Chapter 17

Circular Dichroism of Peptides

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Abstract

Circular dichroism measures the difference between the absorbance of left- and right-handed circularly polarized light, and can be used to monitor the secondary structure of peptides (far UV) and the tertiary structure of larger polypeptides (near UV). This technique is especially useful for helix–coil transitions and other aspects of structural alterations. Data from several low-resolution spectroscopic techniques, including CD, can be combined to generate an overall picture of peptide structure as a function of environmental conditions.

Key words Circular dichroism, Near UV CD, Far UV CD, Helix–coil transitions, Peptide, Conformational integrity, Stability

1 Introduction

Circular dichroism (CD) measures the difference between the absorbance of left and right circularly polarized light. The far-UV CD spectra (260–180 nm) resulting from light absorption by peptide bonds contain information pertaining to the secondary structure of peptides. The near-UV CD spectra (320–250 nm) originate from light absorption by optically active tryptophan, tyrosine, phenylalanine, and cystine residues. These spectra are sensitive to the tertiary and quaternary structure of larger polypeptides. Oligopeptides showing a propensity to form α-helices in solution have been extensively studied by far-UV CD techniques [1]. Peptides having a tendency to self-assemble by aligning β-sheet structures have also been characterized using far-UV CD [2]. Since many peptides are relatively small, however, they may possess far-UV CD spectra more characteristic of disordered structure. A major exception is small peptides that form a variety of β-turn

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structures [5]. Helix–coil transitions and other aspects of structural alterations in peptides have been intensively studied using far-UV CD spectroscopy [5] and provide a major use for this technology.

2 Materials

1. A CD spectropolarimeter equipped with single/multi-position Peltier-controlled cell holder and a xenon lamp (Jasco, OLIS, Applied Photophysics, AVIV, etc.) and temperature control.
2. A set of 0.1, 0.05, 1, and 10 mm quartz cuvettes (Starna, Hellma).
3. Dedicated software for CD data acquisition and processing and Excel and/or Origin software for data analysis.

3 Methods

3.1 Instrument Preparation

1. The CD instrument is purged with pure nitrogen at the manufacturer's indicated flow rate for at least 5 min prior to starting the xenon lamp. Nitrogen flow must be continued until the end of the experiment. Water circulation and Peltier control are started to maintain a constant temperature while data are being collected (*see* **Note 1**).
2. Initial wavelength scans for far-UV (180–260 nm) and near-UV (250–340 nm) CD are usually performed at room temperature (or lower temperatures such as 10 °C) in quartz cuvettes with appropriate path lengths. The run parameters are optimized to obtain good signal-to-noise statistics by adjusting the following experimental parameters according to the desired signal levels: scan rate (10–30 nm/min), integration time (time constant) (1–3 s), bandwidth (<2 nm), and number of spectral acquisitions [2–5]. Examples of typical far-UV CD spectra from the three model peptides are shown in Fig. 1, and representative near-UV CD spectra of the same three model peptides are shown in Fig. 2. In these examples, the three different peptides differ in their content of aromatic amino acid residues (angiotensin I contains one Tyr and Phe, α-melanocyte-stimulating hormone peptide contains one Trp, Tyr, and Phe, while substance P contains two Phe residues) and display some minor differences in their far-UV and near-UV CD spectra (*see* **Note 2**).
3. The spectral bandwidth and the integration time are adjusted for near-UV CD spectra in such a way that the fine structural details typically available in such spectra can be obtained without compromising the signal-to-noise ratio (*see* **Note 3**). Derivative analysis can be used for this purpose. Taking the derivative of a spectrum is the simple process of calculating the

