

SHINING NEW LIGHT ON PROTEIN STRUCTURE AND FUNCTION THROUGH SYNCHROTRON RADIATION CIRCULAR DICHROISM (SRCD) SPECTROSCOPY

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Background

Circular dichroism (CD) spectroscopy has been employed for more than 50 years for the study of the structure and dynamics of proteins. It is now a workhorse of structural biology, finding applications in the determination of protein secondary structures, monitoring and deciphering protein folding, examining macromolecular interactions, and defining and quantitating protein-ligand binding. For the most part, CD studies have used laboratory-based instruments to measure electronic transitions in the far (190-250 nm), near ultraviolet (UV) (250-300 nm) and visible (> 400 nm) wavelength ranges, which have enabled studies of polypeptide backbones, aromatic amino acids and coloured chromophores, respectively. Additional transitions exist at lower wavelengths in the vacuum ultraviolet (VUV) region (<190 nm); however, these transitions tend to be inaccessible to conventional CD instruments, due to the low intensity of their Xenon arc lamp light sources at wavelengths below ~190 nm.

In 1980, the first synchrotron-based CD instruments were constructed (1,2), which took advantage of the high photon flux available from synchrotron light sources at these wavelengths. However, the technique of synchrotron radiation circular dichroism (SRCD) did not really take off until enabling studies had been done to show that additional data were obtainable for proteins in the VUV region (3), that these data were readily accessible with modern beamlines (4), and most importantly, that new applications of these data existed in structural molecular biology (5,6).

Advantages of SRCD Spectroscopy

SRCD has a number of advantages over conventional CD spectroscopy: it enables the collection of lower wavelength data, it produces data with higher signal-to-noise levels, the beamlines have flexible sample and detector geometries, and the high flux enables improved penetration into samples with high absorbance.

The availability of lower wavelength data permits better definition of a protein's secondary structure. This is especially true for proteins containing beta sheet components. In conventional CD spectra at wavelengths above 190 nm (Fig. 1), helical and sheet spectra are relatively similar in shape, except beta sheet spectra are

of much lower magnitude, so in proteins which have a significant helix content, the sheet signal tends to be swamped. This is one of the contributing factors as to why sheet contents are generally more poorly defined by CD than are helical structures. In the VUV region however, helical and sheet spectra have opposite signs (Fig. 1, green background), and thus the definition of sheet structure is greatly improved when these data are included in analyses. A consequence of this is that by including VUV data, it is possible to more accurately monitor folding of beta sheet-rich proteins. Finally, as the information content in a spectrum increases with the amount of data (7) and thus the number of electronic transitions detectable, it has been proposed (5) that the VUV data may contain information on fold motifs. Such information would considerably extend the utility of SRCD measurements.

The higher signal-to-noise ratios in SRCD spectra enable detection of smaller conformational changes (6) and the accurate identification of smaller differences between two samples. As an example of the latter, in a mutant crystallin protein that causes juvenile cataract, spectral changes arising from a single amino acid change have been detected and used to define structural changes associated with the disease-related protein. (8,9) (Fig. 2). An additional advantage of the SRCD high signal-to-noise is that smaller sample sizes and/or volumes are required. We have designed micro cells that use as little as 1 microlitre of solution; consequently, this technique then becomes a feasible means of monitoring the state of expressed proteins within a structural genomics program context.

The flexible sample/detector geometry enables the examination of scattering samples, including membranes (10) and even fibrous samples such as spider silk (11), by increasing the detection angle and decreasing the artifacts normally associated with measuring absorption spectra against a background of light scattering.

Finally, and perhaps most importantly, the high flux of the synchrotron light enables measurements in solutions containing buffers, salts, detergents, and additives that absorb light in the UV and VUV wavelength ranges. This enables, for instance, measurements to be made under both crystallisation conditions, and under 'physiological' conditions (3), thus permitting examination of the biological relevance of crystal structures by comparison with solution conditions more like those found *in vivo*.

