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The role of amphiphilicity and negative charge in glycoprotein 41 interactions in the hydrophobic pocket

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Abstract

The hydrophobic pocket within the coiled coil domain of HIV-1 gp41 is considered to be a hotspot suitable for small molecule intervention of fusion, although so far it has yielded only μ M inhibitors. Previous peptide studies have identified specific hydrophobic interactions and a Lys-Asp salt bridge as contributing to binding affinity in the pocket. Negative charge appears to be critical for activity of small molecules. We have examined the role of charge and amphiphilic character in the interaction, by studying a series of short pocket binding peptides differing in charge, helical content and in the presence or absence of the Lys-Asp salt bridge, and a series of fatty acid salts with varying charge and hydrocarbon length. Quantitative binding analysis revealed that long range electrostatic forces and a greasy non-specific hydrophobic interaction were sufficient for μ M potency. The results suggest that an extended interaction site may be necessary for higher potency. We examined a region of the coiled coil immediately C-terminal to the pocket, and found that specific salt bridge and hydrogen bond networks may reside in this region. Negatively charged groups extended towards or beyond the C-terminus of the pocket could therefore result in improved low molecular weight fusion inhibitors.

Keywords

HIV-1 gp41; hydrophobic pocket; electrostatics; peptide binding; fatty acid salts

The coiled coil domain of HIV-1 gp41 is a trimer of helices from the N-heptad repeat (NHR) region, with a long hydrophobic groove extending 50 residues in length. Amphiphilic C-heptad repeat (CHR) peptides from gp41 bind into the grooves during the process of HIV-1 fusion to form a six-helix bundle ^{1–3}. A new generation of anti-HIV agents are fusion inhibitors derived from the CHR domain. T20, an approved entry inhibitor, is a 36 amino acid peptide formed from residues 638–673 ⁴, which appears to block formation of the fusion pore at the membrane interface ^{5, 6}. C34 is a 34 amino acid peptide consisting of residues 628–661 ^{2, 7}, which spans two thirds of the length of the coiled coil and acts by preventing formation of the six helix bundle ^{7, 8}. The N-terminal end of C34 contains highly conserved hydrophobic residues Trp628, Trp631 and Ile635 which bind in a deep hydrophobic pocket situated approximately midway along the coiled coil (residues 565–579) ⁹. The hydrophobic pocket is considered to be a hot-spot for small molecule inhibition of fusion ⁷. Trp628 and Trp631 are invariant in all viral strains tested, including among mutants which arise in response to C34 exposure ¹⁰.

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Although multiple mutation and binding studies have implicated a critical role for Trp628, Trp31, Ile635, Ile642 and an Asp 632 – Lys574 salt bridge in gp41 processing, stability of the six helix bundle ^{11, 12, 13}, and CHR inhibitory potency ^{7, 9}, there has been limited success in designing potent inhibitors focused on the hydrophobic pocket. At the same time, studies have shown that attachment of 8–12 carbon fatty acids to the N-terminus or C-terminus of a peptide comprising the first 25 residues of T20 restored potency to the equivalent of C34 or T20 ¹⁴. In the former case, the fatty acid segment would be located in the region of the hydrophobic pocket. Other studies have indicated that non-specific amphiphilic phosphorothioate oligonucleotides blocked gp41 six-helix bundle formation in a length dependent manner ¹⁵. Small molecules directed to the hydrophobic pocket also appear to be amphiphilic in nature, and require negatively charged groups for activity ^{16, 17}. In this report, we have investigated the role of charge and amphiphilicity on binding by studying a set of peptides differing in helical content and charge, and a set of low molecular weight fatty acid salts, differing in charge and length of the hydrocarbon chain. We have also studied the role of specific polar interactions that, in conjunction with hydrophobic specificity of the Trp-Trp-Ile-Ile motif, could be used in the design of potent non-peptide fusion inhibitors. We have included studies on the Lys-Asp salt bridge and a polar region contained in a C-terminal extension of the hydrophobic pocket. In this way, we hope to distinguish between non-specific and specific interactions in dictating binding affinity, and obtain information on polar interactions that could be incorporated into the design of low molecular weight inhibitors.

