Thermodynamics of the Interaction of Human Immunoglobulin E with Its High-Affinity Receptor FcεRI†

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ABSTRACT: We have employed isothermal titration calorimetry (ITC) and circular dichroism (CD) spectroscopy to characterize the binding of soluble fragments of IgE (IgE-Fc and Fcε3-4) to a soluble fragment of the high-affinity receptor FcεRI α-chain (sFcεRIα). The thermodynamic parameters for the interaction of IgE-Fc and Fcε3-4 with sFcεRIα, determined using ITC, confirm the earlier conclusion that the Cε2 domain is not involved in the interaction and that the stoichiometry of both complexes is 1:1. For both IgE-Fc and Fcε3-4, the value of $\Delta H°$ is -36.9 ± 4.6 kcal mol^{-1} at 37.3 °C and $\Delta C_p°$ is -820 ± 120 cal mol^{-1} K^{-1}. The temperature at which $\Delta S°$ is zero is 284 ± 1 K, indicating that the entropy contribution to the thermodynamics of association is unfavorable at physiological temperature. Of particular interest is the large value of $\Delta C_p°$. The large surface area of IgE and FcεRIα that is implicated in complex formation from previous mutagenesis studies on the two proteins may account in part for the magnitude of $\Delta C_p°$. Additional contributions may arise from hydration within the binding site and changes in tertiary structure of the individual components of the complex. However, the CD spectra of IgE, IgE-Fc, and Fcε3-4 complexes with sFcεRIα are merely the sum of the spectra of their individual components, indicating that the secondary structure of the immunoglobulin domain folds are preserved on complex formation. Thus, any change in tertiary structure must be limited to the relative disposition of the immunoglobulin domains Cε3 and Cε4 in IgE and the two immunoglobulin-like domains in the α-chain of FcεRI.

The binding of allergens to immunoglobulin E (IgE)[1] antibodies attached to the high-affinity IgE receptor, FcεRI, on the surface of mast cells and basophils triggers the allergic response. Understanding the physical basis of the tight binding ($K_a \approx 10^{10}$ M^{-1}; *1*−*3*) between IgE and FcεRI is of fundamental interest and may facilitate the design of therapeutically effective allergy inhibitors. The stoichiometry of the complex is known to be 1:1 (*4*−*6*), and the complementary binding sites in the two proteins have been localized to the third constant domain of the ε-heavy chain of IgE (Cε3) and, principally, to the second membrane proximal immunoglobulin-like domain of the α-chain of FcεRI (see ref *7* for review). However, the thermodynamics of the binding process has not previously been examined.

In this work, we have used a combination of isothermal titration calorimetry (ITC) and circular dichroism (CD) spectroscopy to characterize the IgE−FcεRI interaction. ITC is a technique ideally suited for a direct thermodynamic investigation of interacting systems involving soluble proteins with affinities higher than 10^8 M^{-1}. Even though a direct measure of the association constant is not possible in such cases (*8*), ITC data provide information on the stoichiometry and enthalpy of reaction. In addition, a study of the temperature dependence of the interaction allows direct determination of the heat capacity change upon association, the significance of which has recently become apparent (*9*). We now describe a complete thermodynamic characterization of the interaction of human sFcεRIα with both human IgE-Fc and Fcε3-4 (a covalent dimer of the Cε3-Cε4 fragment of the ε-heavy chain). CD spectroscopy provides a measure of the secondary structure of the individual components and the complexes, placing constraints on the interpretation of the thermodynamic parameters in terms of any conformational change involved in the interaction.

MATERIALS AND METHODS

Preparation, Isolation, and Characterization of IgE-Fc, Fcε3-4, and sFcεRIα. The preparation of IgE-WT (a mye-

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[1] Abbreviations: IgE, immunoglobulin E; IgG, immunoglobulin G; Fc, C-terminal fragment of immunoglobulins responsible for effector functions; FcεRI, high-affinity IgE receptor; sFcεRIα, soluble extracellular fragment of the FcεRI receptor α-chain; Cε2−Cε4, IgE ε-heavy chain constant regions 2−4, respectively; Fcε3-4, covalent dimer of Cε3-Cε4; sFcγRIII, soluble extracellular fragment of the low-affinity IgG receptor FcγRIII; PBS, phosphate-buffered saline (pH 7.4); ITC, isothermal titration calorimetry; CD, circular dichroism; hGH, human growth hormone; hGHbp, human growth hormone receptor.

