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Core-binding factor β (CBFβ) increases the affinity between human Cullin 5 and HIV-1 Vif within an E3 ligase complex

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Abstract

HIV-1 Vif masquerades as a receptor for a cellular E3 ligase harboring ElonginB, ElonginC, and Cullin5 (EloB/C/Cul5) proteins that facilitate degradation of the antiretroviral factor A3G. This Vif-mediated activity requires human 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 $Vif^{(95–192)}$/EloB/C (K_d = 327 ± 40 nM), which cannot bind CBFβ. A comparison of heat-capacity changes supports a model wherein CBFβ prestabilizes $Vif^{(1–192)}$ relative to $Vif^{(95–192)}$, consistent with a stronger Cul5 interaction with Vif's C-terminal Zn^{2+}-binding motif. An additional interface between Cul5 and an N-terminal region of Vif appears plausible, which has therapeutic-design implications.

Keywords

calorimetry; heat capacity; A3G; HIV-1 Vif; Cul5; EloB/C; CBFβ; buried interface; equilibrium K_d

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 (2)]. In Vif deficient HIV-1 infection, A3G incorporates 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 (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

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

essential for E3-ligase-mediated degradation of A3G, as well as viral infectivity (14, 15). The cellular role of CBFβ is posited to be allosteric stabilization of the DNA-bound form of its cognate α subunits, which form essential α/β transcription factors (16, 17). CBFβ has two isoforms (18) that co-immunoprecipitate Vif and support HIV-1 infectivity (19, 20).

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 human Cul5(N) binding 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 the Cul5(N) interaction 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 *E. 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 ternary and quaternary complexes (Figures S2A and S2B, Supporting Information).

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 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 on 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). Cul5(N) affinity in the presence of CBFβ was on par with the SOCS2$_{SOCS\text{-}box}$/EloB/C interaction (apparent K_d = 8 nM, Table 1 and Figure S2C, Supporting Information), which comprises the human SOCS2 SOCS-box (residues 158–198). The stoichiometry of Cul5(N) binding 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 Vif affinity for Cul5 via conformational stabilization (14, 15, 19, 20). Our results further support this idea since CBFβ did not interact with Vif$_{C}$/EloB/C or Cul5(N) alone (Figures S2D and S2E, 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 Cul5(N) binding to Vif$_{C}$/EloB/C and Vif/EloB/C/CBFβ, respectively (Figure 2 and S3, Supporting Information). ΔC_p for the interaction of Cul5(N) with Vif$_{C}$/EloB/C and Vif/EloB/C/CBFβ was −0.30 ± 0.01 and −0.52 ± 0.02 kcal K^{-1} mol^{-1}, respectively. $\Delta C_p < 0$ can indicate a predominantly apolar interface whereas a $C_p > 0$ suggests a predominantly polar one [reviewed in (22, 23)]. Our findings are consistent with the presence of conserved apolar residues in the Vif HCCH motif reported as crucial for Cul5 binding and HIV-1 infectivity [(10) and Figure S4, Supporting Information]. Notably, our results support a direct interaction of Vif residues with Cul5.

Several interpretations are possible for the near- ly 2-fold difference in ΔC_p for the Cul5(N) interac- tion 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 binding interfaces (Supporting Information). For the Cul5(N) in- teraction with Vif$_C$/EloB/C, we calculated 20 resi- dues in the interface with ~2,100 Å^2 buried (Table 1) – typical of a heterodimeric interface. 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 buried residues to 37 with a buried area of ~3,600 Å^2 (Table 1). As a caveat, any values would be mis-estimated if protein conformational rearrangements accompany binding.

The absence of experimental structures for the Vif complexes in Table 1 leaves the location of putative buried area an open question, especially beyond the well-studied HCCH motif. Possibilities include Cul5(N) interactions 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, 25), albeit direct interactions have not been shown. 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 up-regulates Vif's interaction with Cul5 (20), which is akin to its role in promoting α-subunit binding 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 buried area suggests a substantial region of the Vif N-terminus, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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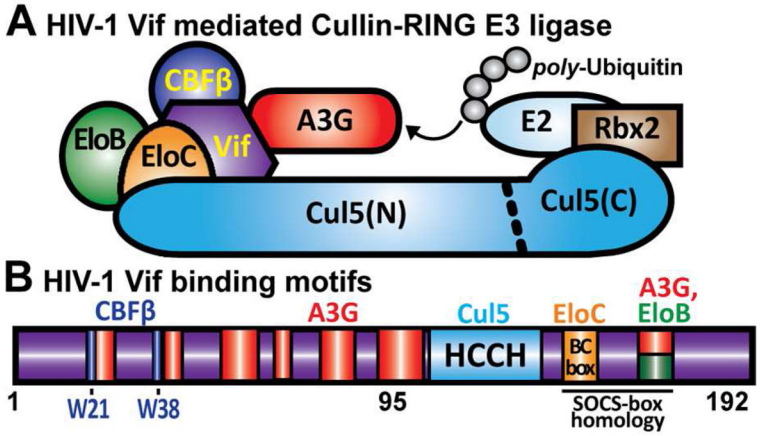

Figure 1.

Schematic of the Vif-mediated E3 ligase and Vif sequence motifs. (A) A3G is recruited by Vif to the N-terminus of Cullin 5 (herein called 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.

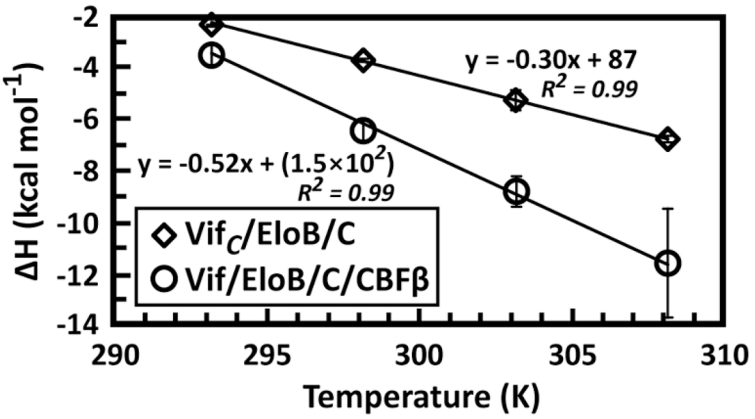

Figure 2.

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

Table 1

Average Thermodynamic Parameters at 303 K for Cul5(N) Binding to Vif/EloB/C/CBFβ

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	~2,100
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	~3,600
Cul5(N)	SOCS2$_{SOCS\text{-}box}$/EloB/C	−11.3 ± 0.1	−5.4 ± 0.2	−5.9 ± 0.0	7 ± 2	N/A	N/A	N/A

[a] The C values of 3,200 and 2,783, 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 (K) 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 C_p.