Results

Selection of peptides

We examined a series of 18-amino acid peptides derived from the gp41 CHR with variations in sequence, helicity and overall charge. Mutations which altered helicity or peptide pI were restricted to amino acids that project away from the binding interface with the coiled coil. All of the peptides were terminated with a cysteine residue, so that they could be labeled for binding assays. Peptides tested included the wild-type sequence, sequences with helix stabilizing mutations, pI variations, a point mutation at Asp632, a scrambled sequence and a sequence from another Class 1 virus (hRSV). Except for the scrambled sequence, the N-terminal half of each gp41 C-peptide contained the residues which bind in the hydrophobic pocket, including the conserved Trp628, Trp631 and Ile635 residues. The C-terminal half of each gp41 C-peptide extended beyond the hydrophobic pocket and included Tyr638 and Ile642 which make hydrophobic contacts with the coiled coil. In the absence of this C-terminal extension, no binding was observed by us and others ¹⁸. Included in the study were longer hydrophobic pocket binding peptides extended either in the N-terminal (C27-e3.0) or C-terminal (C32-e5.0) direction, that were matched to longer receptors env3.0 (discussed below) or env5.0 (manuscript accepted for publication).

Selection of fatty acid salts

A series of sulfated alkyl salts was examined, with variation in the length of the hydrocarbon chain and the charge. Charge was modified by altering the ionic strength of the acidic head group, in the order $-\text{SO}_4^{2-} > -\text{SO}_3^{2-} > -\text{CO}_2^-$. Hydrocarbon chain length was varied between 6–18 C atoms.

Binding constant determination

Binding affinity of selected inhibitors was evaluated using a competitive inhibition fluorescence assay developed recently in our lab ^{16, 19}. It enabled precise measurement of compounds with affinities $\geq 0.3 \mu\text{M}$ for the hydrophobic pocket, and has been verified as accurately representing the hydrophobic pocket interaction in gp41. Figures 1 and 2 show the inhibition curves for the peptides and fatty acid salts, and illustrate the level of precision with

which we could discriminate between inhibitors with similar K_I 's. We were easily able to distinguish K_I 's within a factor of 2. All curves were fit to a 1:1 binding model¹⁶. Results are delineated in Tables 1 and 2. Included in Table 1 is the helicity of the peptides determined by CD, and calculated peptide pI²⁰ and net charge. It is clear that salt-bridge and alanine substitution did not have a readily predictable effect on helical content. Table 2 reports the critical micelle concentration (CMC) for each of the fatty acid salts. Specific binding of these detergent-like molecules was obtained at concentrations several orders of magnitude below the CMC, ruling out denaturation as the cause of inhibition. CD was used to confirm the binding mechanism. The helicity of the metallopeptide Fe(env2.0)₃ remained unchanged upon addition of 20 μ M SDS, a concentration that caused 75% fluorescence recovery in the competitive inhibition assay. Furthermore, the increase in helical content which occurred upon addition of C18-Aib to Fe(env2.0)₃ 19 (a sign of 6-helix bundle formation) was 65% reversed in the presence of 20 μ M SDS, confirming that SDS disrupts the NHR – CHR interaction.

Evaluation of observed trends

The results on both peptides and alkyl salts illustrate the dependence of affinity on both specific and non-specific hydrophobic and electrostatic interactions in or around the hydrophobic pocket, as discussed below.

Specific hydrophobic interactions

The peptide studies confirm the role of specific hydrophobic interactions between the Trp628 – Trp631 – Ile635 – Tyr638 – Ile642 motif and the hydrophobic pocket. Affinity as low as 0.5 μ M was observed for an 18-residue peptide binding to Fe(env2.0)₃. A scrambled peptide did not bind, and an unrelated amphiphilic peptide from Respiratory Syncytial Virus F1 protein, RSV-C28, bound to the gp41 coiled coil structure with a K_I of 13 μ M. The moderate affinity of RSV-C28 likely arises from some sequence similarity to the gp41 peptide, namely at positions Ile635 and Ile642, a phenylalanine at the position of Trp631 and an aspartic acid at position 632, which may form a salt bridge with Lys574¹³.

Non-specific hydrophobic interactions

Interestingly, it was also possible to obtain sub- μ M inhibition of the NHR-CHR interaction with amphiphilic alkyl salts (Table 2). The affinity increased with increase in hydrocarbon chain length and with increase in the ionic strength of the head group. Figure 3 illustrates the distribution of K_I as a function of hydrocarbon chain length. Sub-micromolar inhibition constants were obtained for 16–18C alkyl chains. Thus it appears that a long chain hydrocarbon can substitute for the specific groove-binding non-polar amino acids, as was observed earlier in a study in which a hydrocarbon chain was oriented in the pocket through attachment to an adjacent groove binding peptide¹⁴. The length of an extended 18-carbon alkyl chain matches the distance across the Trp-Trp-Ile_Ile motif in a helical conformation of the C-peptide (~22Å).