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loma protein), IgE-Fc, Fcϵ3-4, and sFcϵRIα has been described in detail elsewhere (*3*, *5*, *10*, *11*). The IgE-Fc comprises residues D222−K547 containing the mutations C225A, N265Q, and N371Q. The Fcϵ3-4 preparation comprises residues C328−K547 and contains the mutation N371Q. Purity and monodispersity were demonstrated by HPLC on a Zorbax GF250 bioseries column in 50 mM sodium phosphate, 150 mM NaCl, 0.5 M arginine hydrochloride (pH 6.0), and 0.05% (w/v) NaN_3 and by electrophoresis in 15% polyacrylamide in SDS under reducing and nonreducing conditions (*12*). Similar preparations were verified by analytical ultracentrifugation and found to be monodisperse (*3*, *5*, *6*, *11*). The concentrations of the IgE-WT, IgE-Fc, Fcϵ3-4 (dimer), and sFcϵRIα solutions were determined using calculated extinction coefficients at 280 nm of 260 610, 95 700, 64 520, and 54 700 M^{-1} cm^{-1}, respectively, based on the amino acid composition (*13*).

Isothermal Titration Calorimetry. ITC was performed using an Omega microcalorimeter (MicroCal, Inc.) fitted with an external MicroCal nanovoltmeter and connected to an external cooling bath set at 5.0 °C. All measurements were performed using samples which had been exhaustively dialyzed against PBS containing 0.05% (w/v) NaN_3 and degassed just before use. Experiments studying the interaction of IgE-Fc with sFcϵRIα were carried out with either IgE-Fc or sFcϵRIα in the reaction cell. In the latter case, a 0.25 mM IgE-Fc solution was loaded in a 250 μL syringe and titrated (10 injections of 10 μL) into a 0.01 mM sFcϵRIα solution. Otherwise, a 0.158−0.169 mM solution of sFcϵRIα was loaded into the syringe and titrated into a 0.005−0.01 mM IgE-Fc solution contained in the cell. Injections were carried out using volumes such that 15% of the initial IgE-Fc was titrated. In a study of the interaction of Fcϵ3-4 with sFcϵRIα, experiments were performed using a 0.174 mM sFcϵRIα solution in the syringe and a 0.00852 mM Fcϵ3-4 solution in the cell. Injection volumes of 12 μL were used. In all cases, injections were carried out over a 5−10 s period, while stirring at 400 rpm. A 3 min equilibration period between each injection was sufficient for the baseline to be re-established.

Data analysis and fitting were performed using the ITC data analysis software supplied by MicroCal (Origin 2.9). Numerical integration of the data and subtraction of the heats of dilution yielded the measured heats of interaction. The experimental data were fitted to a theoretical titration curve with ΔH (the enthalpy of reaction in kilocalories per mole), K_a (the association constant in M^{-1}), and n (the number of sFcϵRIα binding sites per Fcϵ3-4 or IgE-Fc) as floating parameters. In all cases, the value of K_a was large enough ($>10^7$ M^{-1}) such that the parameter $K_aM_t(0)$, where $M_t(0)$ is the initial protein concentration in the cell, was larger than 500. This corresponds to a tight binding regime in which no information about the precise value of K_a can be obtained (*8*).

The "best-fit" values of K_a ranged from 2 to 11 × 10^7 M^{-1}. This range is 3 orders of magnitude smaller than that previously reported (see Table 1), a difference arising from the imprecise values of K_a obtained by ITC for this particular high-affinity interaction. Such imprecision arises from the fact that the concentrations of the reactant in the cell are at least 250 000 times greater than the value of K_d. Despite the imprecision in K_a, the values of ΔH and n are accurate.

Table 1: Thermodynamic Parameters for the Interaction of Human IgE-Fc and Fcϵ3-4 with sFcϵRIα at 22.0 °C[a]

parameter[a]	IgE-Fc	Fcϵ3-4
$\Delta H°$ (kcal mol^{-1})	−22.6 ± 1.3	−24.6 ± 0.2
$\Delta S°$ (cal mol^{-1} K^{-1})	−27 ± 2	−33 ± 0.3
$\Delta C_p°$ (cal mol^{-1} K^{-1})	−815 ± 100	−825 ± 20
$\Delta G°$ (kcal mol^{-1})	−14.5	−14.8
K_a ($\times 10^{10}$ M^{-1})	5.0	9.0
T_H (K)	270	268
T_S (K)	285	283

[a] Enthalpies of reaction $\Delta H°$ and the corresponding errors are obtained from the isothermal calorimetric data, whereas the free energy of reaction $\Delta G°$ is calculated on the basis of the equilibrium association constant obtained from cell binding studies carried out at 22.0 °C (*3*, *6*). The temperature-independent heat capacity change upon association, $\Delta C_p°$, is calculated as $\delta\Delta H°/\delta T$ (Figure 3). $\Delta G°$, $\Delta H°$, and $\Delta S°$ are reported at a temperature of 22.0 °C (295.15 K). T_H and T_S are the temperatures at which the enthalpic and entropic contributions, respectively, are zero.

