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Time-resolved circular dichroism: Application to the study of conformal changes in biomolecules

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Abstract. Circular dichroism (CD) is known to be a very sensitive probe of the conformation of molecules and biomolecules. It is therefore tempting to implement CD in a pump-probe experiment in order to measure ultrarapid conformational changes which occur in photochemical processes. We present two technical developments of such time-resolved CD experiments. The first one relies on the modulation of the probe polarization from left to right circular whereas the second one measures the pump-induced ellipticity of the probe with a Babinet-Soleil compensator. Some applications are described and extension of these techniques towards the study of elementary protein folding processes is discussed.

1 Introduction

Circular dichroism (CD), the difference in absorption for a left or a right circularly polarized light, is with optical rotation a unique optical characteristics of chiral molecules. Because chirality is primarily a geometrical property, CD is in turn very sensitive to the conformation of molecules. This feature makes CD an attractive probe for stereochemistry and especially for the study of biomolecules [1]. Taking advantage of our knowledge in ultrafast optics and nonlinear optical properties of chiral molecules, we have developed new experimental schemes allowing such time-resolved CD (TRCD) measurements.

2 Time-resolved CD

In order to achieve ultrafast measurements, pump-probe experiments have been utilized for a long time and TRCD experiments are based on the same principle. A first intense light pulse (the "pump") is sent onto the sample so as to provoke a change in the molecules which is the monitored by a second, weak, delayed pulse (the "probe"). Our experimental set-up is based on a 1 kHz, 150 fs Titanium-Sapphire laser. The pump pulses are most often obtained after frequency-doubling or tripling the output of the laser (400 nm or 267 nm, 200 nJ). On the other hand, extensive use of nonlinear optics for the generation of new frequencies in frequency-mixing stages or in optical parametric amplifiers allows us to have a very versatile source for the probe [2]. Depending on the experiments, we use probe pulses tunable in the visible (400-500 nm) or in the UV (230-350 nm). Pump and probe pulses are focussed on the sample. The delay between the pump and the probe is computer-controlled and can be varied up to 1.5 ns. The sample consists in a fused-silica, 1 mm thick cuvette which is maintained in constant motion in order to avoid cumulative heating effects. For all the experiments, the sample concentration is chosen so that the optical density at the pump wavelength is of the order of unity. It corresponds to concentration in the range 100-300 μM.

In order to measure the CD of the probe, we have implemented two different techniques : modulating the probe-polarization and measuring the probe ellipticity wirh a Babinet-Soleil compensator.

2.1. Modulation of the probe polarization

The most straightforward technique is to modulate the probe polarization from right circular to left circular. In that case, CD is translated into a modulation of the probe transmission which can be extracted from the signal with a lock-in detection. In our case, the probe polarization is alternately right and left circularly-polarized thanks to a longitudinal Pockels cell on which a ± 1.5 kV voltage is applied. Deconvoluting the signal transmitted through the sample directly yields the absorption and the CD. Measurement is then carried out for various pump-probe delays. This technique is very straightforward but suffers from many artifacts. Indeed, it is very difficult to obtain perfectly circular polarizations and a default in the symmetry of the left and right polarizations yields artifactual signal which are indiscernible from the CD to be measured. To overcome this problem, we have developed a procedure to get a very precise alignment of the Pockels cell in order to obtain perfectly symmetrical circular polarizations [3]. This technique has been successfully applied to the study of the conformational changes in the heme pocket of myoglobin within 100 ps after photoexcitation [4].

2.2. Measurement of the pump-induced change in the probe ellipticity

It is well-known that when a linearly-polarized beam passes through a chiral sample, it acquires some ellipticity which can directly yields the sample CD. The idea is therefore to measure the pump-induced ellipticity of the probe beam. In order to measure the probe ellipticity, we put the sample between a polarizer and a crossed analyzer and insert a Babinet-Soleil compensator. We then insert a mechanical chopper on the pump path and measure the transmitted probe intensity with a photomultiplier tube ((hereafter called the "PM" signal) and its modulated part with a lock-in amplifier (hereafter called the "LI" signal) as a function of the Babinet-Soleil retardation. Calling φ the Babinet-Soleil retardation, very simple algebra, detailed in Ref. [5] yields for the two measured signals :

$$PM = \varphi^2 + C_1 \quad (1)$$

$$LI = -\delta\alpha L\, \varphi^2 + \delta CD/2\, \varphi + C_2 \quad (2)$$

In these equations, C_1 and C_2 are constants, $\delta\alpha L$ is the pump-induced absorption change and δCD the pump-induced CD change. Examination of the formulas shows that comparing the curvature of the PM and LI parabolas directly yields δαL and that the LI parabola is shifted compared to the PM one by $\delta CD/4\delta\alpha L$. This shift is easily measurable (especially when $\delta\alpha L$ is not too strong) and this technique allows $\delta\alpha L$ and δCD to be detected in the 10^{-4} range. The measurements therefore consist in recording the two parabolas for a fixed pump-probe delay and to extract change in absorption and in CD by fitting them.