Examples of New Applications of SRCD

The above-noted advantages have enabled a number of new types of experiments, including: detection of folded constructs of proteins more suitable for NMR and crystallisation studies (12); identification of ligand binding for functional genomics studies of proteins with unknown substrates (13, Wallace & Byrne, unpublished results); detection of spectral features of proteins with similar secondary structures but different folds (8,14); development of assays for detection of lipoproteins in serum (15); detection and definition of structural features involved in macromolecular interactions (Wallace & Hargraves, unpublished results, Wallace & Martin, unpublished results); determination of membrane protein secondary structures (10); examination of isolated sugar moieties (16); and identification of sugar components and their consequences on structure and function in glycoproteins (17).

Complementarity of SRCD to Macromolecular Crystallography

In the life sciences, synchrotrons are generally only thought of in conjunction with macromolecular crystallography, but SRCD is beginning to play a valuable role in structural biology, where it is complementary to crystallographic studies. While it is clear that CD data are no substitute for the high resolution information obtainable by crystallography, CD can provide dynamic and static structural

information that either supplements crystal structure information, or provides information on the secondary structures of proteins that don't crystallise or of precious proteins for which only small amounts of material are available. In addition, CD can provide information that may aid in crystallisation: it has been used to identify conditions which favour specific conformational substates resulting in more homogenous samples for crystallisation (17), to detect disordered regions, thereby enabling the design of well-ordered constructs, and to define ligand binding constants to enable production of monodisperse complexes.

To take advantage of this synergy, some new synchrotrons, such as DIAMOND, which is under construction in the UK, are being organised around a 'village' concept, where beamlines for techniques such as macromolecular crystallography, SRCD, non-crystalline diffraction, and infrared spectroscopy and microscopy, will be located adjacent to each other around the ring, to facilitate interactions and cross-utilisation of facilities in the life sciences.

SRCD Facilities Worldwide and the Australian Synchrotron Project

At present there are only a few working SRCD beamlines worldwide. These include CD12 at the SRS Daresbury in the UK, UV1 at ISA in Denmark, U9b and U11 at the NSLS in the USA, D-12-1B at BESSY2 in Germany, and BL-15 at HiSOR/HSRC in Japan.