The values of ΔH and n correspond to the intercepts and midpoints, respectively, of the titration curves and can be obtained directly without any data fitting. Indeed, identical values for ΔH and n were obtained when the data were reanalyzed with a fixed K_a larger than 10^7 M^{-1}.

Thermodynamic Analysis. The calorimetric data are used to determine the enthalpy of association, $\Delta H°(T)$. The constant pressure heat capacity change upon association, $\Delta C_p°$, was determined on the basis of the temperature dependence of $\Delta H°(T)$. As $\Delta H°(T)$ was found to vary linearly with temperature, the temperature-independent $\Delta C_p°$ was calculated as $-\Delta H°(T)/-T$. The free energy of association, $\Delta G°(T)$, was calculated at 22.0 °C (295.15 K), on the basis of the association constants (K_a) obtained from cell binding studies (*3*, *6*):

$$\Delta G°(T) = -RT \ln K_a = \Delta H°(T) - T\Delta S°(T) \quad (1)$$

$\Delta H°$(295.15 K), obtained by interpolation of the temperature dependence of $\Delta H°(T)$, allowed for the calculation of $\Delta S°$-(295.15 K), and thus a determination of $\Delta S°(T)$ over the temperature range considered (*14*).

Circular Dichroism Spectroscopy. CD spectra were measured using a Jobin-Yvon CD-6 spectrophotometer using cylindrical quartz cells (Hellma) with a path length of 0.5 mm. The spectrophotometer was calibrated for wavelength and ellipticity using *d*-10-camphorsulfonic acid. Samples were measured in the concentration range of 100−500 μg mL^{-1} in 20 mM sodium phosphate buffer at pH 7.2 and 20 °C in a thermostated cell holder.

For each sample, five spectra were recorded in 0.2 nm steps with an intergration time of 4 s, averaged, and corrected by subtraction of the solvent spectrum obtained under identical conditions. The units of $\Delta\epsilon$ are M^{-1} cm^{-1} per backbone amide.

The ellipticity of different molar proportion mixtures of IgE-WT (or IgE-Fc or Fcϵ3-4) and sFcϵR1α was measured at a series of defined wavelengths. The total protein concentrations of the mixtures were as follows: 8 μM IgE-WT/sFcϵRIα, 5 μM IgE-Fc/sFcϵRIα, and 15 μM Fcϵ3-4/sFcϵRIα. The spectrophotometer was set to collect data at the desired wavelength for 3 min with a 30 s integration time for each data point. Six separate data points were taken. and the mean ± the standard deviation was plotted against

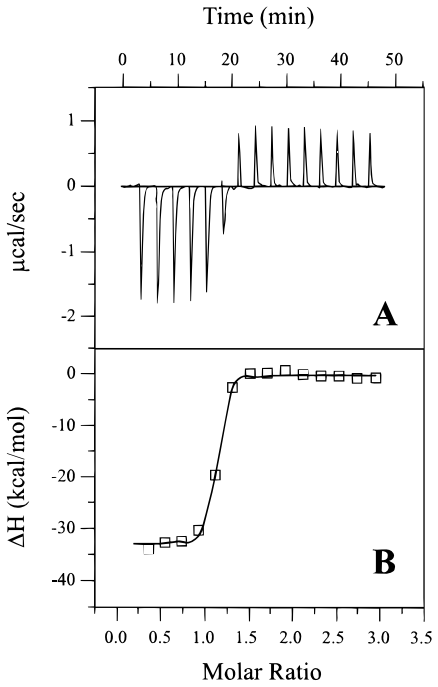

FIGURE 1: (A) Raw ITC data for the interaction of IgE-Fc with sFcεRIα in PBS containing 0.05% NaN_3. The titration consists of fifteen 15 μL injections of a 0.158 mM sFcεRIα stock solution. The solution was injected into a sample cell containing 0.010 mM IgE-Fc at a temperature of 37.2 °C. (B) Integrated calorimetric heats and best-fit curve to a simple single-site binding model.

the changing composition of the mixture. The CD curves were analyzed with and presented by Origin (version 3, MicroCal Software).

