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USE OF CIRCULARLY POLARIZED LIGHT TO STUDY BIOLOGICAL MACROMOLECULES

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Abstract - Differential absorption of circularly polarized light has long been used to study chiral molecules. Here we will emphasize the newer experimental and theoretical methods which are especially applicable to macromolecules. They include fluorescence-detected circular dichroism, the angular dependence of the differential scattering of circularly polarized light, the analysis of the contribution of this differential scattering to differential extinction of circularly polarized light, and the measurement and interpretation of images based on circular differential extinction.

INTRODUCTION

When light is incident on a sample, only a fraction is transmitted. The part that is not transmitted is either absorbed or scattered. It is essential to distinguish between scattering and absorption to interpret properly a transmission experiment. The fraction of light transmitted by a sample is characterized by an extinction coefficient. For small molecules in solution the contribution of scattering of light to the extinction of incident light is usually negligible. However, for macromolecules the scattering contribution to extinction may become dominant. We will discuss methods to separate the contributions of scattering and absorption to circular dichroism. The interpretation of the two contributions provides very different information about the structure of a macromolecule.

The total light scattered by the sample affects the extinction coefficient, but the angular dependence of the scattered light also contains structural information. The measurement and interpretation of the differential scattering of circularly polarized light -- circular intensity differential scattering (CIDS) -- is a new research area. We think it will be valuable for very large, chiral macromolecular systems.

To study individual biological cells a microscope is needed. It can be used to focus the incident light on the cell, or part of a cell, and the circular dichroism can be measured in the usual way. A more intriguing possibility is to obtain an image which depends on the circular dichroism of each part of the cell. That is, the contrast in the image is proportional to the circular differential extinction, instead of the optical density (extinction), of the object.

CIRCULAR DICHROISM AND FLUORESCENCE-DETECTED CIRCULAR DICHROISM

The separation of circular differential absorption from circular differential scattering in a chiral macromolecule can be done using fluorescence to monitor the absorption. Two methods are shown in Fig. 1. A non-chiral fluorophore is added directly to the sample, or is placed in a separate container around the sample. The light not absorbed by the macromolecule -- either transmitted or scattered -- is converted into fluorescent light and detected (Ref. 1-3). In a standard circular dichroism apparatus the circular differential extinction ($\varepsilon_L - \varepsilon_R$) is measured; with fluorescence detection the circular differential absorption ($a_L - a_R$) is measured. The difference is the circular differential scattering ($s_L - s_R$).

$$\varepsilon_L - \varepsilon_R = (a_L - a_R) + (s_L - s_R)$$

Fig. 2 shows the circular dichroism and fluorescence-detected circular dichroism for helical octopus sperm (Ref. 4). Above 300 nm essentially all the circular dichroism is caused by circular differential scattering. The circular differential absorption and the circular

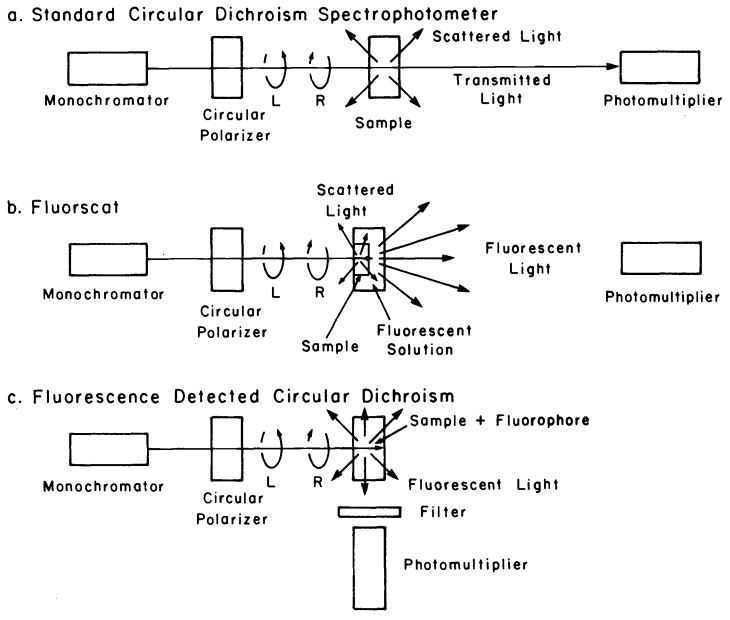

Fig. 1. Fluorescence can be used to measure the contribution of circular differential absorption to circular dichroism. A non-chiral fluorophore converts transmitted or scattered light into fluorescent light, which is detected. Reprinted with permission from Tinoco, et al., Trends in Biochemical Sciences 8, 41-44 (1983).

