1.6 ACTIVATION OF COMPLEMENT

1.6.1 The complement cascade

The complement cascade comprises a series of proteolytic zymogens found in the blood of all vertebrate organisms. The activation of this cascade involves the conversion of these zymogens to biologically active proteases, the characteristic end-product of the pathway being a protein complex of $(C5b\text{-}9)_2$ which inserts into the cell membrane and induces cell death by lysis (Porter, 1979; Porter & Reid, 1979; Biesecker et al., 1979). This complex may be formed via two routes, which are distinguishable by the protein components involved prior to C3, and the nature of the activating species. A schematic diagram of the complement pathways is shown in Fig. 1.10.

1.6.2 The role of component C1

The initial step in the activation of the classical pathway of the complement cascade involves the binding to antibody-antigen complexes of the C1q subcomponent of the C1 macromolecular complex. C1 is a Ca^{2+}-dependent complex of three subcomponents, C1q, C1r, and C1s (Lepow et al., 1963; Naff et al., 1964), having the stoichiometry $C1q.C1r_2.C1s_2$ (Siegel et al., 1981). Recent reviews which give an excellent account of C1 structure and function are Porter & Reid (1979) and Reid (1983).

Subcomponent C1q: C1q is a highly unusual serum glycoprotein, having an amino acid composition which includes two hydroxylysines, five 4-hydroxyprolines and seventeen glycines per 100 residues (Reid et al., 1972). Two types of carbohydrate are found: 6 mol/mol of complex-type

2.2.4 Extinction coefficients

The following extinction coefficients were used: Fv, $E_{280}^{0.1\%}$ = 1.5; V_L, $E_{280}^{0.1\%}$ = 1.0; V_H, $E_{280}^{0.1\%}$ = 2.0; DNP-lysine, E_{360}^{M} = 17,530; DNP-glycine, E_{360}^{M} = 15,900; DNP-aminocaproate, E_{360}^{M} = 17,800; TNP-glycine, E_{360}^{M} = 11,000. Haptens were purchased from BDH Chemicals Ltd.

2.2.5 NMR measurements

Samples (0.45 ml) were prepared by dissolving freeze-dried protein in ^2H_2O (99.8%; Ryvan Chemical Co.) to give a 1-1.5 mM solution. NaCl was added to 0.15 M, and the sample was adjusted to the required pH* by addition of small aliquots of dilute NaO^2H or ^2HCl (Aldrich Chemical Co.) in ^2H_2O.

NOE-difference spectra were recorded on a Bruker 300-MHz spectrometer. All remaining spectra were recorded on the Oxford Enzyme Group 470-MHz spectrometer which uses a Nicolet 1180 computer and an Oxford Instruments magnet. Data were collected in the Fourier transform mode, with the solvent ^2H resonance used to obtain an internal field frequency lock.

1-pulse spectra: Free induction decays were accumulated over 8K data points, using quadrature detection. Spectra were obtained from 1000 scans, with a repetition rate of 1 second and a 70^o pulse angle.

NOE-difference spectra: Spectra were accumulated over 16K data points using the NOE-difference spectrum program of the Aspect 1000 data system package. It was found that continuous irradiation of a resonance was necessary if an adequate signal to noise ratio was to be obtained.

5.1 INTRODUCTION

5.1.1 Aims of this chapter

As was described in section 1.4, IgG contains a conserved N-linked oligosaccharide at Asn-297$_H$ in the C_H2 domain. Since this domain is the site of many of the secondary functions of the molecule (sections 1.5-1.9) the possible involvement of the carbohydrate in these functions is of interest. A method of examining the role of this oligosaccharide is to compare the properties of normal IgG with IgG which is deficient in carbohydrate. This chapter describes the preparation of aglycosyl IgG in sufficient quantities and purity to be useful for such functional studies, which are described in chapter 6.

Carbohydrate depletion may be acheived by two distinct approaches. Firstly the isolated native glycoprotein may be deglycosylated by chemical or enzymic treatment. Alternatively, since the pathways for polypeptide biosynthesis and glycosylation are separate processes, inhibition of the latter leads to production of aglycosylated or incompletely glycosylated proteins.

5.1.2 Deglycosylation of glycoproteins

a) Enzymic Both endo- and exoglycosidases have been used for this purpose. It is important to note that in general the glycosidase(s) used have quite strict linkage / sequence specificities (Kobata, 1979), and therefore it is essential that the detailed sequence of the oligosaccharide is known if the digestion pattern is to be predicted. High-mannose type oligosaccharides can usually be removed in a single