RESULTS

Thermodynamics of the Association of sFcεRIα with IgE-Fc and Fcε3-4. ITC follows the binding of one macromolecule to another by measuring the evolution or uptake of heat due to a change in binding saturation. Figure 1A shows a typical thermogram obtained for the titration of IgE-Fc with sFcεRIα at 37.2 °C. The sign of the measured heat change for each injection is negative up to a molar ratio of 1.0, after which it changes sign and remains constant beyond 3 equiv of sFcεRIα. The endothermic signals represent the heat of dilution of the sFcεRIα and are observed in a control titration, wherein sFcεRIα was titrated into PBS buffer only. Similar data were obtained when the sFcεRIα was titrated with IgE-Fc, showing that a 1:1 equivalence was reached, irrespective of whether the IgE-Fc was the titrant or the reactant. It may be noted that similar calorimetric experiments with the rat neonatal IgG receptor (FcγRn) and IgG indicated a FcγRn:IgG stoichiometry of 2:1, again irrespective of whether IgG was the titrant or the reactant (*15*). Analysis of the processed thermogram (Figure 1B) in terms of a simple single-site model shows that binding is exothermic. The average of three determinations, carried out at temperatures of 37.2−37.5 °C, leads to the following best-fit parameters: $n = 1.02 \pm 0.07$, $K_a = (6.0 \pm 5.0) \times 10^7$ M^{-1}, and $\Delta H° = -36.9 \pm 4.6$ kcal mol^{-1}. We note that the average value of the parameter $K_aM_t(0)$ is larger than 500; accordingly, the value obtained for K_a does not reflect a precise binding constant (*8*). Evaluation of experiments carried out with Fcε3-4 at 37.3 °C in terms of a single site leads to the following values: $n = 0.95 \pm 0.01$, $K_a = (4.9 \pm 0.4) \times 10^7$ M^{-1}, and $\Delta H° = -36.9 \pm 0.2$ kcal mol^{-1} (data not shown); these values cannot be distinguished from those observed for IgE-Fc binding to sFcεRIα in the same conditions.

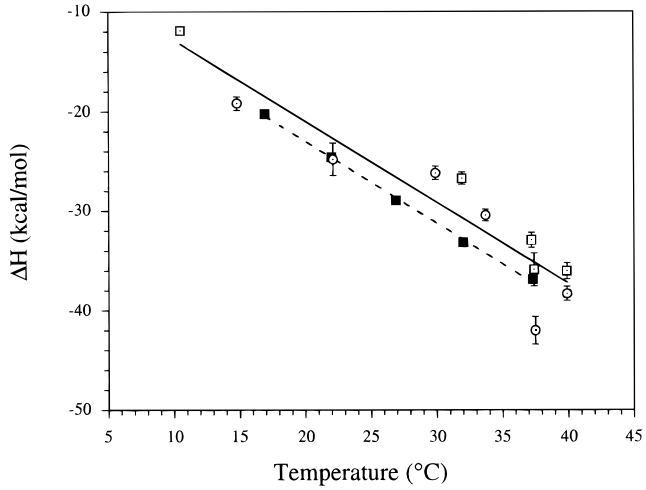

FIGURE 2: Dependence of the enthalpies of binding of sFcεRIα to IgE-Fc (open symbols) and Fcε3-4 (filled symbols) on temperature. Squares represent data obtained with either IgE-Fc or Fcε3-4 in the reaction cell, whereas circles represent data obtained with sFcεRIα in the cell. The enthalpies of reaction were obtained by integration of the isothermal titration calorimetric data, subtraction of the corresponding heats of dilution, and fitting to a simple single-site model. Solid (IgE-Fc, $R = 0.939$) and dashed (Fcε3-4, $R = 0.999$) lines represent best-fit linear least-squares fits to the data, yielding the temperature-independent excess heat capacities of association, $\Delta C_p°$ (Table 1). The errors observed for the titrations of Fcε3-4 with sFcεRIα are smaller than the symbol used to plot the data.

To further investigate the apparent similarities observed for the interactions of Fcε3-4 and IgE-Fc with sFcεRIα, ITC experiments were performed at various temperatures in the range of 10−40 °C. In all cases, a stoichiometry of 1.0, averaged at 1.03 ± 0.04, based on 16 determinations, was noted. Furthermore, the data obtained show that the interactions of both the Fcε3-4 and IgE-Fc with sFcεRIα have enthalpies of reaction which vary in a parallel fashion with temperature (Figure 2). The linear dependence of $\Delta H°(T)$ with temperature yields closely similar values of $\Delta C_p°$, namely, -825 ± 20 cal mol^{-1} K^{-1} for the Fcε3-4−sFcεRIα interaction and -815 ± 99 cal mol^{-1} K^{-1} for the IgE-Fc−sFcεRIα interaction (Figure 2). The values of $\Delta H°(T)$ and $\Delta C_p°$ suggest that these interactions are thermodynamically indistinguishable, confirming results of other workers which have suggested that the Cε2 domain plays no role in the interaction (*6*, *16*−*18*). The large negative $\Delta C_p°$ has been suggested by some to identify an association process in which local (un)folding−rearrangement is coupled to complex formation (*9*) and by others to involve both structural rearrangements and hydration (*19*). The complete thermodynamic characterization of the associations, based on cell binding association constants (*3*, *6*) and ITC data, is summarized in Table 1. The thermodynamics are typical of an enthalpy−entropy compensation process with $|\Delta C_p°| \gg |\Delta S°|$ and are characterized by a favorable enthalpic contribution over the temperature range studied. The temperature at which the enthalpic contribution is zero, T_H, is 269 K (−4 °C). At physiological temperature, the entropic

