A CALORIMETRIC STUDY OF SUBUNIT INTERACTION IN IMMUNOGLOBULIN G

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SUMMARY: The non covalent interaction of the heavy and light chains of immunoglobulin G has been studied in a batch calorimeter and shown to be exothermic. The enthalpy of association ranged from -5.6 to -112.5 kJ/mole of heavy-light chain pairs formed at 25° for subunits derived from eight myeloma proteins. The values for kappa and lambda chains were not significantly different. The enthalpy showed a marked temperature dependance giving changes in heat capacity near -9kJ/deg/mole between 25° and 35° suggesting the involvement of apolar bonds in the association. However the large negative enthalpy of association suggests the presence of additional types of bonding.

INTRODUCTION: The H* and L chains of IgG interact through non covalent bonds and disulfide bridges (1,2). The non covalent interactions play an important role in stabilizing the four-chain structure of IgG since reduction of the interchain disulfide bonds does not result in measureable dissociation of the molecule at neutral pH. The subunits may be separated by gel filtration at acid pH following reduction. Stevenson and Dorrington (3) have shown in low ionic strength buffers at slightly acid pH (eg. 4 mM Na acetate, pH 5.4) isolated H exists as a monodisperse dimer and L as a monomer-dimer mixture. Mixtures of subunits in such solvents rapidly recombine to form an H_2L_2 species indistinguishable by a number of criteria from native IgG (3,4). The overall reaction appears to be (3):

$$H_2 + 2L \rightleftharpoons H_2L_2.$$

Attempts to measure the equilibrium constant and free energy change of this reaction, in our laboratory, have been uniformly unsuccessful because of the extremely high affinity between the subunits.

In the present study we have used direct calorimetic measurements to estimate the enthalpy change ($\Delta H°$) accompanying subunit interaction.

* Abbreviations used: IgG - immunoglobulin G; H - heavy chain; L-light chain.

MATERIALS AND METHODS: Human IgG (subclass 1) was isolated from the serum of patients with multiple myeloma by ammonium sulphate precipitation and chromatography on DEAE-cellulose (3). H and L chains were prepared from reduced and alkylated IgG by chromatography on Sephadex G-100 in 1 M propionic acid, 25 mM NaCl as described previously (3). Only H chain eluting as monomer was used. The separated subunits were dialysed against 4 mM Na acetate buffer, pH 5.4.

A batch microcalorimeter (LKB, Type 10700-2) fitted with 18 caret gold reaction cells was used at 25°, 30° and 35°. The differential voltage signal was amplified by a Keithley 15013 Microvolt Ammeter and the amplified signal was recorded on a Recordall 5000 recorder fitted with a ball and disc integrator (Fisher Sci. Co.). The performance of the calorimeter was checked frequently by measuring the enthalpy of dilution of a standard sucrose solution. In all experiments the solvent was 4 mM Na acetate, pH 5.4. The two compartments of the reaction cell were filled with 4.0 ml of H chain solution and 2.0 ml of L chain solution respectively. The corresponding compartments of the reference cell were filled with 4.0 and 2.0 ml of buffer. Initial protein concentrations ranged from 4.0 to 4.7 x 10^{-5}M for H chain and 2.5 to 14.0 x 10^{-5}M for L chain. Protein concentrations were determined spectrophotometrically at 280 nm using molar extinction coefficients of 75.4 x 10^3 for H (mol. wt. 52,000) and 27.0 x 10^3 for L (mol. wt. 22,500). The heats of dilution for H and L chain solutions determined in separate experiments were negligable.

Binding curves were constructed by measuring the heat (Q) evolved as a function of increasing L chain concentration; the concentration of H being held constant. Although the reactive species of H is the dimer (3) all calculations were based on the equivalent monomer concentration to give the enthalpy of formation of an H-L pair. Following each experiment in which L was at a lower molar concentration than H the contents of the reaction vessel was chromatographed on Sephadex G-100 in 0.2 M NaCl, 0.05 M TRIS HCl, pH 7.8. The absence of protein eluting in the position of L chain confirmed that complete recombination had occurred.

RESULTS AND DISCUSSION: Calorimetric binding curves for two H-L pairs (one kappa and one lambda L chain) at 25° are shown in Figure 1. Using a constant

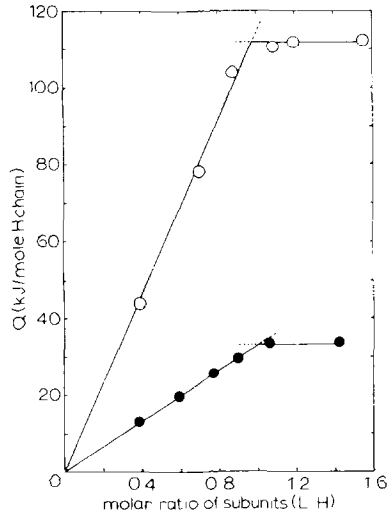

Figure 1. Heat evolved (Q) at 25° as a function of increasing L chain concentration for IgG1 lambda-Co (○——○) and IgG1 kappa -Br (●——●). The final H chain concentration was constant for each binding curve. Each point represents the average value from at least three determinations.

final concentration of H the heat evolved is a linear function of L concentration until equimolar amounts of the subunits are present after which Q remains constant. The characteristics of the binding curves reflect the formation of a strong 1:1 complex between H and L and indicates that essentially all the H chain is capable of binding L, in the buffer system used. This confirms the efficacy of the buffer in inhibiting the formation H chain aggregates since these do not interact with L (unpublished observation). Gel filtration of samples taken from the linear portion of the binding curves consistently failed to show residual free L chain thus confirming that recombination was complete.

