Circular dichroism and synchrotron radiation circular dichroism spectroscopy: tools for drug discovery

Bonnie A. Wallace*[1] and Robert W. Janes†

*Department of Crystallography, Birkbeck College, University of London, Malet Street, London WC1E 7HX, U.K., and †School of Biological Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, U.K.

Abstract

CD spectroscopy is an established and valuable technique for examining protein structure, dynamics and folding. Because of its ability to sensitively detect conformational changes, it has important potential for drug discovery, enabling screening for ligand and drug binding, and detection of potential candidates for new pharmaceuticals. The binding of the anti-tumour agent Taxol to the anti-apoptosis protein Bcl-2 [Rodi, Janes, Sanganee, Holton, Wallace and Makowski (1999) J. Mol. Biol. 285, 197–204] and the binding of the anti-epileptic drug lamotrigine to voltage-gated sodium channels [Cronin, O'Reilly, Duclohier and Wallace (2003) J. Biol. Chem. 278, 10675–10682] are used as examples to show changes detectable by CD involving secondary structure, and are contrasted with the binding of the agonist carbamylcholine to acetylcholine receptors [Mielke and Wallace (1988) J. Biol. Chem. 263, 8177–8182], an example where binding does not involve a secondary structural change. Synchrotron radiation CD spectroscopy offers significant enhancements with respect to conventional CD spectroscopy, which will enable its usage for high-throughput screening and as a tool in 'chemical genomics' or 'reverse chemical genetics' strategies for ligand identification. The lower wavelength data available enable more detailed, sensitive and accurate detection, the higher light intensity permits much smaller amounts of both proteins and drug candidates to be used in the screening, and future technological developments in sample handling and detection should enable automated high-throughput screening to be performed.

CD spectroscopy for drug discovery: why?

CD spectroscopy is a valuable technique for detecting conformational changes in proteins, as the spectra are sensitive to small alterations in structure. Consequently, the technique has been used extensively for both qualitatively and quantitatively examining the binding of ligands and drugs to proteins, as well as for deciphering the nature of interactions between proteins and other macromolecules. The advantages that CD spectroscopy has over other techniques for monitoring conformational changes include the ease and speed at which the experiments can be conducted, the small quantities and low concentrations of protein and drug needed, the absence of 'probe' molecules, and the ability to examine both soluble and membrane proteins. The latter of these is important, given the high percentage of drug targets that are membrane proteins, and the difficulty in obtaining information on this class of protein by other techniques. The changes detected in the CD signal provide structural information about the nature of the binding, information that is an enhancement on that provided by methods that merely detect the presence of binding.

Key words: chemical genomics, conformational change, drug discovery, high-throughput screening, protein structure, synchrotron radiation circular dichroism spectroscopy.

Abbreviation used: SRCD, synchrotron radiation CD.

[1]To whom correspondence should be addressed (e-mail ubcg25a@mail.cryst.bbk.ac.uk).

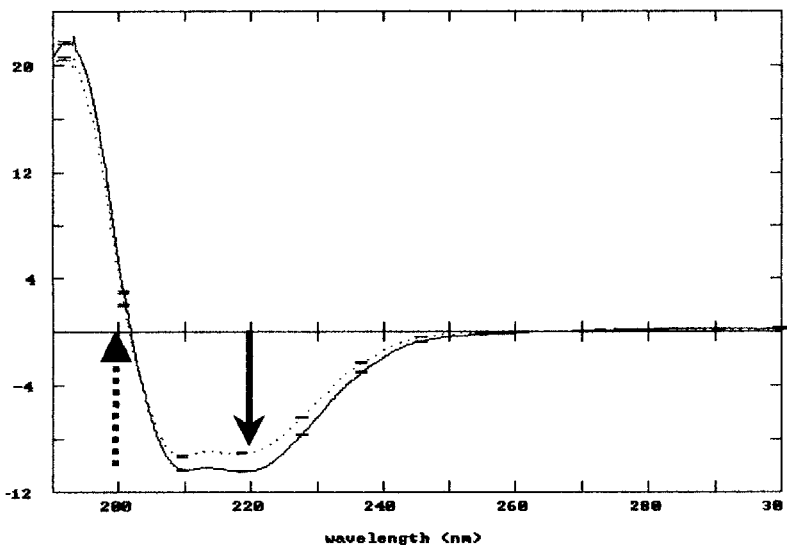
With conventional CD instruments, it has been possible to detect binding using several different wavelength ranges, which monitor different types of structural changes. To date, the most effective region of the spectra is the far-UV region (approx. 240–190 nm), where conformational changes involving secondary structures can be detected. Changes in the far-UV can be interpreted on a molecular level in terms of specific alterations in the polypeptide-backbone structure. The spectral changes can be used to determine binding constants, to quantify the number of amino acids involved in the binding, and, in some cases, to pinpoint the type or region of the structure involved in the binding. Conformational changes involving alterations to side-chain environments can be detected in the near-UV region (300–240 nm) [1], but these transitions are less intense, and thus require the use of substantially more material, and are consequently not as practical for screening and drug discovery. As an alternative to following changes in the protein structure, induced CD signals in achiral drugs themselves can sometimes be used for monitoring protein–ligand interactions at other wavelengths [2].