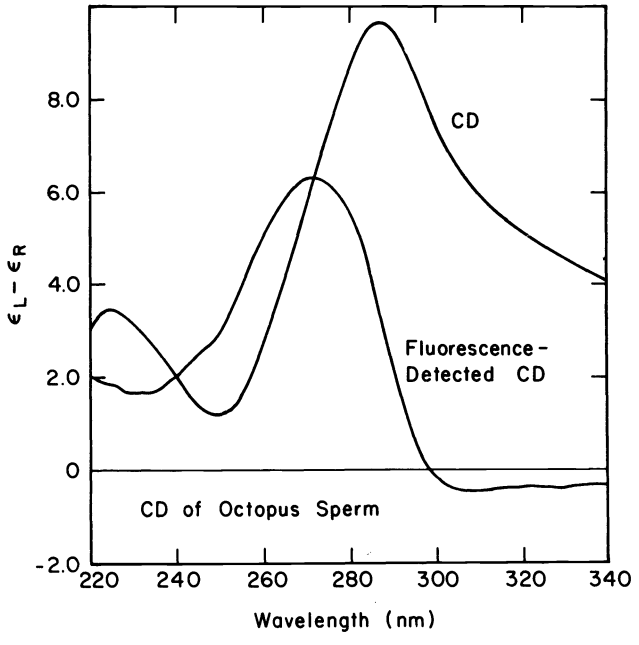

Fig. 2. The circular dichroism and fluorescence-detected circular dichroism of octopus sperm heads in suspension. The main contribution to the circular dichroism above 300 nm is circular differential scattering.

differential scattering can each be interpreted in terms of the structure of the macromolecule (Ref. 5). However, before any quantitative interpretation of a measured circular dichroism is attempted, it is necessary that the separate contributions from absorption and from scattering be assessed.

CIRCULAR INTENSITY DIFFERENTIAL SCATTERING

The angular dependence of the differential scattering of circularly polarized light is called circular intensity differential scattering (CIDS) (Ref. 6). It is defined as $(I_L - I_R)/(I_L + I_R)$ where $I_{L,R}$ are the scattered intensities when left and right circularly polarized light is incident on the sample. For isotropic solutions only one angle is necessary to characterize the scattering -- the angle θ between the incident beam and the detector in the scattering plane. Fig. 3 shows the measured scattering from a suspension of the head of the sperm from the Mediterranean octopus Eledone cirrhosa (Ref. 4). The sperm have a well-characterized helical structure. They are left-handed screws with a pitch of about 650 nm; the threads have an outer radius of about 320 nm and an inner radius of about 125 nm. The measured CIDS shows a maximum in circular differential scattering at about 40° relative to the incident beam. Left circularly polarized light is preferentially scattered in this direction. The maximum value of $(I_L - I_R)/(I_L + I_R)$ is 1.4×10^{-2}. Right circularly polarized light is preferentially scattered to the sides (around 90°).

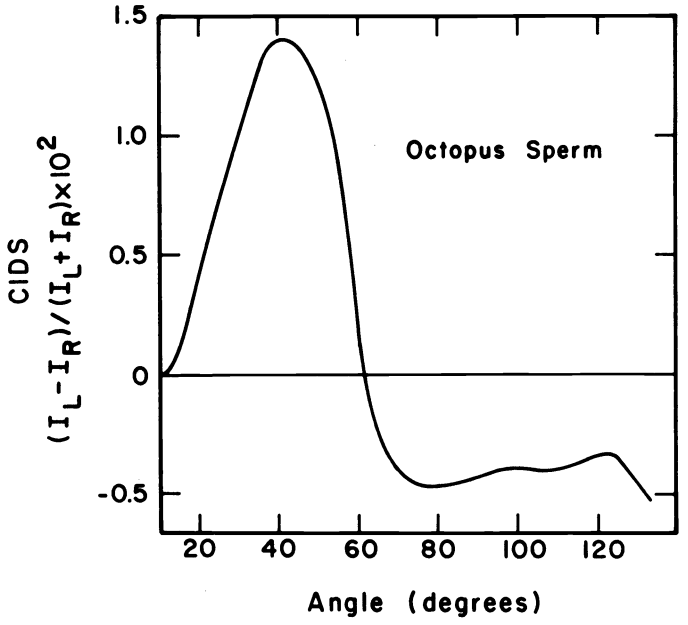

Fig. 3. Measured CIDS as a function of scattering angle for octopus sperm heads in suspension. The wavelength is 442 nm and the angular resolution is 5°. There is an excess of left circularly polarized light scattered in the forward direction.

