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Core-Binding Factor β Increases the Affinity between Human Cullin 5 and HIV-1 Vif within an E3 Ligase Complex

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Supporting Information

ABSTRACT: HIV-1 Vif masquerades as a receptor for a cellular E3 ligase harboring Elongin B, Elongin C, and Cullin 5 (EloB/C/Cul5) proteins that facilitate degradation of the antiretroviral factor APOBEC3G (A3G). This Vif-mediated activity requires human core-binding factor β (CBFβ) in contrast to cellular substrate receptors. We observed calorimetrically that Cul5 binds tighter to full-length Vif$^{(1-192)}$/EloB/C/CBFβ (K_d = 5 ± 2 nM) than to Vif$^{(95-192)}$/EloB/C (K_d = 327 ± 40 nM), which cannot bind CBFβ. A comparison of heat capacity changes supports a model in which CBFβ prestabilizes Vif$^{(1-192)}$ relative to Vif$^{(95-192)}$, consistent with a stronger interaction of Cul5 with Vif's C-terminal Zn^{2+}-binding motif. An additional interface between Cul5 and an N-terminal region of Vif appears to be plausible, which has therapeutic design implications.

Viral infections can be accompanied by the hijacking of cellular pathways to subvert innate defense mechanisms.[1] This is exemplified by HIV-1 in which an essential protein, viral infectivity factor (Vif), neutralizes A3G and related family members inherent to CD4(+) T cells (reviewed in ref 2). In Vif deficient HIV-1 infection, A3G is incorporated into virions and travels to subsequently infected cells where it exhibits antiviral properties, including dC-to-dU deamination of first-strand HIV-1 DNA.[3,4] In wild-type HIV-1 infections, however, Vif masquerades as a SOCS-box substrate receptor that directly binds A3G via conserved sequences (reviewed in ref 5 and Figure 1) and recruits it to a Cullin-RING E3 ubiquitin ligase, resulting in polyubiquitination and proteasomal degradation (Figure 1A).[6,7] Vif binds EloC via a canonical BC-box conserved in cellular SOCS-box proteins[8,9] but utilizes a novel HCCH Zn^{2+}-binding motif to associate with N-terminal Cul5 regions in lieu of the cellular Cul5-box.[10,11] The model for E3 complex formation posits that the SOCS-box/EloB/C interaction precedes Cul5 binding.[12]

Although the interaction between A3G and Vif has been known for a decade,[13] CBFβ was shown recently to associate with N-terminal Vif residues (Figure 1B) and to be essential for E3 ligase-mediated degradation of A3G, as well as viral infectivity.[14,15] The cellular role of CBFβ is hypothesized to be allosteric stabilization of the DNA-bound form of its cognate α subunits, which together form essential α/β transcription factors.[16,17] CBFβ has two isoforms[18] that co-immunoprecipitate Vif and support HIV-1 infectivity.[19,20]

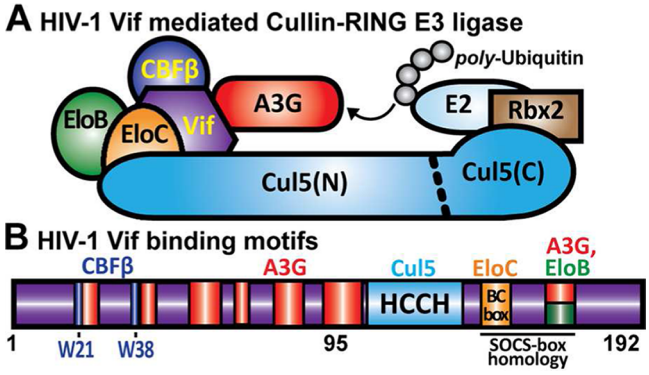

Figure 1. Schematic of the Vif-mediated E3 ligase and Vif sequence motifs. (A) A3G is recruited by Vif to the N-terminus of Cullin 5 [Cul5(N)] in conjunction with the heterodimeric EloB/C substrate adaptor. Cul5(C) and Rbx2 position the E2 ubiquitin conjugase. (B) Conserved Vif binding motifs.

Prior calorimetric analysis showed strong binding of mouse Cul5(N) to Vif$^{(100-192)}$/EloB/C (K_d = 89 ± 26 nM).[11] Despite the importance of CBFβ in fortifying the interaction between HIV-1 Vif and its host-binding partners,[14,15] the affinity of Cul5 for Vif/EloB/C/CBFβ has not been quantified. To assess the effect of CBFβ on the interaction between Cul5 and Vif, we undertook a thermodynamic analysis of binding of human Cul5(N) to (i) the Vif$^{(95-192)}$/EloB/C complex, herein called Vif$_C$/EloB/C, where Vif's N-terminal truncation precludes CBFβ binding,[14] and (ii) a complex with full-length forms of Vif and CBFβ, herein called Vif/EloB/C/CBFβ. The resulting parameters were then compared to those of the interaction of Cul5(N) with a minimal human SOCS2/EloB/C complex, which is representative of cellular SOCS-box affinity.

