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The Binding Thermodynamics of the N-terminal Peptide of the CCR5 Coreceptor to HIV-1 Envelope Glycoprotein gp120[†]

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Abstract

The initial events of HIV-1 cell infection involve the sequential binding of the viral envelope glycoprotein gp120 to the cellular CD4 receptor and the coreceptor, usually CCR5 or CXCR4. Binding to the coreceptor triggers the chain of events that culminates with the entry of the virus into the cell. In this process, the interaction of gp120 with the tyrosine-sulfated N-terminus of CCR5 is critical, however, this interaction has never been characterized at a quantitative or thermodynamic level. Here, we present the first thermodynamic analysis of the interaction of gp120 with the N-terminal peptide of the CCR5 coreceptor. Microcalorimetric titrations demonstrate that measurable binding of S22 peptide, a 22 amino acid tyrosine-sulfated peptide corresponding to the CCR5 N-terminus, requires prior binding of CD4 to gp120. The S22 peptide binds to the gp120/CD4 complex with a binding affinity of $4.5 \times 10^5 \text{ M}^{-1}$ ($K_d = 2.2 \text{ } \mu\text{M}$) in an enthalpically and entropically favorable process. An identical peptide lacking the sulfated tyrosine residues is unable to bind the gp120/CD4 complex. These results indicate that the sulfated tyrosines contribute close to -3.5 kcal/mol to the Gibbs energy of binding. Furthermore, the S22 peptide is a competitive inhibitor of the 17b HIV-1 neutralizing antibody, which is known to bind to the CCR5 coreceptor site in gp120. Together, these results point towards compounds containing sulfated aromatic groups as potential inhibitors of viral entry. In analogy to existing inhibitors that bind to the CCR5 coreceptor directly, these compounds will accomplish the same result by binding to the coreceptor site in gp120.

The entry of human immunodeficiency virus (HIV-1) into CD4 positive cells is the first event in HIV-1 infection, making this process an important target for the development of new antiretroviral therapies against HIV/AIDS (1). Viral entry is controlled by the glycoproteins gp120 and gp41 on the virion surface. The exposed HIV-1 envelope glycoprotein gp120 interacts non-covalently with the transmembrane glycoprotein gp41 anchored in the viral membrane, forming trimers on the virion surface. These trimers interact with target host cell receptors (2,3). Entry of HIV-1 into the human host cell is a cascade of events that involves the sequential binding of the HIV-1 envelope glycoprotein gp120 to the CD4 receptor and to the coreceptor, usually CCR5 or CXCR4, which further triggers gp41 to mediate fusion of the viral and host cell membranes (1,4). Before binding to host cell proteins, gp120 is composed of a structured core and largely unstructured regions (5–7). The coreceptor binding site is poorly defined in the unliganded gp120 (5,7,8). Binding of CD4 induces a large structuring in gp120 that activates the coreceptor binding site (5,7–9). Due to the presence of highly disordered regions in unliganded gp120, crystal structures have only been obtained for HIV-1 gp120 with truncated N- and C-termini, and most of variable loops deleted, in complex with CD4 and the Fab fragment of any of the CD4-induced antibodies 17b, X5, or 412d (10–13).

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Figure 1 illustrates the sequence of events leading to the fusion of viral and cell membranes. Binding of gp120 to CD4 triggers a conformational change in gp120 that leads to the activation of the coreceptor binding site. The nature of this conformational change can be inferred from the unusually large changes in favorable enthalpy and unfavorable entropy, a thermodynamic signature that can be associated to a significant structuring of gp120 (5–7,9). The CD4-induced increase in affinity for the coreceptor has been studied extensively in this and other laboratories using the monoclonal antibody (MAb) 17b that binds to an epitope that overlaps the binding site for the coreceptor (5,7,9,12–14). Although MAb 17b has proven to be a useful coreceptor surrogate, the thermodynamics of gp120 binding to CCR5 or CXCR4 has never been characterized. CCR5 and CXCR4 are G protein-coupled receptors containing seven transmembrane helices, connected through three extracellular loops, and an acidic extracellular N-terminus rich in tyrosine residues (15). The N-terminus of CCR5 contains two sulfated tyrosines critical for the binding to gp120, and plays an important role for the entry of R5 isolates (16–20). Residues in the third variable loop (V3-loop) of gp120 also interact with the second extracellular loop of CCR5 and determine coreceptor specificity (21,22). Mutations in extracellular loop regions that interact with CD4-bound gp120 have been shown to hinder viral entry (23). Contrary to its significance in CCR5, the N-terminus is of only minor importance for the binding of CXCR4 coreceptor (18), which is the preferred coreceptor used by X4 isolates (24).

