Exciton coupled circular dichroism using MOS-500

I – Introduction

The Exciton Coupled Circular Dichroism (EC-CD) results from the interaction between two chiral chromophores (natural or added) in a macromolecule. When the two chromophores are in position to 'be coupled' (short distance, right angle, environment,…) their electronic transitions interacts and generate two distinct CD bands resulting from two distinct Cotton effects with opposite direction.

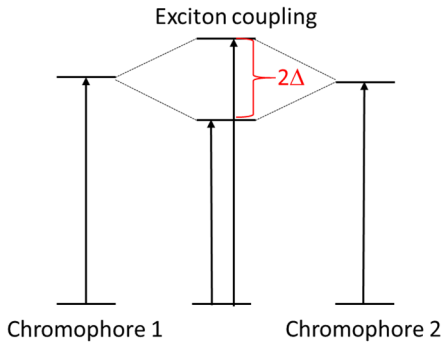

Fig 1: levels of energy induced by exciton coupling

The EC-CD technique is a sensitive and well recognized technique to get information on the absolute configuration of complex structures as the sign of signal is often correlated to a given geometry (stereoisomer). EC-CD is often used in the visible region on polymers, chiral organogels or other chiral macromolecules but can also be applied in near UV range for structural studies of proteins in complement to traditional far-UV CD spectrum.

II–Experimental conditions

2-Aminopurin is traditionally a fluorescent marker that is paired to thymine or cytosine. When two 2-aminopurin are in short distance, these two chiral centers can interact and the macromolecule is locally distorted and changed conformation. This change of orientation between the two chiral centers generates EC-CD.

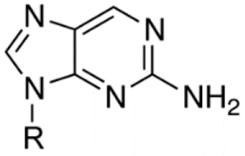

Fig 2: 2-aminopurin

In this note we are giving an example of EC-CD induced by 2-aminopurin, measured during a DNA-protein interaction.

MOS-500 is used to run CD spectra from 300 nm to 360 nm with 2 nm slits and data acquisition speed was set to 0.5s per data point. Concentration of samples was 10 µM and CD spectra were recorded in a 1 cm quartz cuvette at room temperature. It is very important to notice that NO nitrogen purge of optics is required for such measurement. A blank was also measured and automatically subtracted.

III–Results

CD spectrum of DNA-protein complex containing 2-aminopurin is shown in figure 3. In this example only the positive Cotton effect of the exciton coupling is observed due to the remaining absorption of DNA up to 310 nm.

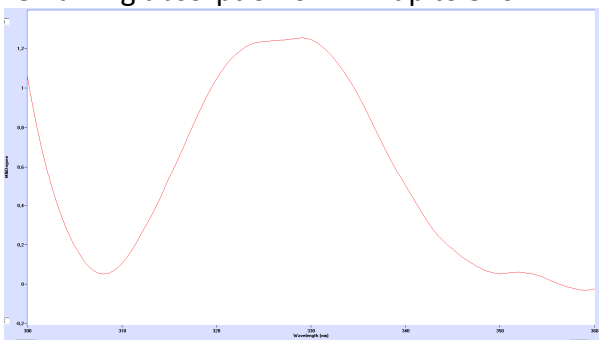

Fig 3: CD spectrum: DNA+protein

A maximum signal is found at 330 nm and total amplitude is only 1.2 mdeg so one can check extreme sensitivity of MOS-500.

A CD spectrum of protein without DNA was recorded in the same conditions (blue scan in Figure 4) to check the absence of exciton coupling.

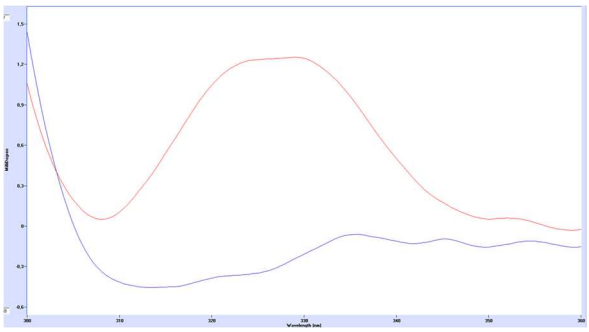

Fig 4: CD spectra with and without DNA

IV–Conclusion

EC-CD spectrum can easily be recorded with MOS-500 in its standard configuration. MOS-500 is the ideal system for such measurement as EC-CD spectra are always done above 195 nm so in a wavelength range where MOS-500 does not require any nitrogen flushing of optics. It thus makes running cost at their lowest.