SIRT1 Contains N- and C-terminal Regions That Potentiate Deacetylase Activity*‡§

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Min Pan‡, Hua Yuan§, Michael Brent§, Emily Chen Ding‡, and Ronen Marmorstein‡§

From the ‡Wistar Institute and the §Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Background: The SIRT1 deacetylase contains a conserved catalytic core domain and variable flanking N- and C-terminal regions.

Results: We show that the SIRT1 N- and C-terminal regions potentiate catalytic activity of the central core domain through formation of an intramolecular holoenzyme.

Conclusion: These studies highlight the unique catalytic properties of SIRT1.

Significance: These studies have implications for the development of SIRT1-specific modulators.

SIRT1 is one of seven mammalian sirtuin (silent information regulator 2-related) proteins that harbor NAD\(^+\)-dependent protein deacetylase activity and is implicated in multiple metabolic and age-associated pathways and disorders. The sirtuin proteins contain a central region of high sequence conservation that is required for catalytic activity, but more variable N- and C-terminal regions have been proposed to mediate protein specific activities. Here we show that the conserved catalytic core domain of SIRT1 has very low catalytic activity toward several known protein substrates, but that regions N- and C-terminal to the catalytic core potentiate catalytic efficiency by between 12- and 45-fold, with the N-terminal domain contributing predominantly to catalytic rate, relatively independent of the nature of the acetyl-lysine protein substrate, and the C-terminal domain contributing significantly to the \(K_m\) for NAD\(^+\). We show that the N- and C-terminal regions stimulate SIRT1 deacetylase activity intramolecularly and that the C-terminal region stably associates with the catalytic core domain to form a SIRT1 holoenzyme. We also demonstrate that the C-terminal region of SIRT1 can influence the inhibitory activity of some sirtuin inhibitors that are known to function through the catalytic core domain. Together, these studies highlight the unique properties of the SIRT1 member of the sirtuin proteins and have implications for the development of SIRT1-specific regulatory molecules.

Sirtuins comprise a family of NAD\(^+\)-dependent protein deacetylase enzymes that catalyze the removal of an acetyl moiety from the \(\varepsilon\)-amino group of lysine residues within protein targets (1, 2) to yield the deacetylated protein product, nicotinamide, and 2'\(^{-}\)O-acetyl-ADP-ribose (3, 4). The founding member of this protein family, Saccharomyces cerevisiae Sir2p, one of five yeast sirtuin proteins including HST1–4, is a limiting factor in yeast aging, because deletion of the SIR2 gene results in reduced replicative lifespan (5), and additional copies of SIR2 result in increased yeast replicative lifespan (6). The sirtuin protein family is broadly conserved in all three domains of life (7), and increased sirtuin expression in higher eukaryotes has been reported to lead to increased lifespan in worms (8) and flies (9), and increased longevity caused by a calorie-restricted diet in some of these organisms is sirtuin-dependent (9). Humans have seven sirtuin proteins (SIRT1–7) (10), several of which have been shown to have distinct biological properties (11). The SIRT1 protein has been the most thoroughly studied of the human sirtuins and has been shown to play roles in many biological processes including cell survival, apoptosis, stress resistance, fat storage, insulin production, glucose homeostasis, and lipid homeostasis through direct deacetylation or regulation of its many known \textit{in vivo} targets including transcription factors such as p53, Forkhead box class O, and peroxisome proliferator-activated receptor \(\gamma\) and histones such as H3 (K9 and K14) and H4 (K16) (reviewed in Ref. 12). SIRT1 has also been implicated to play a role in a number of age-related human diseases including diabetes, cancer, and inflammation (reviewed in Ref. 13).