Tyrosine-sulfated peptides representing the CCR5 N-terminus are powerful tools for assaying critical aspects of the interactions between gp120 and coreceptor. Recently, Huang et al. employed NMR and crystallographic techniques to investigate the binding mode of CCR5 N-terminus and the CD4-induced antibody 412d to gp120 (10). It was revealed that a critical sulfotyrosine residue of the CCR5 N-terminus binds to a conserved pocket at the junction of the bridging sheet and the base of the V3-loop, serving to fix the normally flexible V3-loop as a defined β -hairpin. The implications of sulfotyrosine binding to a distinct cavity in gp120 are important in fully understanding the mechanism of viral entry.

In this study we report the binding thermodynamics of gp120 to the S22 peptide corresponding to the sulfated N-terminal region of CCR5. The measurements show an obligate need for CD4-induced structuring of gp120 as no binding of S22 peptide to gp120 is observed in the absence of CD4. The S22 peptide exhibits enthalpically and entropically favorable binding to CD4-bound gp120, whereas the unsulfated version of this peptide cannot bind gp120 even after activation of the coreceptor site, confirming that sulfated tyrosines are critical components of this interaction. According to the thermodynamic results presented here, the sulfated tyrosines contribute close to -3.5 kcal/mol to the Gibbs energy of binding. Furthermore, the S22 peptide is a competitive inhibitor of the HIV neutralizing antibody 17b, which binds to the CCR5 coreceptor site in gp120 (14). These results suggest that the targeting of this site with sulfated aromatic compounds will define a new generation of cell entry inhibitors that will inhibit binding of the viral envelope glycoprotein gp120 to the chemokine coreceptor CCR5.

Materials and Methods

Production of YU2 gp120

Full-length gp120 from the subtype B YU2 HIV-1 strain was produced using a stably transfected CHO-K1 cell line kindly provided by Dr. J. Sodroski (Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA). CHO-K1 cells expressed YU2 gp120 in 100 mL suspension cultures of ProCHO5 (Lonza, Walkersville, MD, USA) with 2% glutamine synthetase (GS) supplement (SAFC Biosciences, Lenexa, KS, USA), 1% hypoxanthine/thymidine (HT) Supplement (Gibco, Carlsbad, CA, USA), 100 U/L penicillin G with 100 mg/L streptomycin (Gibco), 275 μ M L-methionine sulfoximine (Sigma, St. Louis, MO, USA) and 1 mL/L Protease Inhibitor Cocktail Set III (Calbiochem, La Jolla, CA, USA). CHO-K1 cells

were cultured at 37 °C with 8% CO₂ and 100% humidity, on an orbital shaking platform rotating at 180 rpm.

