

comment

Conformational changes by synchrotron radiation circular dichroism spectroscopy

Circular dichroism (CD) spectroscopy is a valuable technique for detecting conformational changes in proteins as the spectra are sensitive to small alterations in polypeptide backbone structures¹. It has been used extensively for both qualitatively and quantitatively examining the binding of ligands to proteins, as well as for deciphering the nature of interactions between proteins and other macromolecules. Using CD spectroscopy, it has been possible to determine the types of secondary structures involved in conformational changes and, in some cases, to quantify the magnitude of the conformational change (number of amino acids involved)². Some of the advantages of CD as a technique for monitoring conformational changes include the small quantities and low concentrations of protein required, the absence of 'probe' molecules, which could distort the results of any measurements made, and the ease and speed at which the experiments can be conducted. Furthermore, the changes can be interpreted on a molecular level in terms of alterations in the polypeptide backbone.

The technique of synchrotron radiation circular dichroism (SRCD) was first developed in 1980 (refs 3–5), but it was not until the late 1990s that it began to demonstrate its greater potential over conventional CD for studying protein conformational changes^{6–8}. As it uses synchrotron radiation as its light source, SRCD measurements can access much more of the vacuum ultraviolet (VUV) wavelength range than can be accessed using conventional (laboratory based) CD instruments. Although at high wavelengths (~240 nm) the fluxes of SRCD and CD instruments may be roughly comparable, at 180 nm the flux for a SRCD instrument may be as much as 10⁴ times that of a conventional CD instrument. Published conventional far UV CD

spectra generally include the wavelength range from ~240 nm down to ~190 nm, although with a well-tuned instrument data collection to as low as 178 nm is sometimes possible if very short pathlength cells and high protein concentrations (~7 mg ml⁻¹) are used. Under similar conditions, it is now possible to obtain SRCD data down to 160 nm for aqueous solutions of proteins (Fig. 1). As a result, SRCD spectra include electronic transitions of the polypeptide backbone heretofore not seen for aqueous solutions of proteins.

The acquisition of data at lower wavelengths means that the SRCD spectra will be richer, containing more eigenvectors (unique elements) of information than conventional CD spectra due to the additional electronic transitions measured. Consequently, it should be possible to analyze SRCD spectra for a larger number of distinct secondary structural components or folding motifs. Furthermore, as the spectral features in the VUV region for the various types of secondary structures are much more clearly different than they are in the higher wavelength peptide transitions^{7,9}, more accurate secondary structure determinations can be made.

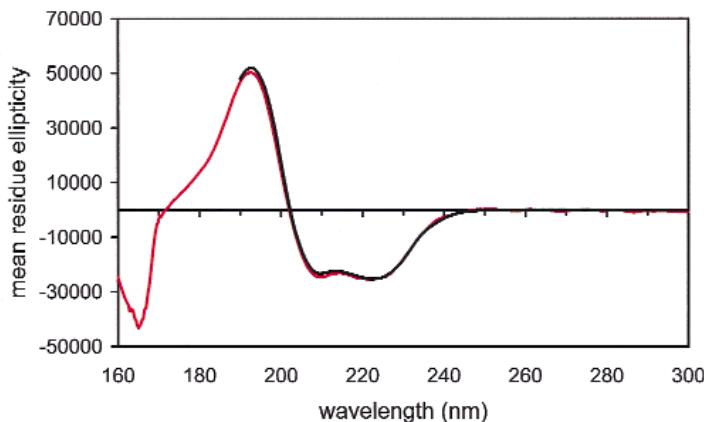
The more intense SR light source also means that the signal-to-noise (S/N) ratios for SRCD measurements are considerably higher in the far UV and VUV region where the peptide backbone transitions occur. A consequence of this is that lower concentrations of protein can be used in SRCD to obtain spectral qual-

ities comparable to conventional CD. Alternatively, shorter pathlength sample cells may be used (0.001 cm or less), thereby decreasing the problem of nonchiral absorption due to the solvent or buffer, and enabling the collection of lower wavelength data. SRCD is particularly valuable for monitoring conformational changes involving ligand binding because the improved S/N ratios mean that more precise definitions of the changes are possible. Also, the resultant smaller error bars on the measurements mean that it is possible to detect significant changes involving smaller numbers of amino acids.

In recent years stopped-flow techniques have shown the value of CD spectroscopy in kinetic studies of conformational changes¹⁰. Faster kinetic measurements can be made with SRCD, even using a conventional mixing apparatus, because the improved S/N ratios decrease the averaging time needed to obtain comparable signals. This means that measurements can be extended into the submillisecond time range, with smaller samples of proteins⁸. The small spot size of illumination of the sample in SRCD relative to that in conventional CD means that in stopped-flow mode much smaller sample volumes are needed, a considerable practical advantage. A further consequence of the higher flux at low wavelengths is that in addition to the 208–240 nm range commonly monitored by conventional CD instruments, the information rich 190–200 nm range can also be sampled by stopped-flow SRCD¹¹.

