Table 2.1 Secondary structure of an IgG2 determined by far UV circular dichroism measurements as a function of temperature

Temperature (°C)	α-Helix	β-Sheet	β-Turn	Random coil	RMS error
50	0	66	22	12	8.5
55	0	59	16	25	8.8
60	4	44	8	44	4.5
65	7	43	6	44	6.7
70	9	39	6	46	7.3
75	9	38	6	47	7.2

Medium, 10 mM phosphate buffer, pH 8.1.
From Vermeer and Norde (2000).

the exposure of the aromatic residues to solvent and increased motion of these residues as the protein unfolds. The inset of Figure 2.4(b) shows the change in CD signal at ~300 nm as a function of temperature, and as can be seen, there is a large decrease in the signal at about 55 °C, which corresponds nicely to the observed secondary structure changes by far UV CD measurements. Although this IgG2 is highly unfolded at 75 °C there still is a significant amount of folded secondary structural elements since 50% of this mAb still has β-sheet, β-turn, and α-helix (Table 2.1). This is in stark contrast to the denaturation of a murine IgG_1 at 6 M guanidinium hydrochloride where only random coil was observed (Buchner et al., 1991).

Fluorescence spectroscopy

The aromatic amino acid residues of proteins have fluorescence in the 300–400 nm range when excited at 250–300 nm. The environment of these residues dictates the fluorescence spectra and therefore this spectroscopy can complement UV and CD spectroscopy for analysis of tertiary structure of proteins. There are several reviews and books on this spectroscopic technique (Eftink, 1998; Lakowicz, 1984, 2002). Examples of application to the study of immunoglobulin structure by fluorescence measurements include investigation of stability of murine antibodies as a function of pH and temperature (Jiskoot et al., 1991; Jiskoot, Beuvery, Dekoning, Herron, & Crommelin, 1990), characterization of a murine mAb at low pH (Buchner et al., 1991), and studies on conformational changes of goat polyclonal anti-human serum albumin antibodies denaturation at low pH (Lin, Andrade, & Chang, 1989).

Infrared spectroscopy

The bending and stretching of bonds in proteins result in characteristic infrared (IR) bands. Application of Fourier techniques to IR spectra has led to Fourier transform infrared (FTIR) spectroscopy which allows for enhanced resolution of the spectra from amide vibrations. Deconvolution of the absorption envelope of the amide vibrations is related to the secondary structure of the amide group in peptide linkages.