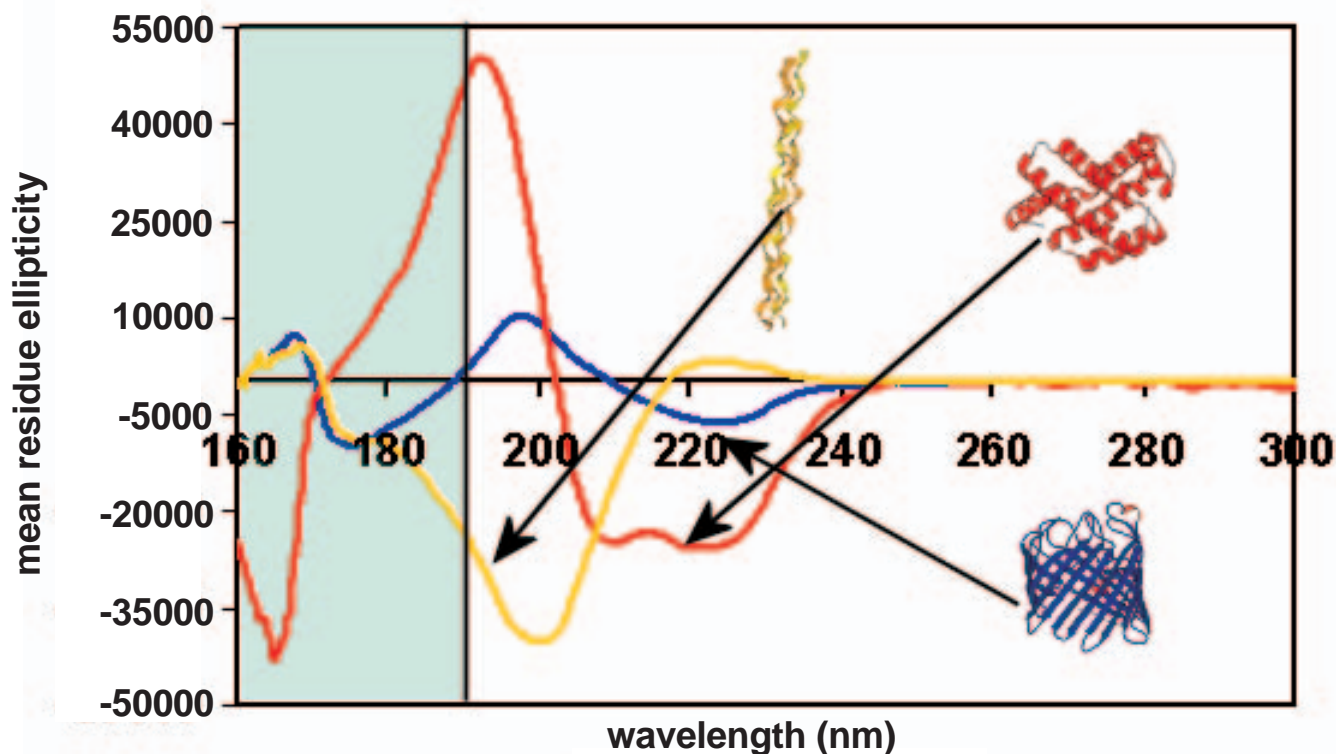


Fig. 1. SRCD spectra of a predominantly helical protein, myoglobin (structure and spectrum in red), a predominantly sheet protein, concanavalin A (blue), and a polyproline helix, collagen (yellow).

The green shaded area shows the data unique to the SRCD spectra (<190 nm), where it can be seen that the helical and sheet spectra are especially well-distinguished from each other. (Data from reference [5]).

Additional beamlines are in the commissioning or planning stages at NSRL (Hefei, China), BSRF (Beijing, China), DIAMOND (UK), and SOLEIL (France). The Australian Synchrotron Project in Melbourne is likely to join this group in the next few years. It is useful to note that SRCD does not require a high energy synchrotron source, since both ISA (at 0.6 GeV) and HiSOR (at 0.7 GeV) (18) have productive SRCD beamlines. Hence, even at low energy sources where macromolecular crystallography beamlines would not be practicable, life science research can be done alongside physical and chemical studies. Higher energy third generation sources, such as those under construction at DIAMOND and SOLEIL, are also able to accommodate SRCD beamlines. Hence, SRCD beamlines are possible on sources with very different energies and could thus be usefully installed on many existing synchrotrons worldwide.

The effort to develop a SRCD beamline at the Australian Synchrotron is being spearheaded by Mibel Aguilar of Monash University. Potential applications of SRCD in structural molecular biology were discussed by this author at the 2004 Lorne Protein Conference and at the 1st Australian Workshop on Membrane Protein Structure (organised by Mibel Aguilar, Ray Norton, Michael Parker and Francis Separovic), where it became apparent that there are many exciting Australian projects that might benefit from its availability. Indeed, as proof of principle, Aguilar and Jenny Martin (Queensland) have both visited the SRS Daresbury to conduct SRCD experiments, the latter in collaboration with my group, and have explored its applications in protein interaction studies.