Preparation and Purification of Protein

CHO-K1 cell supernatants were dialyzed into PBS (Roche Diagnostics GmbH, Mannheim, Germany) and filtered through a 0.22 µm membrane. Approximately 25 mL supernatant was passed through a 5 mL HiTrap NHS-activated HP column (GE Healthcare, Buckinghamshire, UK) linked with MAb 17b (Strategic Biosolutions, Newark, DE, USA) and gp120 was eluted with 100 mM glycine, 150 mM NaCl, pH 2.4. Eluted fractions were immediately neutralized with 4 M Tris, pH 7.4, followed by dialysis into PBS. Approximately 25 mL dialyzed solution was passed through a 5 mL HiTrap NHS-activated HP column coupled to MAb B4a1 kindly provided by Dr. L. Cavacini (Beth Israel Deaconess Medical Center, Boston, MA, USA). gp120 was eluted with 100 mM glycine, 150 mM NaCl, pH 2.4. All column chromatography was performed on an ÄKTA FPLC system (Amersham Biosciences, Uppsala, Sweden). Eluted fractions were immediately neutralized with 4 M Tris, pH 7.4, followed by dialysis into PBS. V3-loop-intact YU2 gp120 was concentrated to approximately 4 µM and stored as 600 µL aliquots at -20 °C. Protein purity and approximate molecular weight of 90 kDa were confirmed by sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE). Protein identity was verified by N-terminal sequencing.

Soluble D1-D2 CD4 (sCD4) was kindly supplied by Dr. R. Wyatt (National Institutes of Health Vaccine Research Center, Bethesda, MD, USA). Monoclonal antibody 17b was produced by Strategic Biosolutions. Following dialysis into PBS, 17b and sCD4 were stored in approximately 6 and 5 mg aliquots, respectively.

CCR5 Coreceptor N Terminus Peptides

S22 and C22 peptides representing the first 22 residues of the CCR5 coreceptor N terminus were synthesized by American Peptide Company (Vista, CA, USA), and were a generous gift from Dr. J. Sodroski (Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA). The amino acid sequence of S22 peptide is the same as the first 22 residues of the CCR5 N-terminus, except that cysteine 20 of CCR5 is replaced with serine in the peptide. The sequences of the S22 and C22 peptides are identical, except that C22 peptide contains tyrosine residues in place of the sulfotyrosine residues present in S22 peptide. The sequences of the two peptides and the CCR5 N-terminus are compared in Figure 1. Stock solutions of S22 and C22 peptides were prepared in water at 8 and 4 mg/mL, respectively, and stored at -20 °C. The concentrations of S22 and C22 peptide stock solutions were determined by total nitrogen assay (25).

Isothermal Titration Calorimetry

Isothermal titration calorimetry experiments were conducted using a high precision VP-ITC calorimetric system from MicroCal Inc. (Northampton, MA, USA). All titrations described in this report were performed by adding the titrant in steps of 10 µL, and were conducted at a constant 25 °C. All solutions contained within the calorimetric cell and injector syringe were prepared in the same buffer, PBS pH 7.4 with 2 % dimethyl sulfoxide (DMSO). For the direct determination of the binding of S22 peptide to gp120, the calorimetric cell containing 2 µM gp120 was titrated with 230 µM S22 peptide contained in the injector syringe. The direct binding of sCD4 and 17b to gp120 was analyzed in experiments where 2 µM gp120 in the calorimetric cell was titrated with a solution of sCD4 or 17b at a concentration of 30 µM or 20 µM, respectively.

In order to assess the binding of S22 peptide, C22 peptide, and 17b to gp120 prebound to sCD4, a titration of gp120 with sCD4 was followed immediately by a titration with either S22 peptide,

C22 peptide, or 17b at a concentration of 200 μM , 150 μM , or 20 μM , respectively. To measure the effect of S22 peptide on the binding of 17b to gp120 in complex with sCD4, gp120 prebound to sCD4 was first titrated with a solution of 200 μM S22 peptide. gp120 in complex with sCD4 in the presence of saturating concentration of S22 peptide was then immediately titrated with a solution of 20 μM 17b. All solutions were properly degassed to prevent bubble formation in the calorimetric cell during stirring. The heat evolved upon each injection of ligand was obtained from the integral of the calorimetric signal. The heat of dilution was subtracted from the heat of reaction to obtain the heat associated with binding of a ligand to the protein in the cell. Nonlinear regression of the data provided the enthalpy change (ΔH) and association constant ($K_a = 1/K_d$). Because of the low affinity, the stoichiometry was fixed to a value of 1 for the binding of S22 peptide to gp120 in complex with sCD4.

