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Synchrotron radiation circular dichroism and conventional circular dichroism spectroscopy: A comparison

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Abstract. Conventional circular dichroism (cCD) spectroscopy is a valuable tool for secondary structure analyses of proteins. In recent years, it has been possible to use synchrotrons as light sources for CD, with the technique being known as Synchrotron Radiation Circular Dichroism (SRCD). In this study, the spectra of two proteins, the primarily helical myoglobin and the primarily beta-sheet concanavalin A, have been collected on both a cCD instrument and on the SRCD at the Daresbury synchrotron and their characteristics were compared. Over the wavelength regions where both instruments are capable of making measurements (from about 300 to 175 nm) the spectra are very similar, except at the low wavelength extreme of the cCD spectra. In this region, the spectra deviate somewhat, due to the limitations of the light source intensity in the conventional instrument. The SRCD spectra extend to much lower wavelengths (160 nm). This additional low wavelength vacuum ultraviolet (VUV) data contains a large amount of extra information, including, for the first time, a number of peaks consistent with previously predicted charge transfer transitions.

1. Introduction

Circular Dichroism (CD) is a popular technique for determining the secondary structural contents of proteins. With modern conventional circular dichroism (cCD) instruments, data can be collected to as low as ∼175 nm for a protein in aqueous solution. Since the absorption of water increases sharply below wavelengths of around 200 nm, and the flux of cCD instruments decreases dramatically over this same wavelength range, this limits the lower bound of the wavelength to which data can be collected. However the flux from a synchrotron light source remains high to much lower wavelengths [1] permitting the measurement of additional electronic transitions. Such measurements are possible because the proteins have been shown to remain undamaged even after long periods of exposure in the Synchroton Radiation Circular Dichroism (SRCD) beam [2]. The information content of CD spectra has been shown to increase with the lower wavelength limit of the data, as more transitions are detected [3]. Recently SRCD spectra collected to 160 nm [4] have been shown to contain new transitions that had been predicted by theory. Due to the extra information content present in the spectra, it has been proposed that SRCD may prove a big improvement over cCD in many areas of biology [5]. For example, structural genomics aims to identify the structure of all the gene products in targeted genomes. In order to make this feasible it is necessary to target novel folds. With sequence-based techniques there is a cutoff of around 35% sequence homology to reliably identify homologues [6]. However most homologues are found at the level of ∼9% sequence homology [7]. SRCD has been proposed as an efficient means of determining if two proteins share the same fold [8]. In addition, the higher information content in the SRCD spectra means that secondary

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structure determinations will be more accurate [5] once an extended reference database of SRCD spectra is available.

In order to demonstrate the utility and comparability of SRCD, it is important to ascertain if the spectra obtained by cCD and SRCD are identical, as they should be, or if any instrumental features give way to spectral anomalies. In this study, a comparison of cCD and SRCD spectra of two proteins, one primarily helical and the other primarily beta-sheet, has been undertaken.

2. Materials and methods

Samples of highly purified horse myoglobin (Mb) and type IV concanavalin A (ConA) were obtained from ICN Biochemicals Ltd., and Sigma, respectively. ConA and Mb solutions were prepared at concentrations of 20 and 5 mg/ml, respectively, in distilled water. To clarify the solutions, they were centrifuged at $\sim$6000 $\times$ g prior to use. For accurate scaling, the precise protein concentrations of the samples were later determined in duplicate by quantitative amino acid analyses.

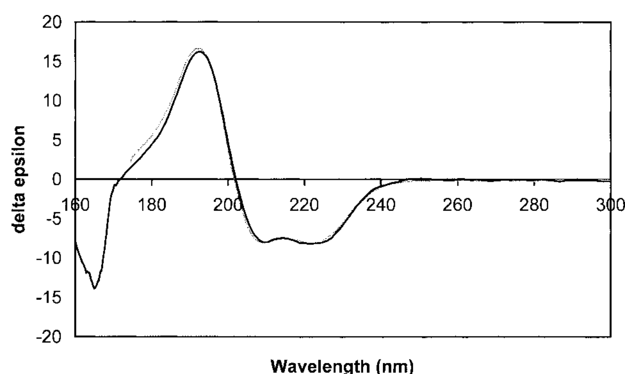
SRCD spectra were obtained on station 3.1 at the SRS Daresbury, which is part of the Centre for Protein and Membrane Structure and Dynamics (CPMSD) and cCD spectra were obtained with an Aviv 62ds instrument. For all spectra, Suprasil cells (Hellma Ltd.) with 0.001 cm pathlengths were used. Camphor sulphonic acid was used to calibrate the instruments (in the case of the SRCD, at the beginning of each beam fill). Measurements were only made down to wavelengths where the dynode voltage indicated the detector was still in its linear range. For the SRCD measurements this was 160 nm; for the cCD measurements, it was 174 nm for Mb and 175 nm for ConA.