Sequence alignment of the sirtuin proteins indicates that they contain an \(\sim\)275-amino acid conserved catalytic core domain (7, 14) (Fig. 1A and supplemental Fig. S1). Consistent with the sequence conservation among the sirtuin proteins, the catalytic core domains of known sirtuin structures show a high degree of structural superposition (supplemental Fig. S1). Several x-ray crystal structures of sirtuin proteins from bacteria (CobB and Sir2Tm), yeast (Sir2 and Hst2), archaea (Sir2-Af1 and Sir2-Af2), and human (SIRT2, SIRT3, and SIRT5) in several different liganded forms reveal a structurally conserved catalytic core domain that adopts an elongated shape containing a large and structurally homologous Rossmann fold domain, characteristic of NAD\(^+\)/NADH-binding proteins; a more structurally diverse, smaller, zinc-binding domain; and several loops connecting the two domains (reviewed in Ref. 15). These loops form a pronounced, extended cleft between the large and small domains where the NAD\(^+\) and acetyl-lysine-containing peptide substrates enter from opposite sides and bind to the enzyme. The amino acids involved in catalysis and the reactive
groups of both bound substrate molecules are buried within a protein tunnel in the cleft between the two domains, the region of the enzyme that contains the highest sequence conservation within the sirtuin enzymes (supplemental Fig. S1).

In addition to the sequence and structural conservation of the catalytic core domain, many eukaryotic sirtuin proteins contain N- and C-terminal flanking regions that are variable in length and sequence and have been proposed to play protein-specific regulatory roles (Fig. 1A). For example, the N- and C-terminal segments of yeast Hst2 have been shown to mediate homotrimer formation that plays an autoinhibitory role in the deacetylase activity of Hst2 (16), whereas the N-terminal region was also shown to contain a nuclear export signal (17). Of the seven human sirtuin proteins, the 747-residue SIRT1 protein contains the most extended N- and C-terminal segments that flank a catalytic core domain within approximately residues 240–500, with the function of these N- and C-terminal segments unknown (Fig. 1A and supplemental Fig. S1).

In this study, we tested the hypothesis that, like yeast Hst2, the N- and C-terminal segments of SIRT1 play autoregulatory functions to modulate SIRT1 deacetylase activity. Consistent with this hypothesis, we find that a SIRT1 protein construct harboring the conserved catalytic core domain contains very low deacetylase activity, whereas the addition of N- and C-terminal segments of SIRT1 can potentiate the catalytic efficiency of the core region by between 12–45-fold, with the N-terminal domain contributing predominantly to catalytic rate and the C-terminal domain contributing significantly to the $K_m$ for NAD$^+$. We also show that the N- and C-terminal regions stimulate SIRT1 deacetylase activity intramolecularly to form a SIRT1 holoenzyme and that the C-terminal domain can influence the activity of SIRT1 inhibitors.

**EXPERIMENTAL PROCEDURES**

**Protein Purification**—A plasmid expressing full-length human SIRT1 was a generous gift from David Sinclair (Harvard Medical School). DNA fragments encoding various SIRT1 constructs (residues 214–583, 160–583, 160–665, and 584–665) were cloned into the first multiple cloning site of the pET-Duet-1 vector containing the gene for yeast SMT3, a ubiquitin-like protein of the SUMO family. BL21-CodonPlus (DE3 RIL) cells harboring the SIRT1 expression constructs were grown at 37 °C in LB medium and induced at log phase with 0.5 mM isopropyl $\beta$-D-thiogalactopyranoside at 18 °C overnight. The cells were harvested and lysed by sonication in a sonication buffer containing 50 mM Tris, pH 7.5, 200 mM NaCl, 5 mM imidazole, 10 mM $\beta$-mercaptoethanol and 5% glycerol with the addition of 0.1 mg/ml PMSF. The cell lysate was centrifuged to remove cell debris, and the protein supernatant was loaded onto a nickel-nitrilotriacetic acid-agarose column and washed extensively with sonication buffer supplemented with 30–600 mM imidazole gradient in sonication buffer. The His-SUMO tag was removed by incubating the SIRT1 protein with ULP1 (ubiqui-
Deacetylase Potentiating Regions of SIRT1