New Software for Conventional CD and SRCD Spectroscopy

In parallel with the development of SRCD hardware, we have produced two new software packages that may aid users of any of the SRCDs (or for that matter, any conventional CD instruments) in processing, analysing and archiving their data. *cdtool* (19) is a PC-based program (<http://cdtools.cryst.bbk.ac.uk>) which takes format-independent input data through the entire data-processing procedure, using data-mining techniques to retain all the information associated with collection and processing in the final output. It also includes the ability to automatically archive the processed data in a local database, a feature which may be valuable in light of recent funding institution directives in a number of countries regarding data sharing and archiving, and requirements for 'good practice' and 'traceability' within the pharmaceutical industry. DICHROWEB (20) (<http://www.cryst.bbk.ac.uk/cdweb>) is a webserver that enables simple online analyses of secondary structures using a wide-range of algorithms and reference databases. The latter will soon be updated to include new specialist databases for membrane proteins (10) and SRCD (low wavelength) datasets (8,14). Access to both is available free to academic users. A further development underway (Wallace and Janes, in preparation) is the creation of the protein CD deposition data bank (PCDDB), analogous to, and linked with the PDB, for the deposition of CD and SRCD spectra, that should provide an enduring and searchable resource for CD spectra of proteins in the future.

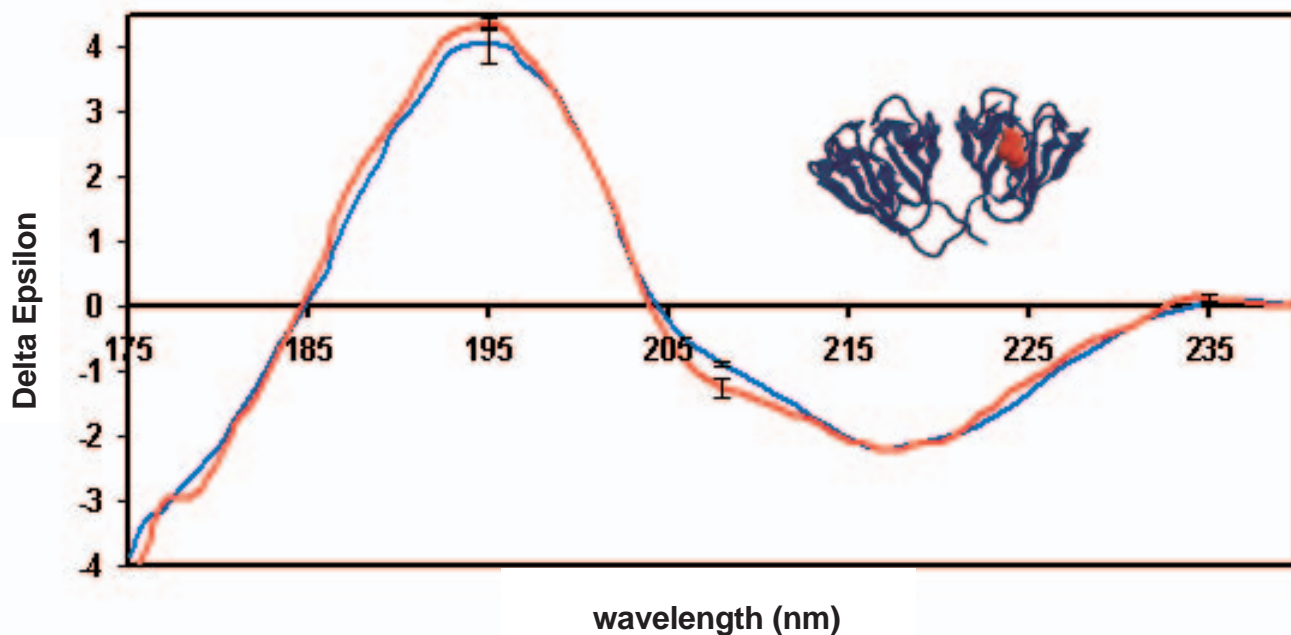


Fig. 2. SRCD spectra of wild type (blue) and cataract-forming mutant (red) crystallins, showing that with the high signal-to-noise levels in the SRCD, it is possible to distinguish subtle structural differences resulting from a single amino acid substitution (P23T).

The crystal structure of the wild type protein is shown in the inset, with the site of the mutant amino acid indicated in red. (Data from reference [9]).

In summary, the future is bright for SRCD research worldwide and the new Australian Synchrotron project should give Australian researchers excellent access to this emerging technique in structural molecular biology.

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