The inability to express Vif as an isolated polypeptide necessitated its production in the presence of its host partners.[21] Efforts to produce a Vif/EloB/C complex comprising full-length Vif, but missing CBFβ, were confounded by poor solubility. As such, we expressed Vif in *Escherichia coli* as Vif$_C$/EloB/C or Vif/EloB/C/CBFβ. Both complexes and Cul5(N) were purified to homogeneity (Figure S1, Supporting Information). We then conducted thermodynamic measurements for the interaction of Cul5(N) with the ternary and quaternary complexes (Figure S2A,B, Supporting Information).

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Table 1. Thermodynamic Parameters at 303.15 K for Cul5(N) Binding to Virus or Host Substrate Receptor Complexes

syringe sample	cell sample	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	K_d[a] (nM)	ΔC_p[b] (kcal mol^{-1} K^{-1})	N_{res}[c]	ΔASA[c] (Å^2)
Cul5(N)	Vif$_C$/EloB/C	−9.0 ± 0.1	−5.2 ± 0.4	−3.8 ± 0.5	327 ± 40	−0.30 ± 0.01	20	∼2100
Cul5(N)	Vif/EloB/C/CBFβ	−11.5 ± 0.3	−8.8 ± 0.6	−2.8 ± 0.9	5 ± 2	−0.52 ± 0.02	37	∼3600
Cul5(N)	SOCS2$_{SOCS\text{-}box}$/EloB/C	−11.3 ± 0.1	−5.4 ± 0.2	−5.9 ± 0.0	7 ± 2	N/A[d]	N/A[d]	N/A[d]

[a]The C values of 3200 and 2783, for titration of Cul5(N) into Vif/EloB/C/CBFβ and SOCS2$_{SOCS\text{-}box}$/EloB/C, respectively, prohibit determination of unique K_a values. Thus, the apparent K_d values represent lower limits of affinity. [b]Heat capacity derived from slope of ΔH vs T (in kelvin) plotted at four temperatures between 293.15 and 308.15 K. [c]Number of residues buried (N_{res}) and change in solvent-accessible surface area (ΔASA) upon interaction derived from ΔC_p. [d]Not determined.

Our results revealed that human Cul5(N) interacts strongly with Vif$_C$/EloB/C, which harbors the conserved HCCH Zn^{2+}-binding motif and the BC-box. The interaction at 303.15 K was favorable enthalpically (ΔH = −5.2 ± 0.4 kcal mol^{-1}) and entropically ($-T\Delta S$ = −3.8 ± 0.5 kcal mol^{-1}) (Table 1), in agreement with results for the closely related mouse Cul5(N).[11] Likewise, the interaction between Cul5(N) and the quaternary complex, comprising full-length Vif and CBFβ, was also favorable (ΔH = −8.8 ± 0.6 kcal mol^{-1}) and ($-T\Delta S$ = −2.8 ± 0.9 kcal mol^{-1}). However, the upper limit of the affinity of Cul5(N) for Vif/EloB/C/CBFβ was 65-fold greater (apparent K_d = 5 ± 2 nM) than for Vif$_C$/EloB/C (K_d = 327 ± 40 nM). The Cul5(N) affinity in the presence of CBFβ was on par with that of the SOCS2$_{SOCS\text{-}box}$/EloB/C interaction [apparent K_d = 7 ± 2 nm (Table 1 and Figure S2C, Supporting Information)], which comprises the human SOCS2 SOCS-box (residues 158−198). The stoichiometry of binding of Cul5(N) to each complex was 1:1 (n = 0.99 and 0.94, respectively), consistent with its binding to EloB/C bound to cellular SOCS-box proteins.[12]

At present, the structural basis of Vif's greater affinity for Cul5(N) in the presence of CBFβ is unknown. The increased affinity of Cul5(N) for Vif/EloB/C/CBFβ over Vif$_C$/EloB/C supports a prior hypothesis that CBFβ acts as a Vif "regulator" that promotes the affinity of Vif for Cul5 via conformational stabilization.[14,15,19,20] Our results further support this idea because CBFβ did not interact with Vif$_C$/EloB/C or Cul5(N) alone (Figure S2D,E, Supporting Information), in accord with prior co-immunoprecipitation data.[14] To probe the influence of CBFβ on the Cul5−Vif interaction, we measured ΔC_p for the binding of Cul5(N) to Vif$_C$/EloB/C and Vif/EloB/C/CBFβ (Figure 2 and Figure S3, Supporting Information). The ΔC_p values for the interaction of Cul5(N) with Vif$_C$/EloB/C and Vif/EloB/C/CBFβ were −0.30 ± 0.01 and −0.52 ± 0.02 kcal mol^{-1} K^{-1}, respectively. A negative ΔC_p can indicate a predominantly apolar interface, whereas a positive ΔC_p suggests a predominantly polar one (reviewed in refs 22 and 23). Our findings are consistent with the presence of conserved apolar residues in the Vif HCCH motif reported as being crucial for Cul5 binding and HIV-1 infectivity (ref 10 and Figure S4, Supporting Information). Notably, our results support a direct interaction of Vif residues with Cul5.