Three runs of each sample and its corresponding distilled water baseline were obtained at 0.2 nm intervals over the wavelength range from 160 to 300 nm in the SRCD, and from $\sim$175 to 300 in the cCD. The spectra were averaged, the appropriate baselines subtracted, and the net spectra smoothed with a Savitsky–Golay filter [9].

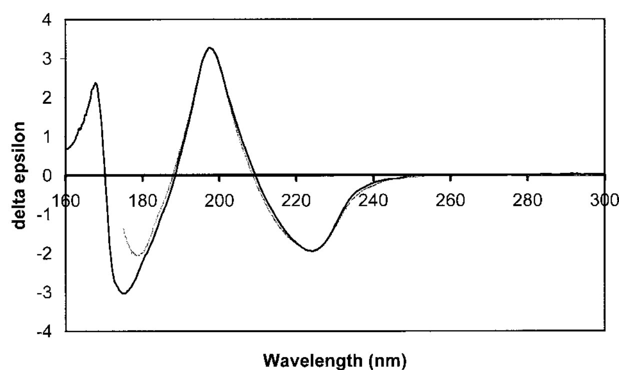
3. Results

By using high concentrations of the proteins and short pathlengths, it was possible to minimise the amount of absorbing solvent in the beam, thereby enabling measurement of the lowest wavelength data possible in both types of instruments. If the solvent had contained salts, buffers or additives, the lowest wavelengths achievable would have been much more limited, especially for the cCD spectra. As the CD signals of beta-sheet proteins are less intense than those of helical proteins, the ConA sample examined was at a higher concentration than the Mb sample. The cCD spectra of the proteins obtained in this study are indistinguishable from cCD spectra present in the existing CD reference databases [10–13] that had been obtained over the past two decades on a wide variety of commercial instruments. Therefore any differences seen here between the cCD and SRCD spectra cannot be attributable to any particular type of cCD instrument.

The SRCD spectra obtained were reliably measured down to 160 nm for both proteins, whereas the low wavelength limit for the cCD data was $\sim$175 nm. The SRCD and cCD spectra are highly similar in their overall shape for both Mb (Fig. 1(a)) and ConA (Fig. 1(b)). The largest differences are seen at the lowest wavelength extreme of the cCD spectra. In cCD at these wavelengths the slit width is fully open to allow more light onto the sample, but this also permits stray light of other wavelengths to penetrate. As the light at the higher wavelengths is more intense, the signal is dominated by this, which results in



(a)



(b)

Fig. 1. (a) Comparison of horse myoglobin CD spectra obtained from instruments using synchrotron (SRCD) (black) and conventional (cCD) (grey) light sources. (b) Comparison of concanavalin A CD spectra obtained from instruments using synchrotron (SRCD) (black) and conventional (cCD) (grey) light sources.

the apparent shift seen relative to the SRCD data. The SRCD data is from monochromatic light and so will better represent the true spectra at these wavelengths.

The spectra of Mb and ConA contain additional transitions at the very low wavelengths. The myoglobin spectrum contains a large peak at 165 nm equivalent in magnitude to the 190 nm peak, and a shoulder at ∼171 nm. The ConA spectrum has two additional peaks at 175 nm and 168 nm. The physical origin of such low wavelength peaks has been debated for some time [14], and various candidate phenomena have been proposed [15]. *Ab initio* studies have shown charge transfer between neighboring amides to give the best fit to the experimental data, using octapeptides in helical and beta-sheet conformations as model systems [16]. Transitions arising from n_0 to σ^* or π bonding (π_b) to π anti-bonding (π_{ab}) orbitals were ruled out [17]. Calculations have shown that charge transfer for the octapeptides should take place in the region of 155 to 175 nm [16], corresponding to the positions of the additional peaks seen in the SRCD spectra of the proteins examined in this study. A π_b to π_{ab} transition is predicted by theory to exist at lower wavelengths, at ∼135 nm [17]. The new beamline CD12 is currently being commissioned at the SRS Daresbury that should in principle permit collection of data down to $<$135 nm, thus enabling detection of this additional charge transfer transition.

4. Conclusion

The cCD and SRCD spectra appear to be very comparable in the near and far UV regions typically used for conventional CD measurements. The differences at low wavelengths in the VUV are attributable to the low light levels in a conventional instrument, that produce spurious results at the extreme end of the cCD spectra. The additional data at in the low wavelength end of the VUV region accessible by SRCD contain important additional information from charge transfer transitions, which may be particularly useful in studies of protein folding. In the future it is anticipated that SRCD will become a valuable technique for studying membrane proteins, fold motifs, protein interactions, amyloidogenesis, and inherently unstructured proteins.

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