Examples of CD studies of protein/drug binding

An example of the use of CD spectroscopy in binding studies is the demonstration that the Bcl-2 anti-apoptosis protein

Figure 1 | Taxol binding to the anti-apoptosis protein Bcl-2

CD spectra of Bcl-2–glutathione S-transferase fusion protein with (solid line) and without (dot–dashed line) Taxol added. Before this study, it was not known that the drug bound to this protein. A large conformational change, involving a loop region, occurs, and can be monitored at the peak wavelength (solid arrow) for screening, with changes in the isosbestic point (dashed arrow) monitored to eliminate false positives. Adapted from the Journal of Molecular Biology, vol. **285**, D.J. Rodi, R.W. Janes, H.J. Sanganee, R.A. Holton, B.A. Wallace and L. Makowski, "Screening of a library of phage-displayed peptides identifies human bcl-2 as a Taxol-binding protein", pp. 197–203, © (2003), with permission from Elsevier.



binds the anti-tumour drug Taxol (paclitaxel) (Figure 1). This study showed that secondary structural changes that occur in the protein upon drug binding involve approx. 10–12 amino acids in a loop region expected to be distinct from the corresponding regions of the homologous Bcl-X_L protein [3], and has led to exploration of new related targets for rational drug design. A second example is the binding of the anti-epileptic drug lamotrigine to voltage-gated sodium channels [4]. The increase in ordered structure associated with maintaining an inactivated, open conformation was shown to be specific to the sodium channels, a control being that no such change occurred with a pooled sample of other ion channels and membrane proteins from the same tissue. This observation provides a means for screening for other anti-epileptic drugs with similar modes of action.

However, it is important to note that there are instances when specific drug binding does occur, but if the binding involves simple active-site blockage or tertiary structural arrangements, no change will be detectable by CD in the far-UV region. Such is the case for carbamylcholine binding to the nicotinic acetylcholine receptor [5] (Figure 2).

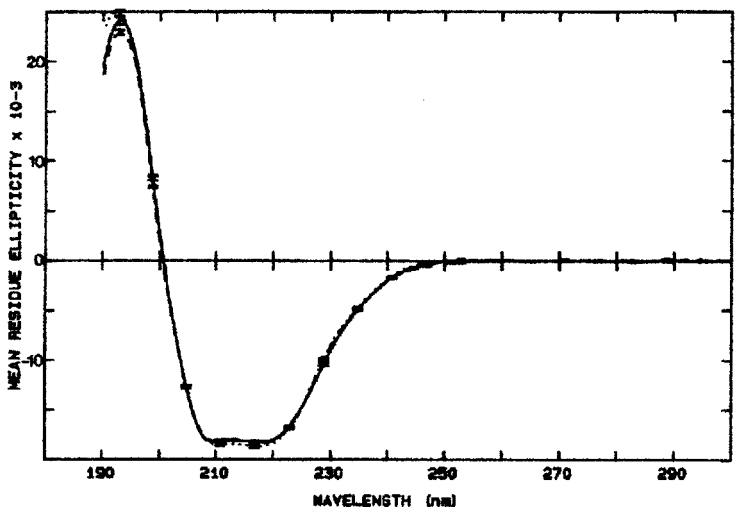
Advantages of synchrotron radiation CD (SRCD) spectroscopy for drug binding and high-throughput screening

SRCD spectroscopy is a variation on conventional CD spectroscopy, which uses the high intensity of a synchrotron as its light source [6]. It is an emerging technique in structural biology, which has the potential for enhancing the utility of CD for monitoring drug binding, and especially for enabling high-throughput screening. The high-flux beam has a number of important spectral consequences for conformational and binding studies [7,8], namely: (1) acquisition of lower-wavelength UV data (which are more sensitive to differences in secondary structure) and, at very low wavelengths in the vacuum UV region below 190 nm, the presence of strong charge-transfer transition signals (which contain information on tertiary structures); (2) higher signal-to-noise ratios (thus enabling the use of even smaller amounts of material); (3) decreased requirements for signal averaging (due to the stronger signal, thereby resulting in faster scans); and (4) collection in the presence of buffers and absorbing components (thereby more closely mimicking 'physiological' conditions). All these features will lead to improved detection of ligand binding and potential drug-candidate identification.

High-throughput methods for sample introduction and detection being developed at the Synchrotron Radiation Source Daresbury Laboratory, Warrington, Cheshire, U.K. (G. R. Jones and D.T. Clarke, personal communication) should facilitate the rapid screening of compounds and the large-scale identification of potential drugs that can bind to known pharmaceutical protein targets. Screening at two wavelengths will enable both detection and elimination of false positives (Figure 1): at the peak wavelength, maximal changes will be observable, whereas any 'hits' that also detect changes at the isosbestic point would be classified as false positives. Thus SRCD should provide a valuable new

Figure 2 | Carbamylcholine binding to acetylcholine receptors

CD spectra of nicotinic acetylcholine receptors from *Torpedo californica* reconstituted into lipid vesicles with (dotted line) or without (solid line) the agonist carbamylcholine. The error bars indicate the S.D. in the measurements. In this example, whereas independent assays demonstrated drug binding, no conformational change involving the polypeptide backbone was detectable. Reproduced from [5] with permission. © (1988) The American Society for Biochemistry and Molecular Biology.



technique for drug discovery, and become a useful tool for the emerging discipline of chemical genomics for functional identification.

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