The CIDS for a macromolecule modeled by an arbitrary arrangement of point polarizable groups has been derived (Ref. 7). Calculations were done for a left-handed helix with a pitch of 650 nm and various values of radius. The helix is a one-dimensional wire and is a very crude model for the sperm. However, a maximum peak of differential scattering near 40° can be obtained (Fig. 4). The sign of the CIDS is correct and the position and magnitude of the forward lobe are reasonable. The calculated CIDS in the side and backward directions show many positive and negative extrema. The sharpness and number of peaks for the simple, model helix indicate the theoretical sensitivity of the method. Obviously, simpler experimental systems are needed to provide a good test of the CIDS method and interpretation.

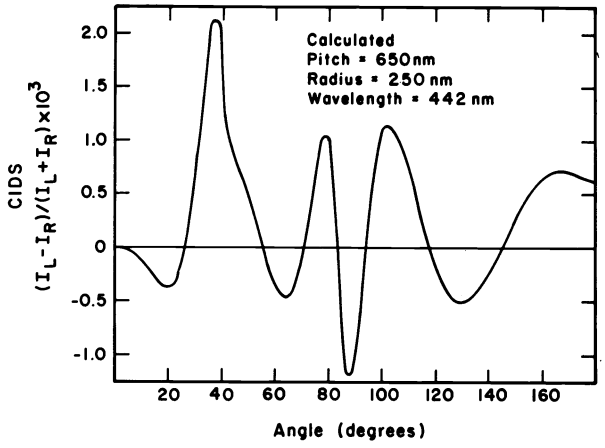

Fig. 4

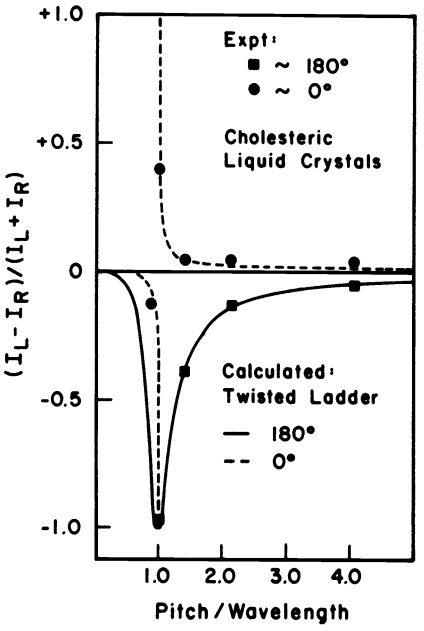

Fig. 5

Fig. 4. Calculated CIDS as a function of scattering angle for a model helix. The helix is represented by uniaxial point polarizabilities tangent to the helix.

Fig. 5. Observed and calculated CIDS as a function of pitch/wavelength for an oriented cholesteric liquid crystal. The light (wavelength = 488 nm) is incident along the helix axis, and the scattering is measured close to 0° (forward) and 180° (backward) relative to the incident beam. The calculation is done for a twisted ladder of polarizabilities with pitch equal to the experimental pitch.

CIDS of chiral cholesteric liquid crystals have been measured as a function of pitch of the liquid crystal (Ref. 8). Mixtures of cholesteric and nematic liquid crystals were used to vary the pitch. Light was incident parallel to the helix axis of the liquid crystal, and the CIDS was measured in the forward ($\theta \cong 0°$) and backward ($\theta \cong 180°$) directions. Figure 5 shows the excellent agreement between observed and calculated results. As the ratio of pitch to wavelength approaches one, the observed and calculated CIDS approaches the minimum value of minus one in the backward direction. This shows that the cholesteric liquid crystal forms a right-handed helix and that only right circularly polarized light is scattered in the back direction. In the forward direction the calculated CIDS is very small except when the ratio of pitch to wavelength is very close to one. Then the CIDS becomes plus one for pitch/wavelength slightly greater than one, and the CIDS becomes minus one for pitch/wavelength slightly less than one. The model used for the calculation here is a twisted ladder; it is a helix with zero radius. The scattering groups are represented by polarizability ellipsoids of revolution. The polarizabilities are placed on a line with the symmetry axes rotated for successive positions on the line (Ref. 9). We hope that measurement of the CIDS as a function of angle for oriented cholesteric liquid crystals will provide new information about long- and short-range order in these interesting chiral materials.