Non-specific electrostatic interactions

The role of negative charge in the interaction with the coiled coil is apparent from Tables 1 and 2. The wild-type peptide C18-WT bound with an inhibition constant of 3.9 μ M. It has a charge of –2 at neutral pH. Peptides C18-SB1 and C18-SB2, containing i – i+4 salt bridge modifications, are neutral at pH 7 and have poor binding affinity to Fe(env2.0)₃. C18-SB2 has twice the helical content of C18-WT, a property that is typically correlated with peptide potency^{21–24}, but it had a six-fold reduced binding affinity. We found that the charge differential between the electropositive coiled coil and negatively charged inhibitors had a measurable effect on the affinity. A C-peptide C18-SB4 with charge –3 has ~1.5-fold improved binding affinity compared to C18-SB3 with charge –2 and similar helical content. Increasing the charge on the receptor by +1 had the same effect. The dissociation constant of peptide C18-Aib (charge

–2) decreased from $1\mu\text{M}$ to $0.51\mu\text{M}$ by making a single Q2R mutation in the receptor Fe (env2.0R)₃. The mutation is situated in the linker region which connects the N-terminal bipyridyl group to the NHR peptide and plays no role in the peptide – peptide interaction. A similar trend was apparent for the alkyl salts, where increased ionic strength of the negatively charged head group resulted in increased affinity (Figure 3). Although these changes in binding constants are small, they are significant based on the precision of our data (Figures 1, 2).

Specific electrostatic interactions

A salt bridge between Asp632 and Lys572 has been identified as forming a key specific electrostatic interaction in the hydrophobic pocket^{12, 13}. We were therefore surprised to find that the peptide C18-DA1, in which Asp632 was mutated to alanine, was the most potent 18-residue peptide in our test set. However, it also had the highest helicity, a property that is known to correlate with peptide potency due to the reduced entropic penalty upon binding. In order to tease out the differential effects of helicity and charge, we examined the free energy of binding as a function of peptide helicity. Free energy of binding was calculated from the K_I according to the equation $\Delta G_{\text{bind}} = RT \ln K_I$. We expanded the test set to include 27 and 32-residue peptides extended in either the N- or C-terminal direction from the hydrophobic pocket, and measured against a correspondingly longer receptor. All peptides contained the hydrophobic groove binding Trp-Trp-Ile-Tyr-Ile motif. The results are shown in Figure 4. ΔG_{bind} of negatively charged gp41 CHR peptides varied linearly with increase in helicity. The trend indicated that observed binding free energy changes were driven mainly by configurational entropy effects, as has been previously observed^{25, 26, 27}. The correlation broke down if the peptides were not charged.

The peptide C18-DA1 is displaced from the trendline, confirming the specific effect of the salt bridge on peptide affinity. A peptide following the observed linear trend as a function of helical content would have a 3-fold lower K_I than that observed for C18-DA1, as illustrated in Figure 4. This is in line with previous mutagenesis studies which showed that the effect on inhibitory potential of reversing the charge at position 574 or 632 is about a factor of 2–4^{12, 13}. The relatively minor effect of removing the salt bridge, compared to substitution of one of the hydrophobic groove binding moieties (a 10–40 fold reduction in affinity^{7, 9}) is likely a reflection of the fact that isolated and exposed salt bridges do not contribute substantially to protein-protein interaction energy²⁸. This is an important result when considering how to mimic the protein-protein interaction with a small molecule.

Studies on extended CHR peptides

The data presented in Table 1 and Figure 4 includes results on two longer CHR peptides, C27-Aib and C32-e5.0, which were extended in the N- or C-terminal direction respectively, and measured against receptors Fe(env3.0)₃ and Fe(env5.0)₃. Both of these peptide – peptide interactions demonstrated the enthalpy – entropy compensation effect that characterizes the protein – protein interaction^{18, 26, 29} (Figure 4). The peptide pair C32-e5.0 / Fe(env5.0)₃ has nM binding affinity, in accordance with that seen for C34 and derivatives, and is the subject of another publication³⁰. As part of our evaluation of electrostatic contributions to inhibitor binding, we were interested in the peptide pair C27-Aib / Fe(env3.0)₃ which include a large percentage of polar residues in their extended segments. The hydrophobic Trp623 of C27-Aib points away from the interaction surface with the coiled coil³¹. Polar residue interactions could be the basis for important electrostatic contacts and hydrogen bonds of inhibitors designed to bind in this region. A recent report has suggested that important NHR – CHR domain contacts reside in this region³².