tin-like-specific protease 1) at 4 °C overnight and then passing the overnight incubation solution through a fresh nickel-nitro-
lotriacetic acid column equilibrated in sonication buffer plus 30
mM imidazole. The NaCl concentration of the collected flow
through was lowered to ~100 mM by diluting with sonication
buffer containing 50 mM NaCl and loading the solution on a
HiTrap Q ion exchange column. SIRT1 protein elution was
wrapped out with a gradient of 100–800 mM NaCl in sonication
buffer. Peak SIRT1 protein fractions were further purified on
Superdex-200 in a buffer containing 20 mM HEPES, pH 7.5, 100
mM NaCl, 5% glycerol, and 10 mM DTT to yield >90% pure
SIRT1 protein (as judged by SDS-PAGE). SIRT1 protein was
concentrated to ~10 mg/ml using a microconcentrator and
either used immediately or stored at -80 °C after flash freezing
prior to use. A 6-liter purification typically yielded 1–3 mg of
purified protein depending on the specific SIRT1 protein
construct.

Deacetylase Assay—The steady state parameters and cata-
ytic efficiency of deacetylase activity of SIRT1 protein con-
structs were determined using a radioactive assay that employs
\[^{14}C\]NAD\(^+\), labeled on the carbonyl group of the nicotinamide
moiety. This assay measures the SIRT1-dependent hydrolysis
of the \[^{14}C\]nicotinamide group, which is isolated from unre-
acted \[^{14}C\]NAD\(^+\) by extraction using ethyl acetate and quanti-
ted by scintillation counting as described elsewhere (2). The
peptide substrates used in this study were purchased from Gen-
script and were centered around the SIRT1 targets
H3(4–14)K9Ac (QTARKK(Ac)STGGK), H4(7–25)K16Ac
(GKGLGKGGAK(Ac)HRHKVLDRN), and p53(372–389)–
K382Ac (KKGQTSRSHKK(Ac)LMFKTEG). Radioactive assays
were carried out for 15 min at 30 °C (in the linear range of enzyme
activity) in a total reaction volume of 100 μl in a buffer contain-
ing 50 mM glycine, pH 9.0, 0.5 mM DTT, 5 mM tetrasodium pyrophos-
phate, and 0.1 mg/ml BSA. The reaction also contained 100 mM
protein, a saturating concentration of NAD\(^+\) of 2 mM (or near
saturating for the CC and N-CC SIRT1 constructs that have rela-
tively high \(K_m\) values for NAD\(^+\)) while varying peptide concentra-
tions (0–500 μM) (see Table 1) or saturating peptide substrate (500
μM) with varying NAD\(^+\) concentrations (0–800 μM) (see Table 2).
The reactions were quenched by adding 67 μl of 0.1 M sodium
borate, pH 8.0, into the reaction buffer before the cleaved \[^{14}C\]ni-
ocotinamide product of the reaction was extracted with ethyl ace-
tate and quantified by scintillation counting. Background control
reactions were performed in the absence of enzyme. All of the
reactions were performed in triplicate, and the data were directly
fit to the Michaelis-Menten equation in GraphPad Prism.

IC\(_{50}\) values for SIRT1 inhibitors (Ex-527, suramin and Ro
31-8220) were determined at room temperature in a 100-μl
reaction volume in the presence of 100 nM SIRT1 protein, 300
μM \[^{14}C\]NAD\(^+\), 1 nM of H3(4–14)K9Ac peptide substrate, and
varying concentrations of the respective inhibitors (76 nM to
167 μM of Ex-527, 6–110 μM of suramin, and 0.1–1000 μM of
Ro 31×8220 stored in Me₂SO) in the same reaction buffer
noted above, and the reaction was quenched and analyzed as
noted above. The IC\(_{50}\) value of nicotinamide was determined
using the Fluor-de-Lys SIRT1 fluorometric drug discovery
assay kit (Enzo Life Sciences) according to the manual. All of
the reactions were performed in triplicate, and the data were fit
to a sigmoidal curve using GraphPad Prism.