Circular Dichroism Spectroscopy

CD experiments were performed using a Jasco J-710 spectropolarimeter (Japan Spectroscopic Corp., Tokyo, Japan). Stock solutions of S22 and C22 peptides in water, at 8 and 4 mg/mL, respectively, were diluted to make 0.1, 0.05, and 0.03 mg/mL solutions in PBS. Wavelength scans were completed for 0.1, 0.05, 0.03 mg/mL S22 and C22 peptide solutions using a 0.1 cm quartz cuvette in a water-jacketed cell at 25 °C. Four consecutive scans were collected from 195 to 240 nm and the data averaged for the complete spectrum. Each individual scan was recorded using a scan rate of 20 nm/min, a bandwidth of 1 nm, and a response time of 2 s/point. Sample scans were corrected by subtracting buffer scans, and the mean residue ellipticity was calculated.

Results and Discussion

Isothermal Titration Calorimetry of the Binding of CCR5 N-terminus peptides to HIV-1 gp120

Isothermal titration calorimetry (ITC) was used to measure the binding thermodynamics of the S22 and C22 peptides to gp120 of the YU2 strain. Figure 2a shows an ITC titration of gp120 prebound to the soluble form of the human CD4 receptor (sCD4) with the S22 peptide at 25° C. The binding of S22 peptide to the gp120/sCD4 complex is characterized by an affinity (K_d) of 2.2 μM in a process that is associated with favorable changes in enthalpy and entropy of -6 kcal/mol and 5.7 cal K^{-1} mol^{-1} , respectively. ITC was also used to examine the binding of the C22 peptide lacking the sulfated tyrosine residues to gp120 prebound to sCD4. Figure 2b shows the ITC results indicating that the C22 peptide is unable to bind gp120 even in the CD4 bound state. Also, no binding could be observed when unliganded gp120 was titrated with the S22 peptide (not shown), indicating that binding of the S22 peptide requires previous binding of CD4 to gp120. Since any binding with a K_d lower than 1 mM would have been detected by the instrument, it can be concluded that CD4 binding enhances the affinity of the S22 peptide by at least 500-fold.

Under similar experimental conditions, gp120/sCD4 was titrated with 17b. The binding of 17b to the gp120/sCD4 complex is characterized by a binding affinity of 1.3 nM, and favorable changes in enthalpy and entropy of -10.1 kcal/mol and 6.7 cal K^{-1} mol^{-1} , respectively. These values are similar to previous results obtained in this laboratory (5,7).

To assess the interactions of 17b with gp120 prior to CD4 binding, unliganded gp120 was titrated with 17b. The antibody binds to unliganded gp120 with a binding affinity of 5 nM in a process characterized by large favorable and unfavorable changes in enthalpy and entropy of -28.9 kcal/mol and -59 cal K^{-1} mol^{-1} , respectively (Figure 3). These values are similar to those reported earlier (5,7). In contrast to the S22 peptide, 17b is able to bind to gp120 in the absence of CD4 (5), albeit with a lower binding affinity. This observation reflects the fact that the binding epitope for 17b is much larger than that of the S22 peptide and contains regions

that are already structured, and therefore binding competent, in unliganded gp120. Even though 17b is commonly used as a coreceptor surrogate (5,7,9,12–14), these experiments suggest that the effect of CD4 is more pronounced with the coreceptor itself.

