Biomolecular Structures Assignment 1 Question 4

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February 17, 2022

1 Part (a) – How do we measure secondary structural character of proteins using the circular dichroism (CD) technique?

1.1 Introduction to the Working of CD technique

Circular dichroism (CD) is a powerful tool for quickly determining the secondary structure and folding properties of proteins purified from tissues or recombinantly obtained. The applications of protein CD include checking whether an expressed, purified protein is folded or if a mutation affects its conformation or stability or to study protein interactions. These are important observations that help us understand protein biology better. What exactly is circular dichroism though? It is defined as the unequal absorption of left handed and right handed circularly polarised light. When asymmetric molecules interact with light, they may absorb right and left handed circularly polarised light to different extents and also have different indices of refraction for the two waves. The result is that the plane of the light wave is rotated and that the addition of the E_R (the vector that rotates the sinusoidal wave obtained after passing the polarised light through a suitable prism clockwise) and E_L (the vector that rotates the sinusoidal wave obtained after passing the polarised light through a suitable prism anticlockwise) vectors results in a vector that traces out an ellipse and the light is said to be elliptically polarized. CD is reported in units of ΔE, the difference in absorbance of E_R and E_L by an asymmetric molecule. Quantitative analysis of CD spectra allows the prediction of the protein secondary structure content. Many enhanced algorithms, based on variable selection, or singular value decomposition of standardized, scaled and calibrated reference spectra have been proposed to predict the secondary structure content. Because the spectra of proteins are so dependent on their conformation, CD can be used to estimate the structure of unknown proteins and monitor conformational changes due to temperature, mutations, heat or binding interactions.

1.2 Measuring secondary structural character of proteins using CD

1. We begin by determining the concentration of protein stock solutions using spectroscopic methods. Time taken for this step is 30 minutes to 1 hour.
2. Preparing protein samples by making solutions. Time taken for this step is 10 minutes to 30 minutes.
3. Preparing the equipment like turning on the nitrogen and flushing the optics compartment 20 minutes before starting the machine. Time taken for this step is 30 minutes to 40 minutes.
4. Turning on the water supply, circulating water bath (for temperature control) and the lamp.
5. Turning on the CD machine and starting the CD program.
6. Setting the various parameters like data path, desired temperature, desired equilibriation time, wavelength range, time parameters and so on.
7. Set the instrument to record the ellipticity and the photomultiplier tube (PMT) voltage.
8. Do some form of testing to make sure there are no unwanted errors and then save the computed data to a file.
9. Smoothing the data and applying the formulae.
10. Saving the data and analysing it.

2 Part (b) – Compare and contrast protein structure determination by X–ray diffraction and NMR techniques.

Let's talk about NMR first. NMR (Nuclear Magnetic Resonance) measures the energy difference that causes the spin flip in nuclei (nuclei are perturbed) depending on their environment in the molecule. This is a very specific technique and thus gives more refined structural information like dihedral / torsional angles inside a protein. It also gives an insight on the protein folding procedure like ligand binding change and side chain rotations.

X–ray crystallography has quite a different approach on the other hand. It uses the X–ray scattered by electron density in the protein to give a static view of the protein. The underlying principle is that crystalline atoms cause a beam of X–rays to diffract into many specific directions and by measuring the angles and intensities of these diffracted beams, we can produce a three dimensional model of the density of the electrons contained in the crystal. This, in turn,

yields certain valuable data for the static model of the protein such as mean positions of the atoms, chemical bonds and so on. This method doesn't account for the multiple conformations a protein might have and gives just one of the conformations as output. This is in contrast with NMR which gives the dynamic structure of protein using a more sophisticated approach.

From this, it can be concluded that the two techniques differ in principle, experiment and analysis that can be done using its results and their applications.

Another difference between the two techniques is that NMR is performed on soluble samples while X–ray crystallography is performed on a diffracting crystal that needs to be built before applying the technique on it. This also means that protein size matters while performing NMR and it doesn't while performing X–ray crystallography.

Another important difference between the two techniques is that X–ray crystallography cannot detect Hydrogen atoms while NMR can.