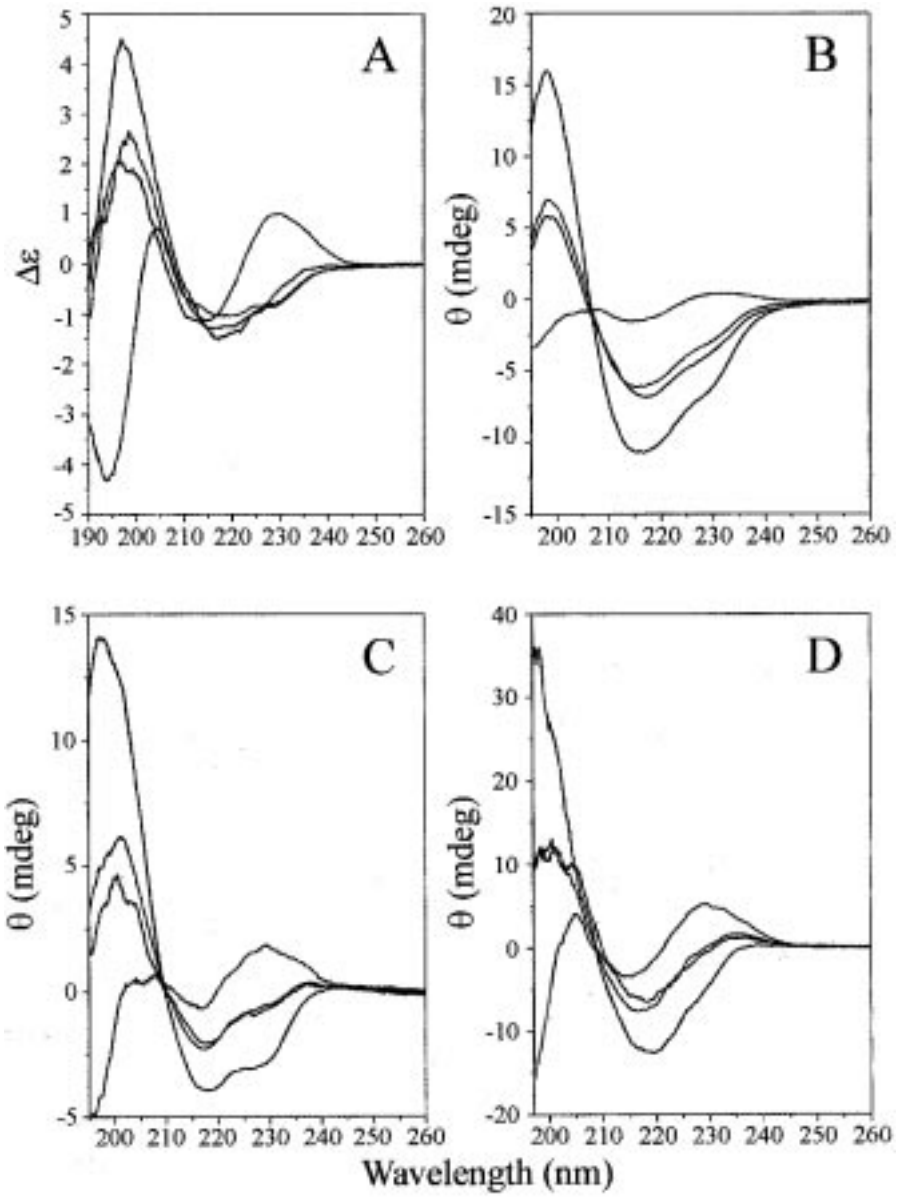

FIGURE 3: Circular dichroism spectra of IgE, IgE fragments, and their complexes with sFcεRIα. (A) Spectra of IgE-WT (black), IgE-Fc (red), Fcε3-4 (blue), and sFcεRIα (green) measured in 20 mM phosphate buffer at pH 7.2. (B−D) Spectra of IgE-WT (B), IgE-Fc (C), and Fcε3-4 (D) and their complexes with sFcεRIα: black, ligand alone; red, sFcεRIα alone; blue, 1:1 complex; and green, arithmetic sum of the individual CD spectra for the ligand and receptor in a 1:1 ratio.

contribution is unfavorable and the corresponding value of T_S, the temperature below which the entropic contribution is favorable, is 284 K (11 °C).