Once CD4 binds gp120 and activates the coreceptor site, the affinity of the coreceptor or antibodies that bind to this region increases significantly because the energy penalty associated with structuring the coreceptor site is already paid by CD4 (5). The thermodynamic signatures presented in Figure 3 demonstrate the effect of CD4 on the binding of 17b or the S22 peptide. In the absence of CD4, the thermodynamic signature of 17b binding to gp120 indicates a binding process associated with significant structuring of gp120 (5). However, the binding of 17b and the S22 peptide to gp120/sCD4 are processes characterized by favorable changes in enthalpy and entropy of a much smaller magnitude and similar to what is normally observed for protein/protein interactions in which only local conformational changes are present (26, 27). While 17b is able to bind unliganded gp120, our results show that the coreceptor site must be structured prior to binding the CCR5 N-terminus, as binding of the S22 peptide to gp120 is only observed after prior binding of CD4. This result confirms previous NMR and surface plasmon resonance (SPR) experiments demonstrating that peptides based on the tyrosine-sulfated N-terminus of CCR5 are capable of binding the gp120/CD4 complex, but not gp120 or CD4 alone (10,16).

Sulfotyrosine residues at positions 10 and 14 of the CCR5 N-terminus are involved in most of the favorable interactions with gp120. Huang and coworkers (10) docked the NMR structure of a tyrosine-sulfated peptide representing residues 2–15 of the CCR5 N-terminus to the crystal structure of CD4-bound gp120 with intact V3-loop. The molecular docking was validated by saturation transfer difference NMR experiments, and revealed that the two sulfotyrosine residues formed a total of two ionic interactions and five hydrogen bonds with residues in gp120 (10). Since, the S22 peptide assumes a helical conformation when it is bound to gp120 (10), changes in helical propensity could contribute to the binding energetics. Circular dichroism (CD) spectra (not shown) of the sulfated, S22, and non-sulfated, C22, peptides show that both peptides lack any helical structure in solution, indicating that the helix is formed upon binding and that the observed binding difference is not due to a higher helical propensity of the sulfated peptide. If we assume an upper limit for C22 binding ($K_d \sim 1$ mM, or the limit of detection of the ITC), it becomes clear that the sulfate groups themselves enhance the binding affinity at least 500-fold or equivalently, they contribute close to -3.5 kcal/mol to the binding energy.

The role of sulfated tyrosines as binding enhancers has been recognized previously for other receptor systems (28–32). To the best of our knowledge, no calorimetric comparison of sulfated and non-sulfated tyrosine peptides or proteins has been reported in the literature, however, a calorimetric analysis of the binding of sulfate to a protein has been reported (33). The binding of sulfate is characterized by an unfavorable enthalpy ($\Delta H = 4.3$ kcal/mol) and a favorable entropy change ($-T\Delta S = -6.0$ kcal/mol) (33). The thermodynamic signature of sulfate binding is dominated by a strong enthalpy of desolvation coupled to an even stronger entropy of desolvation (34,35). If the sulfated tyrosines in the S22 peptide behave in a similar manner, they are expected to contribute unfavorably to the binding enthalpy and favorably to the binding entropy. The experimental enthalpy and entropy changes are favorable, suggesting a significant source of compensatory enthalpy. In addition to direct interactions between S22 peptide and gp120, which are expected to contribute on the order of -12 kcal/mol based upon polar and non-polar surface area changes for the peptide docked by Huang et al (10) ($\Delta ASA_{np} = -593 \text{ \AA}^2$, $\Delta ASA_{pol} = -531 \text{ \AA}^2$) (36–38), two additional important contributors should originate from helix formation in S22 peptide and the ordering of the V3-loop stem of gp120 (10), processes both associated with favorable enthalpies. Helix formation should contribute between -1 and -1.5 kcal/mol per residue to the binding enthalpy (39,40) and at least seven

residues are believed to assume a helical conformation (10). These favorable enthalpy changes carry a conformational entropy penalty that reduces the combined favorable entropy associated with the desolvation of sulfates and non-polar groups upon binding. The final entropic contribution to binding, though favorable, is only -1.7 kcal/mol.