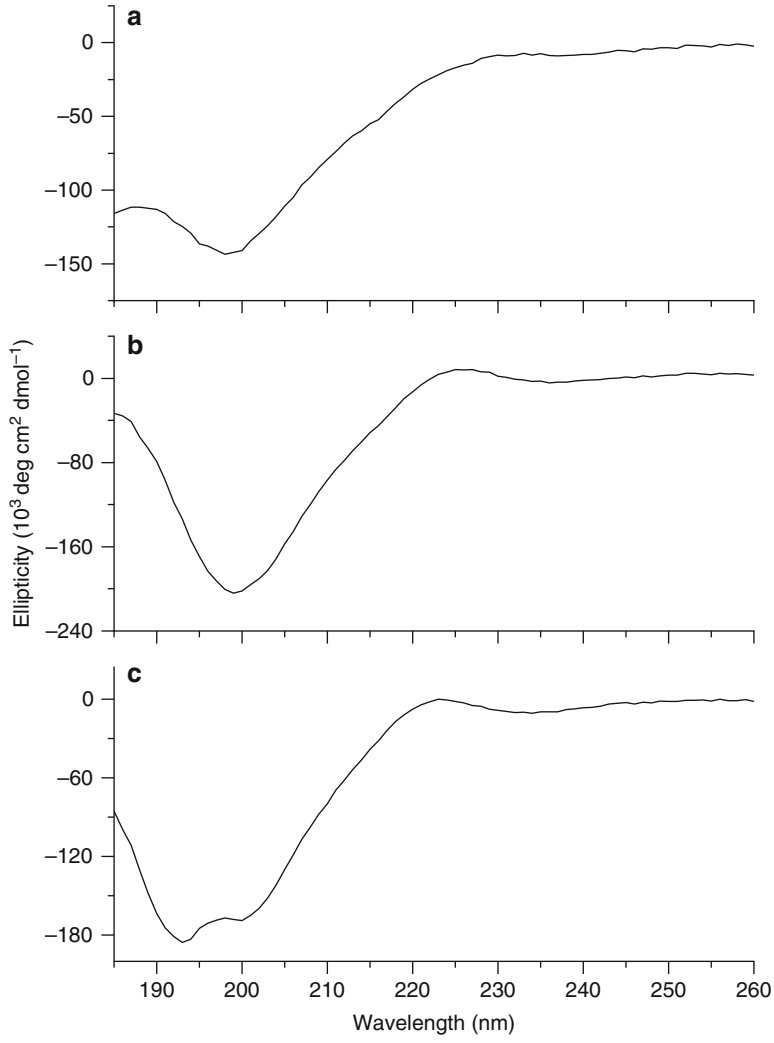

Fig. 1 Far-UV CD spectra of three model peptides: (**a**) Angiotensin I, (**b**) α-MSH, and (**c**) substance P. All three peptides display a far-UV CD spectral minimum near 198 nm characteristic of disordered structure. In addition, substance P has a second minimum near 193 nm presumably reflecting the presence of its β-turn. All three peptide spectra also show a weak positive peak near 220 nm that is usually thought to reflect the presence of some weak helical structure arising from polyproline II-like structure

slope or the gradient of an absorption band. This gradient ($dA/d\lambda$) is plotted against wavelength (λ) to produce a first-order derivative plot. An iteration of this process leads to higher order derivatives ($d^nA/d\lambda^n$). Temperature control parameters are set to the desired starting temperature with a thermal equilibration time of 2–5 min prior to each measurement. It is also possible to obtain thermal perturbation data by