The additional heptad repeat failed to improve the affinity over that of C18-Aib / Fe (env2.0)₃. Since C27-Aib contains a segment which is not helical in the intact ectodomain 1

or in solution, an unfavorable binding entropy occurred upon constraining the extended N-terminal segment, offset by enthalpy gain^{18, 26, 29} (Figure 4). Furthermore, the affinity of C27-Aib for the receptor Fe(env2.0)₃, which does not contain a complete binding site for the added N-terminal segment of C27-Aib, was a full order of magnitude lower. The displacement of the C27-Aib / Fe(env2.0)₃ interaction from the free-energy – helix correlation line is shown in Figure 4. These results imply that stabilizing interactions of at least a factor of 10 occur in the heptad repeat segment of gp41 C-terminal to the pocket. The potential for hydrogen bonding and buried salt-bridges in this region could significantly add to the affinity of an inhibitor extended beyond the hydrophobic pocket and having fewer degrees of freedom than C27-Aib. Networks of buried salt bridges can have a strong cooperative effect²⁸, and taking advantage of polar interactions could improve solubility characteristics of designed inhibitors.

We examined a structure obtained by homology-modeling from the corresponding SIV structure¹ (See Experimental Methods) in order to identify possible electrostatic interactions between N-terminal residues of C27-Aib and the extended heptad repeat in env3.0. Figure 4 shows the interaction of charged residues in and around the hydrophobic pocket, and reveals a network of salt bridges which could be involved in stabilizing the NHR-CHR association beyond the C-terminal end of the pocket. A mesh surface depicts the hydrophobic pocket cavity; the backbone of C27-Aib and residues Trp628, Trp631 and Ile635 are shown in yellow. The figure shows charged and polar residues lining the pocket and NHR N-terminal extension and depicts several potential electrostatic interactions involving atoms < 3.5 Å apart. Arg579 of the NHR could form intermolecular salt bridges or hydrogen bonds to Glu620 and Asn624 on the CHR, as well as an intramolecular hydrogen bond with Gln575. The contacts are buried within the binding interface. In this snapshot, there is also an intramolecular hydrogen bond between Glu620 and Asn624. An alternative snapshot is provided in another homology modeled structure in the literature³¹. Different side chain orientations of Arg579, Glu620 and Asn624 occur in that structure, likely reflecting flexibility of orientation of these groups, but a salt bridge between Arg579 and Glu620 is apparent in that structure as well.

Discussion and Conclusions

Study of a set of 18-residue CHR peptides and fatty acid salts which bind in the hydrophobic pocket of gp41 NHR has revealed that both specific and non-specific interactions can form a basis for inhibitor affinity to the gp41 hydrophobic pocket. In many ways, classical effects such as long range electrostatic forces, specific VdW interactions, entropy and amphiphilicity play a key role in binding. A negative charge on the inhibitor was critical for sub- μ M affinity, and increasing the charge differential between the NHR and CHR peptides caused a small but measurable improvement in affinity. The sensitivity of the interaction to inhibitor charge is not an unexpected result. Charge complementarity has been recognized as a driving force in protein – protein association, increasing the association constant and stabilizing the transition state by long-range electrostatic forces³³. Helical content played an important role both because of its correlation to binding entropy as well as its role in establishing the correct amphiphilic character of the C-peptides with groove binding residues oriented on one face of the helix. The results obtained on peptide studies mirror those obtained using pseudovirus models and mutagenesis, providing confirmation for this rapid biochemical approach to investigating gp41 – inhibitor interactions.