Inhibition of 17b antibody Binding to the gp120/CD4 Complex by S22 Peptide

The site on gp120 where the S22 peptide binds is part of the larger footprint that corresponds to the binding epitope for the 17b antibody (10,41), an antibody commonly used as a surrogate for the coreceptor in biochemical studies (5,7,9,14). A calorimetric approach previously implemented in this laboratory (7), was used to assess if the S22 peptide was a competitive inhibitor of 17b. The effect of the S22 peptide on the binding affinity of 17b was determined by performing a 17b titration of gp120 prebound to sCD4 and S22 peptide. The expected apparent affinity in the presence of a competitive inhibitor is determined using the following equation:

$$K_{a,app} = \frac{K_a}{1 + K_{a,I} [I]} \quad (1)$$

where $K_{a,app}$ is the apparent association constant for 17b in the presence of inhibitor, K_a is the association constant for 17b in the absence of inhibitor, $K_{a,I}$ is the association constant of the inhibitor (in this case, S22 peptide), and $[I]$ is the concentration of free inhibitor. The Gibbs energy of binding, ΔG_{app} , is obtained directly from $K_{a,app}$ using the following equation:

$$\Delta G_{app} = -RT \ln K_{a,app} \quad (2)$$

where R is the gas constant and T the experimental temperature in kelvin. The expected enthalpy change in the presence of a competitive inhibitor, ΔH_{app} , is calculated using the following equation:

$$\Delta H_{app} = \Delta H - F_b \Delta H_I \quad (3)$$

where ΔH is the enthalpy change for the binding of 17b, ΔH_I the enthalpy change for the binding of inhibitor, and F_b the fraction of the gp120/CD4 complex bound to the S22 peptide prior to the experiment. Under the conditions of these experiments and prior to titration with 17b, S22 peptide was present at a concentration of $36 \mu\text{M}$; i.e. 94% of the gp120/CD4 complex was bound to the peptide. At this level of saturation, the expected affinity of 17b is expected to drop 18-fold from $K_d = 1.3 \text{ nM}$ to $K_d = 23 \text{ nM}$ if the S22 were a competitive inhibitor of 17b. Similarly, the enthalpy change is expected to drop from -10.1 to -4.4 kcal/mol. Figure 4 shows the microcalorimetric titrations of the gp120/CD4 complex with 17b in the absence and presence of S22 peptide at 94% saturation. The presence of S22 peptide at 94% saturation reduces the affinity and enthalpy change to 53 nM and -4.7 kcal/mol, respectively, which are close to the expected values for competitive inhibition. The differences between the calculated and experimental values are well within experimental error. In terms of the Gibbs energy of binding (eq 2), the difference between expected and experimental values is 0.5 kcal/mol, and for the change in enthalpy the difference is 0.3 kcal/mol. The experimental and expected thermodynamic values are summarized in Table 2.

Conclusions

The studies presented in this paper demonstrate the role of sulfated tyrosines in eliciting the necessary energy for the binding of gp120 to the cellular coreceptor CCR5. A peptide corresponding to the N-terminus of CCR5 only binds to the gp120/CD4 complex if the two tyrosines at positions 10 and 14 are sulfated. The sulfated tyrosines enhance binding affinity of the S22 peptide to gp120 500-fold or equivalently -3.5 kcal/mol. Furthermore, the S22 peptide is a competitive inhibitor of the neutralizing antibody 17b, which is known to bind to a site that overlaps the CCR5 coreceptor site in gp120 (14). These results provide strong support to the hypothesis that sulfated aromatic compounds can define a new class of HIV viral entry inhibitors. These compounds would inhibit binding of gp120 to CCR5 and block viral entry into the cell. Some novel HIV-1 drugs, including the recently approved maraviroc, also block cell entry by inhibiting gp120 binding to CCR5. They do so, however, by binding to CCR5 itself rather than gp120. Targeting the viral protein rather than the human protein should not only increase the existing arsenal of antiviral drugs, but also address HIV tropism issues (CCR5 or CXCR4 coreceptor) and any potential side effects arising by the targeting of a human protein receptor.

Abbreviations

HIV-1, human immunodeficiency virus, type 1; ITC, isothermal titration calorimetry; CD, circular dichroism; sCD4, soluble form of the human CD4 receptor; MAb, monoclonal antibody; GS, glutamine synthetase; HT, hypoxanthine/thymidine; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide electrophoresis; DMSO, dimethyl sulfoxide.