Equilibrium Sedimentation—Analytical ultracentrifugation
of the SIRT1(160–665) construct was performed at 4 °C using
absorbance optics with a Beckman Optima XL-1 analytical
ultracentrifuge, using a four-hole rotor. The partial specific vol-
ume, buffer density, and viscosity were estimated using Sden-
terp (18). Analysis was performed using six-channel center-
pieces with quartz windows, spinning at 18,000, 22,000, and
26,000 rpm and at protein concentrations of 1.3, 0.9, and 0.6
mg/ml in 20 mM HEPES, pH 7.5, 100 mM NaCl, 5% glycerol
(v/v), and 5 mM β-mercaptoethanol. A global fit of the data,
using the program Heteroanalysis, fit best to a single species
SIRT1(160–665) monomer. The quality of the fit was assessed
from examination of root mean square deviation.

RESULTS

Catalytic Core Domain of SIRT1 Is Significantly Less Active
than Extended SIRT1 Protein Constructs—We successfully pre-
pared recombinant full-length SIRT1 protein in bacteria but
found that the protein was highly prone to aggregation and
degradation and that its catalytic activity was highly variable
(data not shown). To prepare a more stable and catalytically
active SIRT1 protein construct, we used a sequence alignment
of sirtuin proteins to aid in the design of SIRT1 protein con-
structs containing the catalytically active region (supplemental
Fig. S1). This alignment suggested that the catalytic core
domain of SIRT1 mapped to within residues 240–510. The
preparation of several different SIRT1 protein constructs con-
taining this region resulted in the identification of three SIRT1
protein constructs that could be expressed to high levels in
bacteria and purified to >95% homogeneity and isolated as a
monodisperse, nonaggregated species on gel filtration chroma-
tography. These protein constructs were SIRT1(214–583)
(herin called SIRT1-CC for containing the catalytic core
domain), SIRT1(160–583) (herin called SIRT1-N-CC for con-
taining the catalytic core domain plus an extended N-terminal
region), and SIRT1(160–665) (herin called SIRT1-N-CC-C
for containing the catalytic core domain plus extended N- and
C-terminal regions).

The deacetylase activity of these three SIRT1 protein con-
structs were analyzed using saturation kinetics to determine
steady state parameters (\(k_{cat}\) and \(K_m\)) and catalytic efficiency
(\(k_{cat}/K_m\)). For these initial studies, varying concentrations of a
peptide substrate centered around histone H3(4–14)K9Ac was
used with saturating levels of NAD\(^+\). These studies revealed
that the shortest of the SIRT1 protein constructs harboring the
catalytic core domain, SIRT1-CC, exhibits steady state param-
eters of \(K_m = 132.6 \mu M, k_{cat} = 3.4 \text{ min}^{-1}\) and a catalytic effi-
ciency of 25,900 min\(^{-1}\) M\(^{-1}\) (Fig. 2A). Surprisingly, the SIRT1
protein constructs that contained extended N- and C-terminal
segments showed significantly greater activity with SIRT1-
N-CC and SIRT1-N-CC-C constructs exhibiting catalytic effici-
ciencies of 313,000 min\(^{-1}\) M\(^{-1}\) and 648,000 min\(^{-1}\) M\(^{-1}\), respec-
tively (Fig. 2). This elevation in catalytic efficiency of ~12-fold
for the N-terminally extended protein construct and of ~25-
fold for the N- and C-terminally extended construct suggests
that regions N- and C-terminal to the conserved catalytic core
The activity of SIRT1-N-CC in the C-terminal region, SIRT1(584–665) (SIRT1-C) to potentiate the ability of a SIRT1 fragment containing the isolated extended domain of the catalytic core domain by directly binding to the catalytic—To determine Activity by an Intramolecular Mechanism

Deacetylase activity of SIRT1 protein constructs against an H3K9Ac-containing substrate. A, saturation kinetics is shown by varying H3(4–14)K9Ac substrate concentration while keeping NAD+ at a saturating concentration for SIRT1-CC (residues 214–583), SIRT1-N-CC (residues 160–583), and SIRT1-N-CC-C (residues 160–665). All of the measurements were carried out in triplicate. B, comparison of catalytic efficiency (kcat/Km) of SIRT1 protein constructs is shown in bar graph form.

domain of SIRT1 potentiate deacetylase activity on a histone H3(4–14)K9Ac-containing substrate.

