Measured IgG and monovalent anion binding

Practically, IgG charge can contribute significantly to thermodynamic nonideality, hence to solubility and viscosity. Biologically, IgG charge isomers exhibit differences in clearance and potency. It has been known since the 1930's that all immunoglobulins carry a weak negative charge in physiological solvents. However, there has been no systematic exploration of this fundamental property. Accurate charge measurements have been made using membrane confined electrophoresis in two solvents (pH 5.0 and pH 7.4) on a panel of twelve mAb IgGs, as well as their F(ab')2 and Fc fragments. The following observations were made at pH 5.0: 1) the measured charge differs from the calculated charge by ~40 for the intact IgGs, and by ~20 for the Fcs; 2) the intact IgG charge depends on both Fv and Fc sequences, but does not equal the sum of the F(ab)'2 and Fc charge; 3) the Fc charge is consistent within a class. In phosphate buffered saline, pH 7.4: 1) the intact IgG charges ranged from 0 to -13; 2) the F(ab')2 fragments are nearly neutral for IgG1s and IgG2s, and about -5 for some of the IgG4s; 3) all Fc fragments are weakly anionic, with IgG1 < IgG2 < IgG4; 4) the charge on the intact IgGs does not equal the sum of the F(ab')2 and Fc charge. In no case is the calculated charge, based solely on H^+ binding, remotely close to the measured charge. Some mAbs carried a charge in physiological salt that was outside the range observed for serum-purified human poly IgG. A thermodynamically rigorous, concentration-dependent protein-protein interaction parameter is introduced.