Virtually all aspects of spectroscopic studies of protein conformational changes undertaken by conventional CD are potentially enhanced by the use of SRCD. Existing facilities for SRCD are located at the Synchrotron Radiation Source (SRS; Daresbury, UK)¹² and the National Synchrotron Light Source

Fig. 1 SRCD (red) and CD (black) spectra of horse myoglobin in aqueous solution. The SRCD spectrum was obtained on Station 3.1 at the SRS, Daresbury Laboratory over the wavelength range from 160 to 300 nm. Additional transitions and information content are present at low wavelengths that cannot be detected by conventional CD spectroscopy.



(NSLS; Brookhaven National Laboratory, USA)¹³. Two new facilities are expected to come online within a year; one at the Centre for Protein and Membrane Structure and Dynamics (CPMSD)¹⁴ at the SRS¹⁵ and one at the Aarhus Storage Ring in Denmark (ASTRID; Aarhus, Denmark)¹⁶.

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B.A. Wallace

Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, UK, and Centre for Protein and Membrane Structure and Dynamics, Daresbury Lab, Warrington WA4 4AD, UK.

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history

Killer conformations

In 1996 the public was shocked to learn that ten cases of a rare brain disease in the UK might be linked to a similar disease in cattle. In humans the disease is a variant form of Creutzfeldt-Jakob Disease (vCJD) while in cattle it is known as bovine spongiform encephalopathy (BSE) or mad cow disease. These diseases slowly attack the brain, often leaving spongiform holes. They also have the dubious distinction of being fatal within about one year of the onset of symptoms.

In the 1980s an epidemic of BSE in England that killed over 140,000 cows seems to have been caused by feed containing the remains (including spinal cords and brains) of cattle and/or sheep that may have died of a related disease such as scrapie. Presumably, the variant form of CJD was spread to humans through the consumption of meat products from BSE-infected cattle.

Unlike typical disease-causing agents such as bacteria, viruses, fungi and parasites, the infectious agents thought to be the cause of these neurodegenerative diseases lack any nucleic acid. Instead, they seem to be composed exclusively of a modified form of a protein — the prion protein. According to the prion hypothesis, the normally harmless, cellular prion protein (PrP^{c}) is converted into a protease-resistant form (scrapie PrP or PrP^{s}). This aberrant form of the prion protein has infectious properties and acts as a template upon which the normal prion protein is refolded into the aberrant form. The two prion isoforms are an extreme example of how a protein can exist in two different

conformations with dramatically different, and deadly, consequences. The importance of conformational changes in many biological processes is featured in this issue (see the set of Reviews). Since the ability of prions to convert their structures into aberrant conformations is strongly linked to the disease state, we chose to review how the prion hypothesis was developed here.

The idea of a disease-causing protein was first proposed by Griffith in 1967 (ref. 1). Building on the work of many other scientists, in 1982 Stanley Prusiner coined the term “prion” from proteinaceous and infectious. The notion of a protein-only infectious agent has been controversial from the start. Although evidence for the existence of such infectious proteins has increased and earned Prusiner the Nobel Prize in Physiology or Medicine in 1997, a direct causal link between these proteins and the diseases they are believed to cause remains to be established.

Prusiner’s work in this field began in 1972 when, as a resident in neurology, he was attending to a patient dying of CJD (ref. 2). He was “most impressed by a disease process that could kill [his] patient in 2 months by destroying her brain while her body remained unaffected by this process.” Because scrapie, vCJD, and kuru (from a Pacific Islander word meaning “tremble”), another human prion disease, are pathologically and clinically alike, it suggested a similar causative agent. Prusiner’s attention shifted to scrapie when it was shown that it was extremely resistant to inactivation by ultraviolet and ionizing radiation^{3,4}. These

results suggested a number of possibilities for the identity of the scrapie agent — from small DNA viruses, membrane fragments and polysaccharides, to proteins.

After many failed attempts, in 1981 Prusiner was able to purify the scrapie agent ~100- to 1,000-fold with respect to protein⁵. He did this using the golden Syrian hamster system where scrapie could be produced in ~70 days after inoculation rather than the ~1 year required in mice. The shorter incubation times allowed a large number of experiments to be done in parallel and led to the development of effective purification protocols for enriching fractions for scrapie infectivity. Prusiner showed that the scrapie agent contained a protein that was required for infectivity⁶. The agent was inactivated by a number of treatments that destroy protein such as exposure to sodium dodecyl sulfate, phenol, and urea, digestion with proteinase K and trypsin, and chemical modification with diethyl pyrocarbonate.

Subsequently, a number of findings continued to eliminate alternate hypotheses for both the prion structure and its mechanisms of action^{7–11}. In 1985, the gene encoding the prion protein was found to be constitutively expressed in all animals tested, including humans, and the PrP mRNA levels were found to be similar in normal uninfected and scrapie-infected tissues. These findings eliminated the possibility that PrP^{s} induced its own transcription. The structure of the gene showed that the entire protein-coding region was contained within a single exon, thus excluding the possibility that the two forms of the prion protein result from alternatively spliced mRNAs. In 1993, Weissman and coworkers showed that PrP -deficient mice were