LOCALISATION OF ACCUMULATED CHLOROPHYLL CATION IN REACTION CENTRE OF PHOTOSYSTEM II

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Photosystem II reaction centre absorbance and circular dichroism spectra were calculated using point-dipole approximation and compared with experimental data. Light induced difference spectra and their calculated counterparts revealed the location of accumulated chlorophyll cation at the position Chl1 - the accessory chlorophyll at D1 subunit.

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SPECTRAL TECHNIQUES FOR RAPID QUANTIFICATION OF PROTEIN STRUCTURE IN SOLUTION

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Development and improvement in techniques that can be used for characterization of proteins is becoming increasingly important in the rapidly expanding field of proteomics. NMR techniques can provide high-resolution structures of proteins in solution essentially equivalent in quality to those determined by crystallography. Unfortunately, these methods are still time consuming, require milligrams of protein and are limited to proteins of low molecular weight.

Optical spectra such as electronic [1-2] and vibrational [3] circular dichroism (ECD and VCD), Fourier transform infrared (FTIR) [4], as well as Raman [5] spectra have been extensively used to obtain estimates of the average fractional components of secondary structure in a protein. Moreover, these optical methods, which sample structure on an inherently rapid time scale, are particularly appropriate for studying protein-folding processes [6,7], since the intermediate species one wishes to study are often unstable. Such dynamic structures are poorly suited to more precise, but slower time scale, NMR structural techniques or to X-ray diffraction analyses of the crystal-stabilized distribution of structures. Optical spectra can rarely yield the structural detail of those techniques but remain vitally useful for qualitative monitoring of the structure, particularly for relative changes in a single or related protein.

For most applications, no single spectroscopic technique can provide all the information needed, and multitude of methods must usually be employed in order to meet all these needs. For instance, ECD results can often be difficult to interpret since aromatic amino acids can interfere. A reasonable strategy in this case is to confirm the CD results with one of the vibrational spectroscopies - Raman, FTIR or VCD - owing to spectral separation of structurally characteristic vibrational modes. Both Raman and FTIR rely on vibrational modes but selection rules are different (relative band intensities are different) and the influence of aqueous solvent is significant in IR but quite low for Raman. Overall, by applying both techniques it is possible to increase the confidence in a particular estimate of the secondary structure.

VCD provides alternative views of protein conformation with advantages over ECD and FTIR spectroscopy. The important aspect is that VCD, being sensitive to short-range order, senses sheets and other structural elements (including turns) differently than does ECD, which in turn is superior for helix determination. Combination of VDC with ECD and/or FTIR improves determination of all fractional components.

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