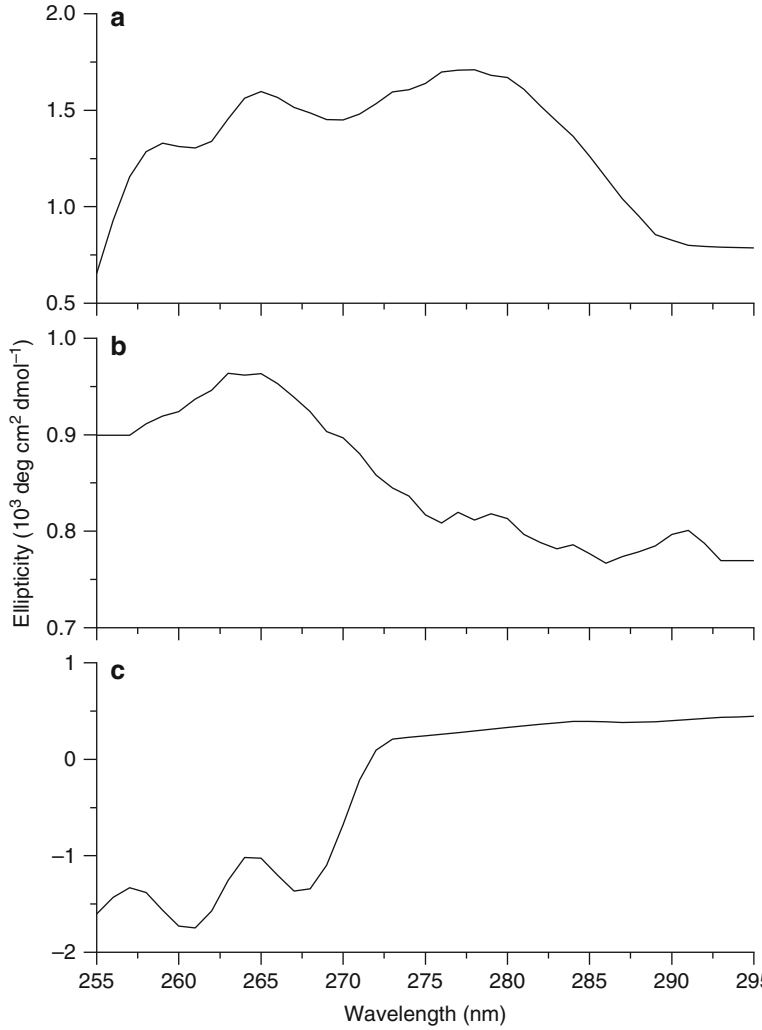

Fig. 2 Near-UV CD spectra of three model peptides: (**a**) Angiotensin I, (**b**) α-MSH, and (**c**) substance P. All three peptides display a near-UV CD spectra with weak transitions found primarily in the regions where the individual aromatic side chains most strongly absorb. Although not the situation here, local environments can sometimes result in the induction of enhanced optical activity in these transactions

slowly elevating the temperature in a continuous mode but temperature equilibrium may not be reached in such analyses. Data can be recorded at one or more fixed wavelengths or the entire spectra can be recorded at each temperature. The base line is recorded by employing a cuvette containing only the sample buffer (without peptide) and this spectrum is subtracted from each spectrum of the peptide-containing solution.

3.2 Sample Preparation

1. Peptide samples are filtered though suitable filters to remove any scattering particles. Peptide concentration must be accurately measured, preferably spectrophotometrically using UV absorbance and a previously determined molar extinction coefficient at 280 nm (using the Beer–Lambert law, which states that absorbance is directly proportional to path length, concentration, and extinction coefficient). Peptide concentrations must be maintained such that the total absorbance at measured wavelengths stays below unity to prevent saturation of the detector. For far-UV CD measurements, cells with path lengths from 0.05 to 0.2 cm and peptide concentrations of 0.1–1 mg/ml are typically employed. The sample volumes are dependent on the design of the cell and can range from 1 ml to 50 μl (*see* **Note 3**). For near-UV CD measurements, longer path lengths and higher peptide concentrations must be used due to the lower molar concentrations and optical activities of the chromophoric entities. Thus cuvettes with path lengths of 5–20 mm and peptide concentrations of 0.5–2 mg/ml are typically used for collecting near-UV CD spectra [4, 6] (*see* **Note 4**). Modern instruments often permit near and far UV spectra to be acquired in a single scan [7].
2. A cleaning step is necessary after every CD experiment using a rigorous protocol to ensure that the cuvettes are clean for the next experiment. Cuvettes are filled with a strong acid cleaning solution (e.g., Nochromix) and allowed to stand for at least an hour. The cuvettes are then emptied and rinsed with water followed by a detergent (10 % Neutrad) cleaning step. Finally, the cuvettes are rinsed thoroughly with nano pure water (*see* **Notes 5** and **6**).

3.3 Data Analysis and Interpretation

1. The CD signals are conveniently converted to the concentration-independent parameter of mean residue molar ellipticity $[\theta]$ using the following formula (Eq. (1)):

$$[\theta] = \frac{[(\theta / 10) \times m]}{l \times C} \quad (1)$$

where θ is the measured value from the instrument in millidegrees after subtracting the value of ellipticity associated with the buffer; m is the mean residue molecular mass determined for the peptide, obtained by dividing molecular weight of the peptide by the number of amino acids in the peptide chain; l is the cell path length in cm; and C is the concentration of the sample in g/ml. Alternatively, molar ellipticity based on the molecular weight of the peptide can be used.

2. Results from CD thermal experiments are usually presented by plotting the molar or the mean residue ellipticities at corresponding wavelengths versus the temperature.