In addition to classical thermodynamic effects, there was also evidence of inhibitor binding via a diffusive transition state lacking specific hydrophobic or electrostatic contacts. Sodium octadecylsulfate had a 0.33 μ M affinity for Fe(env2.0)₃. It is possible that the protein-protein interaction that occurs in gp41 upon fusion is one of the targets of surfactants that show anti-HIV activity in vitro^{34–36}. Direct use of fatty acids as gp41 inhibitors is unlikely because of the toxic effect of these molecules on cells^{37–39}. Sodium octadecylsulfate induced a large

increase in fusion in an HIV gp41 mediated cell-cell fusion assay, presumably by disrupting the cell membrane. Alkyl sulfates with 8 and 12 carbon atoms displayed IC_{50} 's for fusion very similar to their K_I 's. The μM potency in both binding affinity and inhibition against cell-cell fusion demonstrated that μM binding affinity to the gp41 NHR core can be achieved by nonspecific electrostatic and hydrophobic interactions with a simple amphiphilic structure. Interestingly, our observed micromolar potency of surfactant molecules is in the same range as currently available small molecule gp41 inhibitors. These small molecule fusion inhibitors are assumed to bind in the gp41 deep pocket, although direct structural evidence to verify the binding site is lacking even after more than 10 years' struggle. One possibility is that most of these small molecule fusion inhibitors have the same mechanism as the surfactants.

A longer C-peptide with a binding footprint which extended to the region immediately C-terminal to the hydrophobic pocket on the NHR bound with $\sim 1 \mu M$ affinity despite lower helical content (12%). We propose that a network of ionic interactions involving residue Arg579 of the NHR contributes favorably to the energetics of the interaction in this region. Coupling of salt bridges and hydrogen bonds has a cooperative effect on their contribution to the free energy^{28, 40}. On the other hand, isolated and exposed salt-bridges such as that formed between Asp632 and Lys574 are predicted and observed to have limited energetic contribution. This result is important in the design of small molecules to mimic the interaction of peptides in the hydrophobic pocket and vicinity. To date, small molecules targeting the hydrophobic pocket have demonstrated affinities no lower than a few μM . Extending their reach to include the C-terminal groove and the charge on Arg579, and incorporating the concept of networks of buried salt bridges, may provide the key to the design of small molecules which can be effective fusion inhibitors.

Experimental Section

Peptide preparation

Peptides were prepared by solid phase synthesis (Biosynthesis, Inc., TX). The N-terminal metal coordinating moiety 2,2'-bipyridyl carboxylic acid (bpy) was appended to the NHR peptide env2.0, env2.0R, env3.0 or env5.0 on the resin. The sequences of these peptides are as follows:

env2.0: bpy-GQAVEEAQQHLLQLTVWGIKQLQARILAVEKK; residues 560–584

env2.0R: bpy-GRAVEEAQQHLLQLTVWGIKQLQARILAVEKK; residues 560–584

env3.0: bpy-GQAVEEAQQHLLQLTVWGIKQLQARILAVERYLKDQKK; residues 560–593.

env5.0: bpy-GQAVSGIVQQNNLLRAIEAQQHLLQLTVWGIKQLQARILAVEKK; residues 546–584.

Underlined residues occur in wild-type gp41 (HXB2). Ferrous complexes of these peptides were prepared by addition of freshly prepared ferrous ammonium sulfate at 1/3 of the peptide concentration. All peptides were amidated at the C-terminus. C-peptides were acetylated at the N-terminus. C18-Aib and C27-Aib were labeled with fluorophore Lucifer Yellow (LY).

CD studies

CD studies were performed on a DSM20 CD spectrophotometer from OnLine Instruments Systems, Inc., Bogart, GA, using $20 \mu M$ solutions of peptides in 15mM Tris-acetate buffer, pH 7.0 at 25°C.

K_D determination

K_D's for the interaction between Fe(env#0)₃ receptors and their cognate C-peptides were determined by fluorescence. C-peptides C18-Aib and C27-Aib were labeled with fluorophore Lucifer Yellow as previously described¹⁶ and C32-e5.0 was labeled with fluorescein. The dissociation constants of C18-Aib-LY with receptor constructs Fe(env2.0)₃ and Fe(env2.0R)₃ were determined by measuring the fluorescence of 1 μM C18-Aib-LY as a function of metalloprotein concentration, and fitting the data to standard 1:1 binding. K_D's for the C27-Aib / Fe(env3.0)₃ and C32-e5.0 / Fe(env5.0)₃ interaction were measured using 1 μM C27-Aib-LY or 0.15 μM C32-e5.0-FL in a similar fashion.