N- and C-terminal Regions of SIRT1 Potentiate Deacetylase Activity by an Intramolecular Mechanism—To determine whether the C-terminal region of SIRT1 potentiates the activity of the catalytic core domain by directly binding to the catalytic core domain or through some indirect mechanism, we tested the ability of a SIRT1 fragment containing the isolated extended C-terminal region, SIRT1(584–665) (SIRT1-C) to potentiate the activity of SIRT1-N-CC in trans. As can be seen in Fig. 3 and Tables 1 and 2, a 10-fold molar excess of SIRT1-C added to SIRT1-N-CC increases the catalytic efficiency of this domain by ~1.5–5-fold, depending on the nature of the substrate, and very close to the activity of the intact SIRT1-N-CC-C protein construct. Importantly, the addition of a 10-fold molar excess of SIRT1-C to SIRT1-N-CC-C did not further increase the activity of SIRT1-N-CC-C (Fig. 3, A and B). Taken together, these data are consistent with a direct interaction between the catalytic core domain of SIRT1 with the C-terminal segment and possibly also the N-terminal segment to potentiate the catalytic activity of SIRT1.

Because the data described above is consistent with an interaction of the N- and C-terminal segments of SIRT1 with the catalytic core domain to potentiate catalytic activity through the same or possibly another molecule of SIRT1 (for example through dimer formation), we subjected SIRT1-N-CC-C to sedimentation equilibrium studies using analytical ultracentrifugation to establish the oligomerization state of SIRT1. We carried out these experiments at three protein concentrations (1.3, 0.9, and 0.6 mg/ml) and three centrifugation speeds (18,000, 22,000, and 26,000 rpm) (Fig. 3C). A global analysis of this data fit extremely well to a single species monomer model with a molecular mass of 53.3 kDa (actual mass = 57.0 kDa) and root mean square deviation of 0.0105 Abs280. Taken together, these data suggest that the N- and C-terminal segments of SIRT1 potentiate catalytic activity by an intramolecular mechanism.

The Degree of Catalytic Potentiation by the N- and C-terminal Segments of SIRT1 Is Independent of Acetyl-lysine Protein Substrate—We were curious to determine whether the SIRT1 catalytic potentiation observed against the H3(4–14)K9Ac peptide substrate might also be observed for other SIRT1 substrates. To address this, we assayed the SIRT1 constructs SIRT1-CC, SIRT1-N-CC, and SIRT1-N-CC-C against peptides containing H4(7–25)K16Ac and p53(372–389)K382Ac, two other known SIRT1 substrates. As can be seen in Fig. 4A, deacetylation of the H4(7–25)K16Ac substrate shows ~6.7- and ~14.4-fold enhancements in catalytic efficiency of the SIRT1-N-CC and SIRT1-N-CC-C constructs relative to the SIRT1-CC construct, respectively. This is comparable with the ~12.1- and 25-fold enhancement that was observed for the corresponding protein constructs against the H3(4–14)K9Ac substrate (Table 1). Similar levels of potentiation by N- and C-terminal regions was also observed on p53(372–389)K382Ac substrate (9.6- and 11.8-fold, respectively). Taken together, these studies reveal that catalytic potentiation by the N- and C-terminal segments that flank the SIRT1 catalytic core domain is relatively independent of the nature of the acetyl-lysine protein substrate and therefore likely does not participate in protein specific acetyl-lysine recognition.