Circular Dichroism Studies. CD spectra of sFcεRIα, Fcε3-4, IgE-Fc, and IgE-WT were measured in 20 mM sodium phosphate (pH 7.2) (Figure 3A), and the amount of secondary structure was calculated using CONTIN (*20*). The percentages of β, α, and residual elements for Fcε3-4 are 64 ± 3, 4 ± 1, and 32 ± 2, for IgE-Fc are 47 ± 2, 5 ± 1, and 48 ± 1, and for sFcεRIα are 57 ± 2, 0, and 43 ± 2, respectively. The data obtained indicate that these species contain a high percentage of β-sheet, as predicted for correctly folded immunoglobulin domains and immunoglobulin-like domains. Spectra of sFcεRIα, Fcε3-4, IgE-Fc, and IgE-WT were also measured in PBS (the solvent used for the calorimetry studies) and found to be indistinguishable from data collected in 20 mM sodium phosphate (pH 7.2) (data not shown). CD spectra of the 1:1 complexes of Fcε3-4, IgE-Fc, and IgE-WT with sFcεRIα were also measured and found to be composed of the sum of the component spectra (Figure 3B−D); this indicates that there is no net change in secondary structure upon complex formation. This result is confirmed by the titration of IgE-WT, IgE-Fc, and Fcε3-4 with FcεRIα and the observation of a linear variation of θ_{215} with the mole fraction of ligand (Figure 4). At this wavelength, the CD is most sensitive to secondary structure and the linearity of the increase of θ_{215} with receptor concentration indicates that no change in secondary structure occurs. At longer wavelengths approaching 230 nm, the curves become nonlinear. This effect will be discussed below.

DISCUSSION

A study of the association of IgE-Fc and Fcε3-4 with sFcεRIα by isothermal titration calorimetry in the temperature range of 10−40 °C shows that these species interact

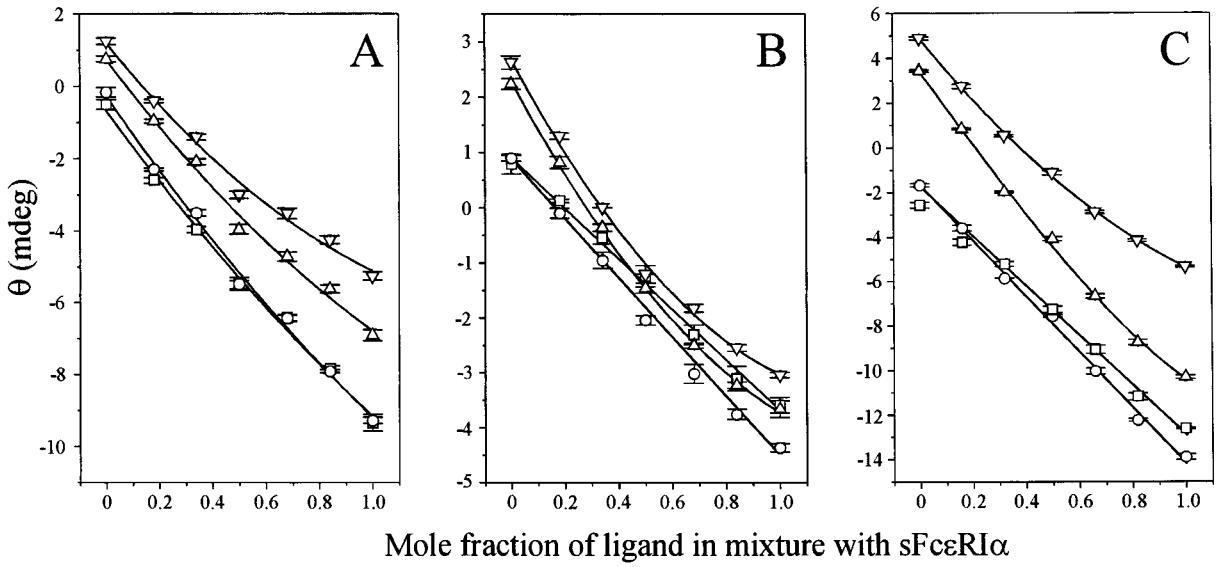

FIGURE 4: Titration of IgE and IgE fragments with sFcεRIα as measured using circular dichroism spectroscopy. Titrations were performed at a constant protein concentration: (A) IgE-WT, (B) IgE-Fc, and (C) Fcε3-4. Ellipticity was measured at 215 (□), 220 (○), 225 (△), and 230 (▽) nm. Data are plotted as mean ± standard deviation for six readings and fitted by linear regression (215 and 220 nm) or polynomial (third-order) regression (225 and 230 nm).