A CIRCULAR DIFFERENTIAL MICROSCOPE AND CIRCULAR DIFFERENTIAL IMAGES

A microscope with quartz optics has been used to measure the circular dichroism as a function of wavelength of single cells. Figure 6 gives the circular dichroism measured for individual Chinese hamster ovary (CHO) cells in tissue culture. Measurements are made on individual cells, but each curve shown is the average of 20 cells. The curves are characteristic for each phase of the cell cycle. In metaphase the chromatin is most condensed, and there is a large scattering contribution to the circular dichroism. Above 280 nm the circular dichroism is mainly circular differential scattering. As the cell cycle progresses through G-1, S and G-2, the circular differential scattering decreases and the circular dichroism decreases. The maximum in the circular dichroism near 230 nm indicates that the

main contribution is from the protein. Circular dichroism spectra of individual cells may be useful for identifying and characterizing cells without the need for staining them. A non-destructive method for monitoring the growth and division of a single cell is thus possible.

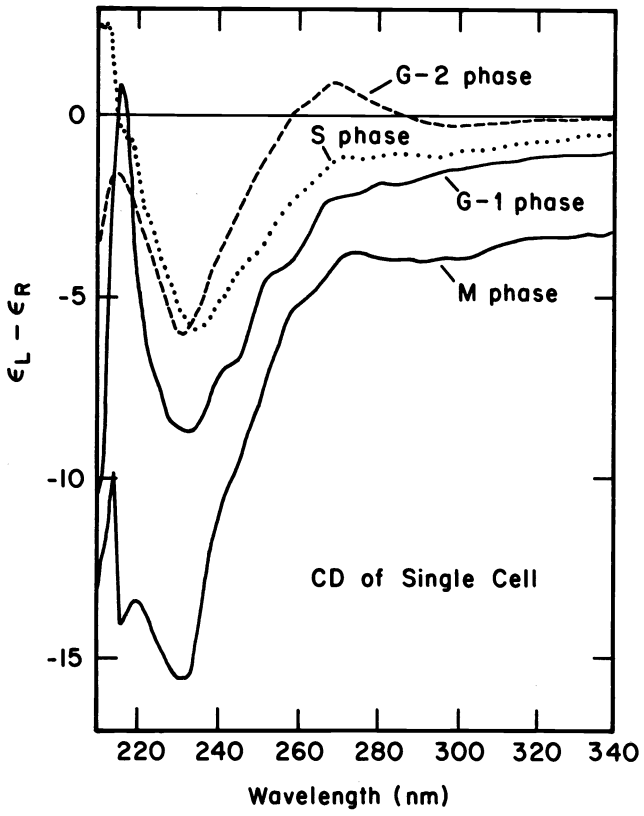

Fig. 6. Measured circular dichroism for single Chinese hamster ovary cells as a function of the cell cycle. Unpublished work of M.F. Maestre, G. Salzman, C. Bustamante and R. Tobey.

An apparatus to measure images dependent on the circular dichroism of the object has been built. A schematic is shown in Fig. 7. Left and right circularly polarized light is incident on the object, and the intensity at each point in the image plane is measured by an array of detectors. The difference in intensity for incident left and right circularly polarized light is a measure of the chirality at each point in the object. For light transmitted by the object (bright-field illumination) the image depends on the circular dichroism of each part of the object. For illumination from the side (dark-field illumination) the image depends on the CIDS of the object. The instrumentation for producing circular differential images, and theoretical analysis of these images, are both at a very early stage.

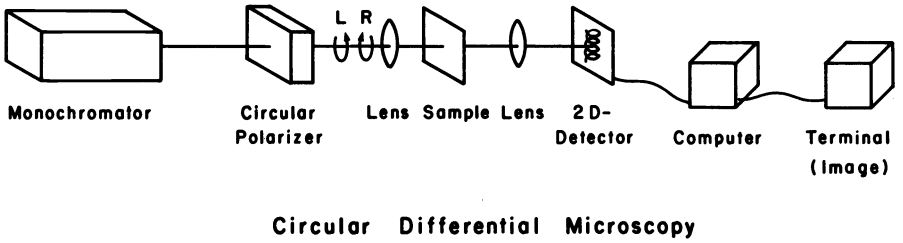

Fig. 7. A schematic diagram of a circular differential imaging microscope. The intensity at each point in the image plane is measured when left and right circularly polarized light is incident on the sample. Only chiral objects will have a difference in intensity, therefore only chiral objects will be evident in the image.

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