Spectroscopic Probes of Protein Structure

With few exceptions, the goal of most protein purification efforts is to obtain a sample that is not only pure, but that also maintains the protein in its native (i.e., biologically active) conformation. The ability to describe the conformation of a protein in solution, and to relate changes in conformation with biological activity, is thus a major focus of protein science. The most detailed description of protein structures come from the determination of the complete three-dimensional arrangement of protein components in space, from x-ray crystallographic or nuclear magnetic resonance (NMR) studies. Despite their power, however, these methods are not without their attendant drawbacks. X-ray diffraction studies of proteins are dependent on obtaining protein crystals of sufficient size and quality to yield usable diffraction patterns. This can often be a time consuming, and not necessarily successful, undertaking. Even when high quality crystals are obtained, solving the structure from the resulting diffraction patterns is a laborious and time consuming effort. Add to this the fact that certain classes of proteins, such as integral membrane proteins, are inherently difficult to crystallize, and one soon realizes that x-ray crystallography, while an extremely powerful method, is not a panacea for protein structural problems. Multidimensional NMR spectroscopy likewise suffers from certain difficulties that restrict its utility. Perhaps the greatest limit to the use of NMR spectroscopy for solving protein structures is that the complexity of the multidimensional data is such that the size of a protein that can reasonably be solved is limited to about 100 amino acids or so. While significant efforts are currently being...
put forth to push up the size limit for NMR spectroscopy, at least for the present this method is limited to relatively small proteins.

How then can the generalist glean information on the conformation of a protein in an expeditious fashion? Fortunately, there are a number of simple spectroscopic methods that have been developed over the years for assessing different aspects of protein structure. While none of these methods alone gives the same level of structural detail as do x-ray crystallography or NMR spectroscopy, in combination these methods can provide a reasonable description of the solution conformation of most proteins.

Since all of the methods described in this chapter rely on specific interactions between components of the protein and light energy, we shall begin with a brief description of the types of interactions between matter and electromagnetic radiation that are important in the spectroscopy of proteins.

INTERACTIONS OF ELECTROMAGNETIC RADIATION WITH MATTER

Spectroscopy can be most broadly defined as the interaction of electromagnetic radiation with matter (see Cantor and Schimmel, 1980; Campbell and Dwek, 1984; and Freifelder, 1982 for reviews). Any electromagnetic wave propagating along the z-axis in a laboratory fixed coordinate system can be considered to be composed of two wave trains at right angles to each other. One of these wave trains represents the electrical component (E), and the other represents the magnetic component (H) of the radiation. The spectroscopic methods we shall discuss here are concerned with the interactions of matter with the electrical component of the radiation only (figure 9.1). Restricting our attention for the moment to this electrical component, we have represented this component in figure 9.1 as a wave train defined by the xz plane. However, in fact, an electromagnetic wave propagating in the z direction can have its electrical component oscillating in any direction perpendicular to the z-axis (figure 9.2).

If we were somehow able to select only that electrical component that does oscillate in the xz plane (by, for example, using a polarizing filter), the resulting radiation would be referred to as plane polarized to denote the fact that we are dealing now with an electrical component restricted to a specific plane. Plane polarized light can be effectively used for defining the direction of transition dipoles in molecules (as in polarized absorption spectroscopy) and for estimating the rotational freedom of