K_I determination

K_I's of 18-residue peptide and alkyl salts were determined in a fluorescence experiment, by measuring competitive inhibition of a metalloprotein - C18-Aib-LY complex, according to a previously described protocol¹⁶. Briefly, a dose response titration of each of the peptides was performed using an assay reagent containing C18-Aib-LY (1 μM) and Fe(env2.0)₃ (7 μM). The fractional fluorescence change was obtained by comparing the data to a second titration in which Fe(env2.0)₃ was omitted from the assay solution. This control ensures that there is no spurious fluorescence increase due to the peptide inhibitor. The increase in residual fluorescence associated with increased peptide inhibitor concentration was fit to a 1:1 binding model to obtain the K_I. Experiments were repeated in quadruplicate, and done twice for each peptide. Compatibility of different data sets was ensured by checking the K_I of the positive control C18-Aib in each data set. All fluorescence experiments were conducted in 25 mM Tris-acetate buffer containing 0.01% Tween and 4% DMSO at pH 7.0.

Computational calculations—A homology modeled structure of the HIV-1 gp41 ectodomain was obtained from the NMR solution structure of SIV gp41¹ by mutating the residues to the HIV gp41 sequence, and performing energy minimization and molecular dynamics with backbone restraints to obtain low energy conformations of the side chains. The 4-residue insert 610-WNAS-613 absent from the SIV structure³¹ was omitted from the model. 10 ps of restrained Molecular Dynamics simulations were performed in vacuo at 300 K using the program Amber 7⁴¹ with 5 kcal/mol.Å² backbone constraints, followed by 300 cycles of conjugate gradient energy minimization.

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Abbreviations

NHR	N-heptad repeat
CHR	C-heptad repeat
bpy	5-carboxy-2,2'-bipyridine
SDS	sodium dodecyl sulfate

K_D	dissociation constant
K_I	inhibition constant

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Figure 1.

Inhibition curves of the $\text{Fe}(\text{env}2.0)_3$ – C18-Aib interaction with several peptides from Table 1. Data shown is the average of 4 experiments per peptide, completed on two different days, which are distinguished by solid line or dashed line fits. The y axis is scaled for overlap of the minimum fluorescence (See Experimental Methods). The error bars are the standard deviation of the measurement. (○) C18-DA1; (●) C18-SB4; (■) C18-Aib; (●) C18-WT; (●) RSV-C28; (○) C18-SB1; (●) C18-SB2; (●) C18-Scr.

Figure 2. Inhibition curves of the $\text{Fe}(\text{env}2.0)_3$ – C18-Aib interaction with alkyl – sulfite salts from Table 2. The number of carbon atoms n in the alkyl chain is 6(●), 8(□), 10(△), 12(◆) and 16(○). The error bars are the standard deviation of four measurements.

Figure 3.

Figure 3. Affinity (K_I) of various surfactant molecules to the gp41 NHR binding pocket as a function of carbon chain length and head group ionic strength. (●)R-SO₄⁻¹; (■)R-SO₃⁻¹; (▲) R-COO⁻¹; ■, R is saturated hydrocarbon chain of indicated length. Error bar is standard deviation of 4 independent measurements.

Figure 4.

Plot of the C-peptide helicity against free energy of binding to metallopeptide trimers. HIV-1 C-peptides have a charge of 0 ($\circ$), -2 ($\bullet$, $\bullet$) or -3 (SB4, $\bullet$) and are measured binding to Fe (env2.0)₃ unless otherwise indicated. The arrows indicates the change in binding energy of C27-Aib due to extending the length of the metallopeptide receptor, and the effect a threefold improvement of affinity would have on the binding free energy of DA1. A linear fit is shown through the data points C27Aib / env3.0, WT, Aib, SB3, SB4 and C32-e5.0 / env5.0.

Figure 5.

Details of the electrostatic interactions in a homology-modeled structure of the HIV-1 gp41 ectodomain. The region shown corresponds to the segment represented by Fe(env3.0)₃ (two helices forming the interaction groove are shown in gray) and C27-Aib (shown in yellow). The cavity of the hydrophobic pocket, defined by residues L566, V570, I573, K574 and Q577 of one chain and L565', L568', T569', W571', G572', Q575' and L576' of the second chain, is shown as a mesh surface in magenta. The Trp-Trp-Ile motif of the C-peptide and polar or charged residues lining the pocket and in the C-terminal segment are shown explicitly. N – O pairs which could form intermolecular salt bridges or hydrogen bonds are shown as solid green lines and have the following distances: Trp631 NE1 – Leu568' O(carbonyl) 2.94Å; Asp632 OD1 – Lys574 NZ 2.98Å; Trp628 NE1 – Q577 OE1 3.44Å; N624 OD1 – Arg579' NH2 2.96Å; E620 OE1 – Arg579' NH1 2.96Å. In dashed lines are two intramolecular hydrogen bonds, between E620 and N624 (3.12Å) and between R579' and Q575' (3.15Å).