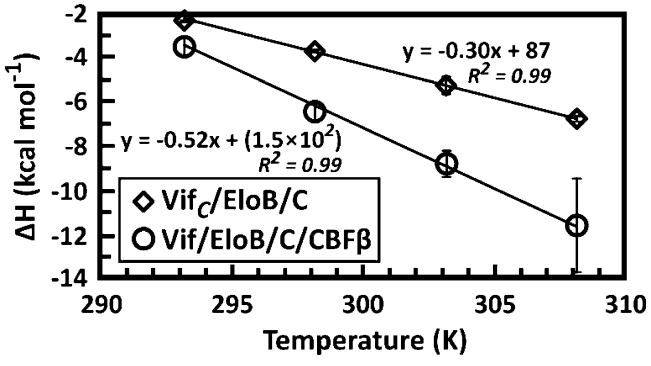

Figure 2. Heat capacity changes (ΔC_p) for the interaction of Cul5(N) with Vif$_C$/EloB/C and Vif/EloB/C/CBFβ taken as the slope of best-fit lines.

Several interpretations are possible for the nearly 2-fold difference in ΔC_p for the interaction of Cul5(N) with the respective ternary and quaternary Vif complexes in Table 1. Proton transfer effects were ruled out by conducting measurements in buffers with disparate deprotonation enthalpies, which revealed negligible ΔH changes (Table S1, Supporting Information). Other possibilities include ion transfer or protein conformational changes upon complex formation, which cannot be dismissed at present. Notably, large negative ΔC_p values, as in Table 1, correlate highly with burial of hydrophobic area.[24] As such, we used an empirical approach to estimate the size of the binding interfaces (Supporting Information). For the interaction of Cul5(N) with Vif$_C$/EloB/C, we calculated 20 residues in the interface with ∼2100 Å^2 buried (Table 1), typical of a heterodimeric interface.[25] These values may represent interfaces between Cul5 and the combined surface of Vif's HCCH motif and EloC.[11,12] By contrast, Cul5(N)'s interaction with Vif/EloB/C/CBFβ nearly doubles the number of buried residues to 37 with a buried area of ∼3600 Å^2 (Table 1). As a caveat, any values would be incorrectly estimated if protein conformational rearrangements accompany binding.

The absence of experimental structures for the Vif complexes in Table 1 leaves the location of the putative buried area an open question, especially beyond the well-studied HCCH motif. Possibilities include interactions of Cul5(N) with N-terminal regions of Vif, CBFβ, or both. While a direct interaction between Cul5 and CBFβ in the context of EloB/C/Vif/CBFβ cannot be ruled out, it is unprecedented in E3 ligases. By contrast, several conserved N-terminal residues of Vif (^{86}SIEW^{89}, T^{96}, A^{103}, and D^{104}) have been implicated in Cul5 binding,[5,26] but direct interactions have not been elucidated. Alternatively, the added buried area could arise from CBFβ's ability to prestabilize Vif's HCCH motif, making it more receptive to subsequent Cul5 binding. In either case, our data support a prior hypothesis that CBFβ upregulates Vif's interaction with Cul5,[20] which is akin to its role in promoting the binding of its α-subunit partner to DNA.[17]

Despite the fact that CBFβ is not required for the Cul5−Vif$_C$/EloB/C interaction, and that Cul5 and CBFβ bind to disparate regions of Vif (Figure 1B), our results demonstrate that CBFβ, and the N-terminal half of Vif, enhance the affinity of Cul5(N) for Vif. These factors nearly double the buried area for this host−virus interaction. Importantly, the increased

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buried area suggests a substantial region of the N-terminus of Vif, in addition to the HCCH motif, may become buried upon Cul5 binding. Whether this results from internal reorganization of Vif or a novel, direct protein interface remains to be seen. Overall, our results quantify Cul5 affinity and have implications for therapeutics designed to disrupt the Cul5−Vif interface.

■ ASSOCIATED CONTENT

Supporting Information

Supporting methods, Table S1, and Figures S1−S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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J.D.S. and G.M.L. contributed equally to this work.

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Notes

The authors declare no competing financial interest.

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