to form a 1:1 complex with $\Delta H°$ values which vary in a linear fashion with temperature (Figure 2). The values of $\Delta H°$ for both interactions are identical, within the experimental precision of the method, as are the values of $\Delta C_p°$. No structural changes, as determined CD thermal stability studies and differential scanning calorimetry, were observed for IgE-Fc, Fcε3-4, and sFcεRIα between 10 and 40 °C (R. Ghirlando, A. J. Henry, and M. B. Keown, unpublished results). The values of $\Delta H°$ and $\Delta C_p°$ on formation of the complexes therefore represent the changes in enthalpy and heat capacity of association between the proteins. These observations, along with the virtually identical values of K_a reported for IgE-Fc and Fcε3-4 using a cell binding assay (*3*, *6*), indicate that the Cε2 domains of IgE-Fc (i.e., residues 222−327) are not involved in the recognition of FcεRI. This result is consistent with the conclusion from other studies that the FcεRI binding site is located in the third constant domain, Cε3 (*2*, *10*, *18*, *21*−*23*) and also demonstrates a similar mode of binding for the interaction of IgE-Fc and Fcε3-4 with sFcεRI. It is significant that both the cell binding data and thermodynamics lead to the same conclusion. Recent studies on the interaction of human growth hormone (hGH) and the soluble extracellular domain of its receptor (hGHbp) have shown that, despite similar K_a values describing the interaction of hGH variants with hGHbp from cell binding data, significant differences in $\Delta H°$ and $\Delta C_p°$ were noted in the thermodynamic studies (*24*). The large compensating changes observed in the enthalpy and entropy of binding in that system lead to similar association constants, which mask subtle mechanistic differences.

The change in the heat capacity upon association, $\Delta C_p°$, provides information concerning the recognition process. In the case of a rigid body association, the value of $\Delta C_p°$ can be directly correlated to the burial of nonpolar surface area resulting from complex formation (*9*). The interaction of IgE-Fc with sFcεRIα does not appear to be a simple rigid body association, as indicated by the large magnitude of $\Delta C_p°$ and the low value of T_S. In an attempt to understand the nature of $\Delta C_p°$, we have considered the contributions of the hydrophobic effect and the loss of translational and rotational degrees of freedom to the entropy of association at T_S. At this temperature, the overall entropic contribution to the association process is zero. Spolar and Record (*9*) have shown that the favorable entropic contribution arising from the hydrophobic effect and burial of nonpolar residues can be approximated by $1.35\Delta C_p°\ \ln(T_S/386)$, namely, 344 ± 50 cal mol^{-1} K^{-1} for the IgE-Fc−sFcεRIα interaction. This is not wholly compensated for by the unfavorable contribution arising from the loss of rotational and translational degrees of freedom (approximately −50 cal mol^{-1} K^{-1}; *9*), implicating a possible contribution to $\Delta S°$ from induced conformational changes, decreased mobility of the interfacial polar side chains, and possible solvent interactions at the IgE-Fc−sFcεRIα interface. Assuming that this excess $\Delta S°$ arises solely from induced conformational changes, it is possible to determine the maximum number of residues involved. Assuming that each residue contributes a value of between −5.6 (*9*) and −9.6 cal mol^{-1} K^{-1} (*25*) to $\Delta S°$, the excess $\Delta S°$ of −294 ± 50 cal mol^{-1} K^{-1} implies that a maximum of 31 ± 1 to 52 ± 9 residues are involved in this rearrangement. These values correspond to between $^1/_3$ and $^1/_2$ of the number of residues comprising a single immunoglobulin or immunoglobulin-like domain. The potential involvement of such a large number of residues would imply that there is a very large area of contact between IgE-Fc and sFcεRIα or a significant structural rearrangement within one or both of the components of the complex.

A large $\Delta C_p°$ and positive $\Delta S°$ for association were also found for the high-affinity interaction ($K_a = 10^9$ M^{-1} at 20 °C) of the *Escherichia coli trp* repressor with its cognate DNA target, in which the observed value of −950 cal mol^{-1} K^{-1} for $\Delta C_p°$ could not be totally accounted for in terms of the surface area of interaction and the 16 protein residues known to be involved in a conformational change upon complex formation (*19*). It has been proposed that this excess decrease in the heat capacity of the complex that is not accounted for arises from the decreased mobility and/or flexibility of the interfacial polar side chains, nucleotide

bases, solvent-exposed backbone elements, and associated water molecules (*19*). Since the IgE−FcϵRI interaction appears to be mediated primarily by electrostatic interactions involving polar side chains (*10*, *22*), it is therefore possible that a contribution to ΔC_p° arises from the nature of the interfacial surface and from trapped water molecules (*19*). However, the thermodynamic parameters alone do not allow us to distinguish between structural changes that may contribute to the large value of ΔC_p° and effects due to the decrease in mobility of interfacial polar side chains and trapped water molecules at the interface.