N- and C-terminal Regions of SIRT1 Potentiate Deacetylase Activity through Distinct Mechanism—An analysis of the activity of SIRT1 constructs on several acetyl-lysine-containing protein substrates clearly shows that the potentiating effects of the N- and C-terminal segments on SIRT1 catalysis is largely driven by an enhancement of kcat of ~6–8-fold for the N-terminal segment and of another ~2–3-fold for the C-terminal segment, with relative minor effects on Km for acetyl-lysine substrate binding (Table 1). To explore whether the N- and/or C-terminal segments of SIRT1 contributed to the Km for NAD+ catalysis, we carried out similar saturation kinetics under conditions of saturating acetyl-lysine substrate peptide and varying concentrations of NAD+ substrate (Fig. 5). We employed both the H4(7–25)K16Ac and p53(372–389)K382Ac substrates for these studies. We observed that the catalytic efficiency is enhanced ~5.7- and ~44.8-fold for SIRT1-N-CC and SIRT1-N-CC-C relative to SIRT1-CC, respectively, against the H4(7–25)K16Ac substrate and similarly ~4.6- and ~26.7-fold, respectively, against the p53(372–389)K382Ac substrate. Interestingly, this degree of catalytic potentiation is ~3-fold higher than that observed when varying the acetyl-lysine protein substrate (compare Tables 1 and 2). A comparison of the steady state parameters reveals that this increase in catalytic potentiation on the NAD+ substrate over the acetyl-lysine protein substrate is largely due to a 3–5-fold increase in the Km for NAD+ of the SIRT1-N-CC construct relative to SIRT1-N-CC-C. This observation suggests that the C-terminal segment of SIRT1 also contributes to NAD+ binding for catalytic potentiation. We note that in the absence of the SIRT1 C-terminal segment, relatively high Km values for NAD+ are obtained for SIRT1-CC.
and SIRT1-N-CC (489–888 μM) so that 2 mM NAD$^+$ is at a
subsaturating concentration for these protein constructs. This
suggests that the apparent $k_{cat}$ values obtained for these protein
constructs at 2 mM NAD$^+$ (Table 1) are marginally smaller than
the actual values (because the enzymes only have 70–80% of
their maximal activity), and therefore the reported potentiation
by the C-terminal segment reported in Table 1 is a slight over-
estimate. Taking the data together, the extended N- and C-ter-
minal segments of SIRT1 both contribute to potentiating the
catalytic efficiency of the SIRT1 catalytic domain. The N- and
C-terminal segments contribute to an elevation in $k_{cat}$, with the
N-terminal segment playing a significant role. In contrast, the
C-terminal segment of SIRT1 plays an additional role in lowing
the $K_m$ for NAD$^+$.

**N- and C-terminal Segments of SIRT1 Can Influence SIRT1 Inhibitor Potency**—Several sirtuin inhibitors have been
described in the literature (reviewed in Ref. 19) including sura-
min (20) and EX-527 (21), the most potent SIRT1 inhibitor with
an IC$_{50}$ value in the mid nanomolar range. Less potent sirtuin
inhibitors, such as Ro 31-8220, an adenosine mimic (22), and
nicotinamide, a product and noncompetitive inhibitor of sir-
tuins (23, 24), have also been described. Although the structure
of suramin bound to SIRT5 suggests that the inhibitor binds
competitively with both NAD$^+$ and acetyl-lysine to the cata-

![FIGURE 3. Stimulation of SIRT1 deacetylase activity in trans.](https://example.com/figure3.png)