Table 1
CHR peptide properties and inhibition constants for the interaction with
metallopeptide coiled coils

Peptide [§]	Sequence	% helix ^a	pI [†]	Net charge	K _i (μM)
C18-WT	Ac-MTWMEWDREINNYTSLIC-NH ₂	13	4.14	-2	3.0 ± 1.0
C18-SB1	Ac-MTWMEWDRKIEEYTKKIC-NH ₂	12	6.01	0	15 ± 2.0
C18-Aib	Ac-MTWBEWDREIBNYTSLIC-NH ₂	17	4.14	-2	1.0 ± 0.2
C18-SB2	Ac-MTWEAWDKRIEEYTKKIC-NH ₂	28	6.01	0	25 ± 5
C18-SB3	Ac-MTWEAWDKIEEYTKKIC-NH ₂	23	4.58	-2	0.6 ± 0.2
C18-SB4	Ac-MTWEWDKIEEYTKKIC-NH ₂	26	4.42	-3	0.5 ± 0.05
C18-DA1	Ac-MTWEWAKIEEYTKKIC-NH ₂	44	4.65	-2	0.4 ± 0.1
C27-Aib	Ac-KSLEQBWNHTTWBEWDREIBNYTSLIC-NH ₂	12	4.83	-2	10.8 ± 1.3 1.2 ± 0.21*
C32-e5.0	Ac-TTWEAWDRAIAEYAAIEALIRAAEQQEKNC-NH ₂	68	4.71	-2	0.007 ± 0.003 [§]
RSV-C28	Ac-FASDEFDASIBQVNEKIBQSLC-NH ₂	n.d. [¶]	3.92	-3	13 ± 1.3
C18-Scr	Ac-MMIWNTDEILTNESYRWC-NH ₂	n.d. [¶]	4.14	-2	191 ± 35

^a receptor used is Fe(env2.0)₃ unless otherwise indicated;

* receptor is Fe(env3.0)₃;

[¶] receptor is Fe(env5.0)₃;

[§] WT=wild-type (HXB2, residues 626–643), Aib=α-aminoisobutyric acid (B) substituted, SB1 – SB4 = salt-bridge substituted, with negative and positive residues involved in the salt bridges shown in red and blue respectively; DA1 = Asp632-substituted, Scr=scrambled;

^a from CD assuming 100% helix at a molar residue ellipticity of –33,500 at 222nm; standard deviation of the measurement is 3% helix;

[†] Calculated using ExPaSY;

[¶] not determined.

Table 2

Observed inhibition constants for sulfated alkyl salts binding to the gp41 hydrophobic pocket

Compound	Mol. Wt. *	CMC (μ M)	K_I (μ M)
$C_8H_{17}OSO_3^-Na^+$	209	30,000	110 ± 17
$C_{10}H_{21}OSO_3^-Na^+$	237	15,000	$21 \pm 5^{\dagger}$
$C_{12}H_{25}OSO_3^-Na^+$	265	2000	2.0 ± 0.4
$C_{13}H_{27}OSO_3^-Na^+$	279	600	$1.5 \pm 0.3^{\dagger}$
$C_{14}H_{29}OSO_3^-Na^+$	293	150	$1.6 \pm 0.6^{\dagger}$
$C_{18}H_{37}OSO_3^-Na^+$	349	<100	0.33 ± 0.05
$C_8OO^-Na^+$	121	200,000	2380 ± 200
$C_{12}OO^-Na^+$	177	2500	20.8 ± 5.2
$C_6H_{13}SO_3^-Na^+$	165	>250,000	$10,070 \pm 1185$
$C_8H_{17}SO_3^-Na^+$	193	>250,000	994 ± 308
$C_{10}H_{21}SO_3^-Na^+$	221	25,000	62 ± 27
$C_{12}H_{25}SO_3^-Na^+$	249	5000	6.7 ± 1.1
$C_{14}H_{29}SO_3^-Na^+$	293	600	1.6 ± 0.3
$C_{16}H_{33}SO_3^-Na^+$	305	≤ 100	0.6 ± 0.4

* Molecular weight of anion;

 † average of several experiments