To assess whether changes in secondary structure may contribute to the large negative value of ΔC_p° (−815 and −825 cal mol^{-1} K^{-1} for IgE-Fc and Fcϵ3-4, respectively), we have analyzed the CD spectra of the three components and the two complexes. There is no evidence for any significant change in the secondary structure, either by comparison of the spectra of the complexes with the sum of their individual components (Figure 3) or in the linear variation of the signal at 215 nm (θ_{215}) with the fractional composition of the mixtures of IgE-WT, IgE-Fc, or Fcϵ3-4 with sFcϵRIα (Figure 4). At this wavelength, the signal should be very sensitive to any changes in β-structure. If any change in tertiary structure does occur, therefore, it must be limited to the arrangement of the domains within one or both proteins on formation of the complex. Moreover, since the value of ΔC_p° is the same for the IgE-Fc and Fcϵ3-4 interactions with sFcϵRIα, we conclude that only the Cϵ3 and Cϵ4 domains of IgE or the immunoglobulin-like domains of FcϵRIα may be involved in any such rearrangement.

There is, however, a nonlinear variation of θ_{230} with the composition of the mixtures (Figure 4). The signal at this wavelength is sensitive to changes in the environment of aromatic residues and to change in the disulfide dihedral angle. The latter would require a global conformational change around the core disulfide, an event that is unlikely in the compact immunoglobulin domain, especially in the absence of any evidence for a change in secondary structure. However, the IgE-binding face of sFcϵRIα (CC′FG strands) contains an unusually large number of aromatic residues that are predicted to be buried in the IgE−sFcϵRIα complex (*26*), and it is more likely that the change in θ_{230} is due to the change in environment of these surface-exposed aromatics, and possibly also the aromatic amino acids on the complementary surface of IgE.

We note that our CD results are not consistent with the data of Sechi and co-workers (*27*), who reported a substantial conformational change in IgE upon binding to sFcϵRIα. This discrepancy could be due to the use of an underglycosylated form of sFcϵRIα in their study. In the case of another protein (CD2) containing a very similar C2-type immunoglobulin-like domain, it has been shown that carbohydrate stabilizes the structure of the domain; indeed, the CD spectrum of the unglycosylated CD2 reveals that it is substantially unfolded (*28*). Alternatively, the discrepancy in CD results between the two studies could be due to the use of very low protein concentrations in the far-UV CD measurements of Sechi et al. (0.1 μM, cf. 5−15 μM in our study), which would have resulted in a poor signal-to-noise ratio (*27*). We would certainly have detected a change in secondary structure of the magnitude reported by Sechi et al. (*27*) had it occurred in our study.

We have previously studied the thermodynamics of the interaction of human IgG$_1$-Fc with sFcγRIII using the analytical centrifuge, the method of choice for the relatively low affinity between these two species. These species interact reversibly in vitro to form a 1:1 complex with an affinity of 1.7×10^5 M^{-1} at 22.0 °C and a ΔC_p° of −360 ± 40 cal mol^{-1} K^{-1} (*29*). This interaction appears to be localized to the lower hinge region of IgG$_1$-Fc, as shown by site-directed mutagenesis studies, which have shown that residues L234−G237 in the lower hinge (*30*−*32*) and K274−E294 on the proximal bend of Cγ2 (*33*) are critical for huFcγR recognition (*34*). In contrast, deletion and site-directed mutagenesis of the IgE-Fc have implicated several widely separated regions of Cϵ3 in the contact with FcϵRI, including the N-terminal linker region analogous to the lower hinge region of IgG (*10*, *21*, *35*), the CD, FG, and EF loops (*22*), and the AB loop (*23*). Together, these mutations define a large continuous contact surface for FcϵRI that spans both Cϵ3 domains (*10*). This must make a significant contribution to the large value of ΔC_p° for the IgE receptor system (−820 cal mol^{-1} K^{-1}) compared with that for IgG (−360 cal mol^{-1} K^{-1}).

Conclusions. This work describes a thermodynamic characterization of the IgE−FcϵRI high-affinity interaction by isothermal titration calorimetry and circular dichroism spectroscopy. We have shown that this interaction is characterized by a very large and negative ΔC_p° of −820 cal mol^{-1} K^{-1}. Upon complex formation, no net change in secondary structure can be discerned from the circular dichroism studies, and hence, we interpret this large and negative ΔC_p° to signify a large surface area of interaction, with possible contributions from the ordering of side chains and water molecules at the interface. If, in addition, tertiary structure changes do occur, they must be limited to the relative disposition of the Cϵ3 and Cϵ4 domains of IgE-Fc, or the immunoglobulin-like domains of FcϵRIα.

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