1. CD spectra for Mn\(^{2+}\) at various molar ratios](image)

Once the molar ratio increases in the positive domain, the specific ellipticity decreases so that for greater molar ratios negative values are reached. A special characteristic for Mn\(^{2+}\) is that in the positive domain the curves present two modulations. It was considered by Zimmer (1971) that conformational modifications at the pairs of bases G-C take place.

Two possible effects of manganese ions on DNA structure can be observed: a) the neutralization of the negative charge of the phosphate backbone and stabilization of the double helical structure; b) the prevention of DNA renaturation by interaction with sites of the bases not involved in base pairing (Siddesh, 2000).
Theoretical studies presented in literature confirm the discussed aspects showing that there is a linear and monotone dependence on the different geometrical parameters which define the double helix of the deoxyribonucleic acid.

Conclusions

Studying the interactions of DNA with metal ions, which in some conditions can induce mutagene and oncogene processes, interesting results are obtained regarding the destabilization of the macromolecule, followed by destructive processes and even cytological modifications. Circular dichroism becomes a valuable method that helps the investigations in pharmacology, molecular biology, toxicologie with applications in human and veterinary medicine. Metallic cations Mn$^{2+}$ which bind to the phosphate group enhance the negative ellipticity.

References

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