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Figure 1.

Viral entry is mediated by the envelope glycoproteins gp120 and gp41 which are assembled as trimers on the virion surface. Prior to interaction with CD4 (green), gp120 contains a structured core and largely unstructured regions, represented by the light blue texture in left panel. Binding of CD4 induces a large conformational structuring of gp120 resulting in a defined coreceptor site. The structuring of gp120 is symbolized by the change in shape and darker blue color of gp120 in the CD4-bound state. Following structuring of the coreceptor site, the CCR5 coreceptor (red) binds gp120. The CCR5 N-terminus contains two sulfotyrosine residues critical to viral entry that interact with a conserved pocket at the base of the V3-loop of gp120. The V3-loop also associates with the second extracellular loop of CCR5. Subsequent events leading to conformation changes in gp41 and fusion of the viral and host cell membranes (magenta and orange, respectively), are not illustrated in the figure. The sequences of the CCR5 N-terminus and the S22 and C22 peptides used in this study are shown in the expanded window on the right.

Figure 2.

Microcalorimetric titrations of gp120 prebound to sCD4 with CCR5 N-terminus peptides at 25 °C in PBS (pH 7.4) with 2% DMSO. gp120 (2 µM) in the presence of saturating concentration of sCD4 was titrated with (a) 200 µM S22 peptide and (b) 150 µM C22 peptide. The minor monotonic changes in the size of the injection peaks in (b) are due to the concentration dependence of the heat of dilution of C22 peptide, and are not representative of a binding event.

Figure 3. Thermodynamic signatures for binding of: (a) MAb 17b to unliganded gp120, (b) MAb 17b to gp120 prebound to sCD4, and (c) S22 peptide to gp120 prebound to sCD4.

Figure 4.

(a) Microcalorimetric titration of 2 μM gp120 prebound to sCD4 with MAb 17b. (b) Microcalorimetric titration of 2 μM gp120 prebound to sCD4 and S22 peptide with MAb 17b. The concentration of MAb 17b in the syringe was 20 μM in both experiments.

Table 1
The Binding Thermodynamics of N-Terminal Peptides of CCR5 to gp120^a

Component in the ITC cell ^b	Titrat	K_d (μ M)	ΔG (kcal/mol)	ΔH (kcal/mol)	$-T\Delta S$ (kcal/mol)
gp120	sCD4	2.0×10^{-2}	-10.5	-34.6	24.1
gp120	17b	5.0×10^{-3}	-11.3	-28.9	17.6
gp120/CD4	S22	2.2	-7.7	-6.0	-1.7
gp120/CD4	17b	1.3×10^{-3}	-12.1	-10.1	-2.0
gp120	S22	No binding	--	--	--
gp120/CD4	C22	No binding	--	--	--

^a Isothermal titration calorimetry experiments were performed at 25 °C in PBS (pH 7.4) and 2% DMSO.

^b gp120 prebound to sCD4 in the cell is depicted as gp120/CD4.

Table 2
Experimental and Expected Thermodynamic Values for 17b Binding to the gp120/CD4 Complex in the Presence of S22 Peptide

Component in the ITC cell ^a	Titrant	Experimental Values		
		$K_{d,app}$ (μ M)	ΔG_{app} (kcal/mol)	$-T\Delta S_{app}$ (kcal/mol)
gp120/CD4/S22	17b	5.3×10^{-2}	-9.9	-5.2
		Expected Values for Competitive Inhibition ^b		
		2.3×10^{-2}	-10.4	-6.0

^a gp120 prebound to sCD4 and S22 peptide is represented by gp120/CD4/S22. Under the conditions of these experiments (see Text) gp120/CD4 is 94% saturated by S22 peptide.

^b Expected thermodynamic values were calculated by assuming competitive binding according to eqs 1, 2, and 3.