One of the most striking observations made in this study was the wide range of enthalpy values. There was a twenty fold difference between the highest and lowest value in a series of eight proteins (Table I). No segregation

Table I: Enthalpy of association for H and L chains derived from eight IgG proteins

Protein	Classification	$\Delta H^\circ_{25^\circ}$ (kJ/mole HL[a])
Te	IgG1 kappa	19.0
Br	IgG1 kappa	33.5
En	IgG1 kappa	75.0
Gl	IgG1 kappa	88.4
Pi	IgG1 lambda	5.6
No	IgG1 lambda	10.5
Ma	IgG1 lambda	50.0
Co	IgG1 lambda	112.5

a) The ΔH° for the formation of the H_2L_2 species would be twice these values.

on the basis of L chain type was apparent. This observation suggests that there may be significant variability in the types of non covalent bonds contributing to the overall binding energy in different H-L pairs. It is tempting to suggest that such differences reflect interactions between the variable regions of H and L. We are currently studying the interaction of isolated variable and regions of L chain with H in an attempt to evaluate this possibility. From consideration of basic thermodynamic relationships, i.e.

$$\Delta F^\circ = \Delta H^\circ - T\Delta S^\circ \quad \ldots\ldots\ldots (1)$$

it is clear that the free energy of interaction (ΔF°) may be made up of both enthalpic and entropic contributions. Since we cannot evaluate ΔF° from measurements of association constants (K_A) it is instructive to calculate K_A using equation 2 making the assumption that ΔS° is negligable.

$$\Delta F^\circ = -RT\ln K_A \quad \ldots\ldots\ldots\ldots (2).$$

For the enthalpy values given in Table I the estimated values of K_A range from

10^{1} to $10^{20}M^{-1}$. The lower values of K_A are not consistent with either the calorimetric binding curves or other available information. For example, reduced and alkylated [^{125}I] IgG showed no evidence of dissociation when chromatographed on Sephadex G-100 at neutral pH at a final concentration of 10^{-12}M (B.R. Smith, unpublished data). It seems likely, therefore, that subunit association is accompanied by significant entropy changes. Clearly the relative importance of enthalpy and entropy as driving forces in association may differ significantly between proteins.

Studies on the temperature dependence of ΔH° can provide useful information regarding the types of non covalent bonds involved in protein association phenomena (5). The H-L binding curves for protein Co at 25° and 35° are shown in Figures 2. Although the shape of the curves are similar at both temperatures, with sharp inflection points at equimolar concentrations of subunits, considerably more heat is evolved at the higher temperature. This protein and protein En showed a linear dependence of ΔH° upon temperature between 25° and 35° (Figure 3). The change in heat capacity (ΔCp) calculated from the slope of the dependence was

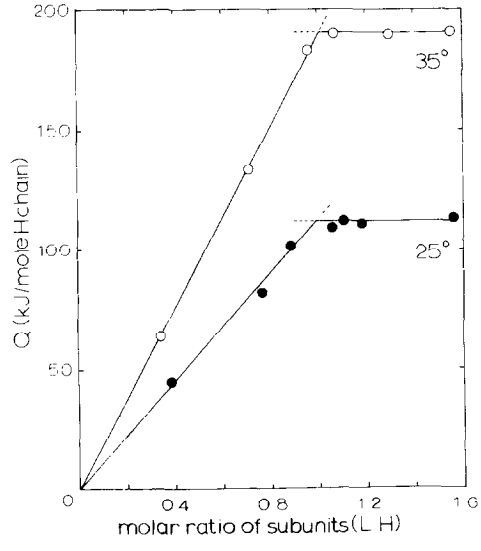

Figure 2. Calorimetric binding curves for H and L chains of IgG-Co determined at 25° and 35°. Other details as in Figure 1.

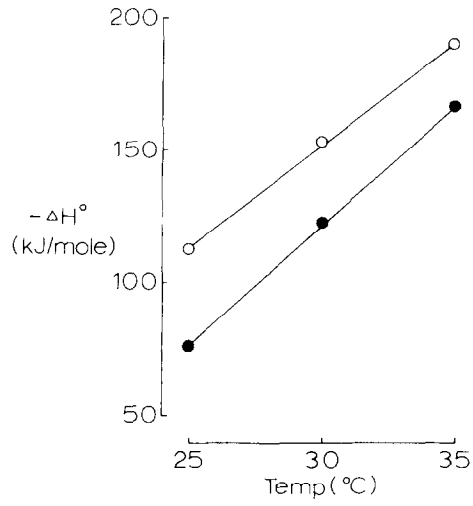

Figure 3. Temperature dependence of the enthalpy (ΔH°) of association for H and L chains of IgG-Co (○——○) and IgG-En (●——●) between 25° and 35°. The change in heat capacity (ΔCp), calculated from the slopes of the lines, was -8.7 kJ/deg/mole for IgG-En and 10.0 kJ/deg/mole for IgG-En. Each point represents the average value from at least five determinations.

similar for the two proteins (i.e. approximately-9kJ/deg/mole). The large value of ΔCp argues strongly for the involvement of apolar bonds in the interaction of H and L. The large negative value of ΔH° clearly suggests the involvement of electrostatic and/or hydrogen bonds at least for some of the subunits (5). The stability of these latter interactions could be enhanced by an apolar environment. The requirement for an alphatic acid (e.g. propionic) as well as low pH to optimize the yields of H and L chains during gel filtration also points to contributions from various types of bond to the overall interaction energy.

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