This new technique presents several advantages. It is free from most of the artefacts since there is no longer modulated circular polarizations for the probe, which represents a remarkable improvement. Use of a mechanical chopper on the pump further increases the signal-to-noise ratio and allows weak signals to be detected with much less averaging than with the other technique. The major drawback of this technique is that it is efficient for the measure of the pump-induced CD on the condition that the change of absorption is not too strong. Fortunately, it is often the case, especially in the UV.

3 Examples of applications

We present here very briefly some applications of this technique to the study of conformational changes in molecules or biomolecules.

In figure 1, we display the experimental results obtained in binaphthol for two solvents. The pump wavelength is 267 nm and the probe one is 237 nm. In this wavelength range, we excite and probe the π–π* transitions of the naphthol moieties. Pump-induced absorption (not shown) displays no dynamics on this time-scale whereas CD clearly shows a solvent-dependent dynamics. In a non viscous solvent like ethanol, a 100 ps relaxation is observed whereas no relaxation is seen in viscous solvent like ethylene-glycol. This signal is a consequence of the change of the dihedral angle in the excited state of binaphthol [6]. Considering that the CD originates in the coupling of the electronic transitions of the two naphthol moieties (excitonic coupling), we can infer from this measurement that the dihedral angle decreases when the p-electrons are excited, as expected for this diaryl coumpound [7].

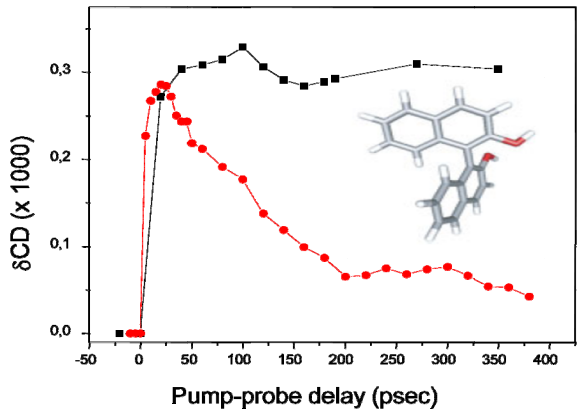

Fig. 1. Time-resolved CD for binaphthol in ethanol (dots) and in ethylene-glycol (squares).

Figure 2 shows an application on the chromoprotein of *Blepharisma japonicum*, a ciliated protozoan known to exhibit a strong photomovement. The chromophore responsible for the light sensitivity is studied as part of the protein or directly in solution. We observe that the dynamics of the CD at 230 nm after photoexcitation at 400 nm displays a rapid dynamics in the protein which is absent in solution [8]. This comforts the idea that there exists an electron transfer from the chromophore to the protein after photoexcitation [9] and that conformational changes in the chromophore play an important role in the transmission of the excitation from the chromophore to the protein backbone.

4 Conclusion and perspectives

Investigating rapid conformational changes in biomolecules is particularly appealing because many biochemical processes rely on the capacity of proteins to change their shapes and to modify or to adapt to their

environment. Such is the case in most enzymatic reactions or in transmembrane signalling for example.

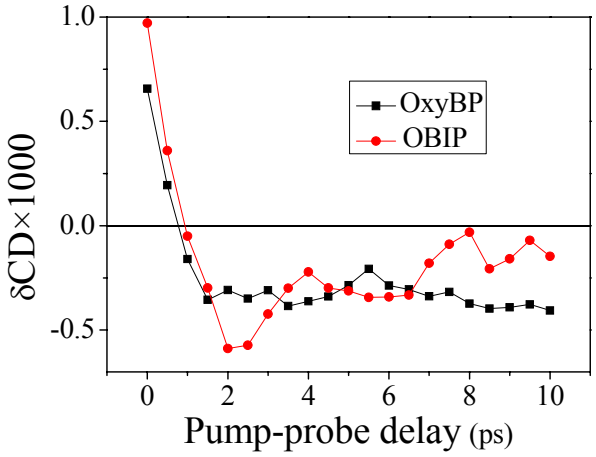

Fig. 2. Time-resolved CD for the chromophore of *B. Japonicum* in the protein (OBIP) and in solution (OxyBP).

Among the issues most studied by biophysicists, protein folding is of paramount importance. The fundamental mechanisms at stake in the formation of globular protein are still strongly debated and much work is currently developed to decipher folding or unfolding processes in small peptides or proteins [10]. CombiningTRCD in the far UV with phototriggering of folding such as T-jump or photoinduced charge transfer could be an alternative technique to probe the formation of secondary or tertiary structures.

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