**TABLE 1**

Summary of steady state parameters and catalytic efficiency of SIRT1 protein constructs on acetylated peptide substrates

| Peptide substrate | SIRT1 construct | $k_{cat}$ | $K_m$ | $k_{cat}/K_m$ | Relative $k_{cat}/K_m$
|-------------------|-----------------|----------|-------|--------------|---------------------|
| H3(4–14) K9Ac     | CC$^a$          | 3.4 ± 0.1| 132.6 ± 9.0 | 25.900 | 1               |
|                   | N-CC$^a$       | 26.7 ± 0.5| 85.3 ± 5.8  | 313,000| 12.1             |
|                   | N-CC + C       | 69.6 ± 1.5| 111.8 ± 7.1 | 623,000| 24.0             |
|                   | N-CC-C         | 64.9 ± 2.4| 100.2 ± 11.4| 648,000| 25.0             |
|                   | N-CC-C + C     | 67.1 ± 2.0| 80.5 ± 8.2  | 833,000| 32.2             |
| H4(7–25) K16Ac    | CC$^a$          | 2.5 ± 0.1| 54.5 ± 4.4  | 45,000 | 1                |
|                   | N-CC$^a$       | 19.2 ± 0.6| 63.2 ± 7.3  | 304,000| 6.7              |
|                   | N-CC + C       | 64.2 ± 1.9| 101.6 ± 9.1 | 632,000| 14.0             |
|                   | N-CC-C         | 64.9 ± 2.4| 100.1 ± 11.4| 648,000| 14.4             |
| p53(372–389) K382Ac | CC$^a$        | 4.7 ± 0.1| 69.1 ± 3.4  | 67,700 | 1                |
|                   | N-CC$^a$       | 26.8 ± 0.6| 41.1 ± 4.0  | 653,000| 9.6              |
|                   | N-CC + C       | 43.1 ± 0.8| 43.9 ± 3.6  | 981,000| 14.5             |
|                   | N-CC-C         | 66.8 ± 2.0| 83.6 ± 8.1  | 799,000| 11.8             |

$^a$k$_{cat}$ and $K_m$ for SIRT1-CC and SIRT1-N-CC are apparent values because the NAD$^+$ concentration is subsaturating.
lytic domain (25), docking studies with Ex-527 suggests that it
binds competitively with only NAD$^+$ (26). Ro 31-8220 and nic-
otinominate are also proposed to bind parts of the conserved
NAD$^+$ binding pocket. We were curious to determine whether
the N- and C-terminal segments of SIRT1 could influence the
potency of sirtuin inhibitors. To do this, we assayed the four
inhibitors suramin, Ex-527, Ro 31-8220, and nicotinamide
against the SIRT1-N-CC and SIRT1-N-CC-C protein con-
structs using the H3(4–14)K9Ac peptide substrate. Because of
the low activity of the SIRT1-CC construct, we could not obtain
reliable inhibition data using this protein construct. The IC$_{50}$
curves were generated for all inhibitors revealing that although
Ex-527, Ro 31-8220 and nicotinamide inhibited with compara-
tible IC$_{50}$ values for both the SIRT1-N-CC and SIRT1-N-CC-C
protein constructs (Fig. 6), suramin showed more than a 10-fold
greater potency against SIRT1-N-CC (IC$_{50}$ = 0.52 $\mu M$) relative
to SIRT1-N-CC-C (IC$_{50}$ = 7.8 $\mu M$) (Fig. 6). These results dem-
onstrate that the N- and C-terminal segments of SIRT1 can
have an influence on the potency of some sirtuin inhibitors.

**DISCUSSION**

Here we show that the SIRT1 protein deacetylase contains
nonconserved N- and C-terminal segments that potentiate the
catalytic activity of a central conserved catalytic region. The
degree of potentiation is relatively independent of the nature of
the acetyl-lysine-containing protein substrate, with the N-ter-
minal segment contributing predominantly to the catalytic rate
and the C-terminal domain contributing significantly to the
K$_{m}$ for NAD$^+$. The contributions of the N- and C-terminal seg-
ments of the SIRT1 catalytic core domain is schematized in Fig.
7. We also show that the N- and C-terminal segments interact with
the catalytic core domain through an intramolecular mechanism
to form a SIRT1 holoenzyme and that they can influence the inhib-
itory activity of some sirtuin inhibitors that are known to function
through the catalytic core domain. The observation that the sura-
min sirtuin inhibitor has a higher IC$_{50}$ value for SIRT1-N-CC-C
over SIRT1-N-CC (by more than 10-fold) further suggests that the
C-terminal segment of SIRT1 might bind along part of the inter-
face that suramin makes with sirtuins (25).