4 Notes

1. Purging with nitrogen is required to create an oxygen-free environment to prevent conversion of oxygen to ozone by far-UV radiation, which in turn can damage the optical surfaces. This purging with nitrogen also permits CD measurements below 200 nm. Ozone absorbs UV radiation from 200 to 300 nm, obstructing observation at these wavelengths. A noble gas, such as He and Ar, is not suitable for purging since it will leak into the interior of the PMT and reduce its vacuum, causing impaired performance. The system will stabilize in about 30 min and then the nitrogen flow rate can be reduced to 5–7 L/min. It also takes 5–10 min for the system to stabilize each time the sample compartment is open and closed.
2. A description of the signature absorbance bands typically belonging to different secondary structures of peptides is described below: (a) α-helix possesses three major absorbance bands with a positive maximum between 190 and 195 nm and two negative minima at approximately 208 and 222 nm; (b) the features of β-sheet are mainly limited to two major absorbance bands including a negative band in the spectral range of 217–218 nm and a positive one near 195–197 nm; (c) random coil structures of peptides feature a strong negative band below 200 nm. Some peptides also manifest a positive/negative weak shoulder at 220 nm, and (d) several different types of β-turns may be present in the secondary structure of peptides. Frequently observed types include type I and type II displaying multiple bands in far-UV CD spectra as discussed in detail elsewhere [8].
3. The spectral bandwidth should be set under 2 nm to minimize spectral distortion. This threshold is especially critical in near-UV CD measurements for resolving fine structural information in this region of the spectrum. The product of scan speed and integration time should be kept below 0.5 nm to minimize possible distortion of the spectrum [3].
4. Light absorption due to buffers, solvents, and other supporting electrolytes should be minimized by choosing appropriate buffers and other components. Absorption due to a wide variety of aqueous components arises in the far-UV region. A thorough description of absorption profiles of commonly used buffers and other reagents can be found in the literature [3]. It should be noted that one of the most interfering buffer components is chloride ion due to strong absorption below 195 nm. Therefore, chloride ions are highly undesirable as a buffer counterion in CD experiments. Salts with fluoride and sulfate ions are better choices to maintain the ionic strength since they possess better

UV transparency. Due to buffer and other components, it is often not possible to obtain data below 200 nm.

5. Artifacts in CD spectra: Similar issues to those seen with UV absorbance spectra are relevant with absorption flattening especially problematic for particulate samples (*see* **Note 6**). In addition, differential scattering of left- and right-handed circularly polarized light can produce artifactual signals. Simply moving the sample cell closer to the PMT to gather more of the transmitted or the scattered light can sometimes minimize such artifacts, although they are relatively rare [9].
6. As the size of particles in a sample becomes larger, light scattering corrections may not be sufficient. Absorption flattening is a phenomenon in which high local concentrations of peptides (due to their aggregation) create an inhomogeneous distribution of sample chromophores in the light path. Absorption flattening typically leads to reduced signal intensities and red shifts in absorption bands and may be erroneously interpreted as peptide structural changes [9]. The use of short-path-length cuvettes or disruption of aggregates (reduction in size) by a technique such as sonication can often be used to reduce or eliminate this artifact.

References

1. Dyson HJ, Wright PE (1991) Peptide conformation and protein folding. Annu Rev Biophys Biophys Chem 20:519–538
2. Aggeli A, Bell M, Boden N, Keen JN, Knowles PF, McLeish TCB, Pitkeathly M, Radford SE (1997) Responsive gels formed by the spontaneous self-assembly of peptides into polymeric β-sheet tapes. Nature 386:259–262
3. Martin SR (1996) Circular dichroism in proteins labfax (Price N.C., Ed.) BIOS scientific publishers Ltd., Oxford, p 195–204
4. Kelly SM, Price NC (2000) The use of circular dichroism in the investigation of protein structure and function. Curr Protein Pept Sci 1:349–384
5. Kallenbach NR, Lyu P, Zhou H, Tlstra L, Mattice WL, Perczel A, Hollósi M (1996) CD spectroscopy and the helix-coil transition in peptides and polypeptides. In: Fasman GD (ed) Circular Dichroism and the conformational analysis of biomolecules. Plenum Press, New York, NY, pp 201–380
6. Martin SR, Bayley PM (2002) Absorption and circular dichroism spectroscopy. Methods Mol Biol 173(2):43–55
7. Hu L, Olsen C, Maddux N, Joshi SB, Volkin DB, Middaugh CR (2011) Investigation of protein conformational stability employing a multimodal spectrometer. Anal Chem 83(24): 9399–9405
8. Sreerama N, Woody RW (2000) Circular dichroism of peptides and proteins in circular dichroism: principles and applications, 2nd ed. (Berova N, Nakanish k, & Woody R.W.,Ed.), John Wiley & Sons, Inc., New York. pp 601–620
9. Schneider AS (1973) Analysis of optical activity spectra in turbid biological suspensions. Methods Enzymol 27:751–767