These studies demonstrate that the SIRT1-N-CC and SIRT1-
N-CC-C constructs show a range of potentiation between ~12-
and 45-fold relative to SIRT1-CC, respectively, which suggests
that either the N-terminal region plays a more important role in
potentiation or that the N- and C-terminal regions have a coop-
erative effect on potentiation. We were unfortunately not able
to prepare a soluble SIRT1 construct to test between these two
possibilities, although the fact that we were not able to prepare
a stable SIRT1-CC-C construct does suggest that the C-termi-
nal segment requires the N-terminal segment for protein sta-
Deacetylase Potentiating Regions of SIRT1

A sequence alignment of the N- and C-terminal catalytic potentiating regions of SIRT1 orthologs shows a high degree of conservation between residues 188–228 at the N terminus and residues 621–664 in the C terminus (Fig. 1, B and C), implicating these specific regions to be important for catalytic potentiation. Interestingly, secondary structure predictions of these regions suggest that although the N-terminal segment is highly helical, the C-terminal region is largely unstructured. It is possible that the C-terminal segment might adopt structure when bound to the central catalytic core domain and/or the N-terminal segment. In this study, we have shown that the C-terminal segment can potentiate the activity of the SIRT1-N-CC construct in trans. It would be interesting to determine whether isolated peptides containing these conserved segments might function as modulators (activators or inhibitors) of SIRT1 activity. If this was the case, then small molecule mimics of these regions might be particularly selective SIRT1 modulators.

FIGURE 5. Deacetylase activity of SIRT1 protein constructs at saturating peptide concentration with varying concentrations of NAD+. A, kinetics is shown for SIRT1 protein constructs against the H4(7–25)K16Ac peptide substrate along with a comparison of the catalytic efficiencies. B, same as A except that the p53(372–389)K382Ac substrate was used. All of the measurements were carried out in triplicate.

FIGURE 6. Potency of SIRT1 inhibitors toward different SIRT1 constructs. A, IC50 curve for the suramin SIRT1 inhibitor against the SIRT1-N-CC and SIRT1-N-CC-C protein constructs. B–D are the same as A except that the Ex-527, Ro 31-8220, and nicotinamide inhibitors were used, respectively. All of the measurements were carried out in triplicate.
Because of the association of SIRT1 with several human diseases, there has been considerable effort in developing small molecule sirtuin modulators. Toward this end, several compounds have also been identified and characterized as small molecule sirtuin inhibitors. In addition to suramin (27), Ro 31-8220 (22), and Ex-527 and its analogs (28), the compounds sirtinol (29), splitomycin (30), cambinol (31), tenovin (32), and surfactin (33) have also been described as sirtuin inhibitors. It is not clear where most of these compounds bind to the sirtuin enzymes or how they exert their inhibitory effect, although the observation that several of these inhibitors have different potencies against different members of the sirtuin family suggest that they do not exclusively target the conserved catalytic core domain. There have also been reports of sirtuin activators including resveratrol (27) and a family of compounds unrelated to resveratrol (34), although several other reports suggest that the SIRT1 activation observed for these compounds is an artifact of the assay method used (28, 35–37). The studies presented here suggest new avenues for the development of SIRT1-specific modulators that take advantage of the SIRT1-specific N- and C-terminal catalytic potentiating segments. In addition to preparing molecules that might inhibit the ability of the N- and C-terminal segments to potentiate catalytic activity, it may be possible to design molecules to promote this potentiation to serve as SIRT1-specific activating compounds. For example, molecules that disrupt the interaction of the C-terminal SIRT1(584–665) segment with the conserved catalytic core domain might inhibit SIRT1 activity, and molecules that promote this interaction might serve as SIRT1 activators.

Other sirtuin proteins, such as yeast Hst2 have also been shown to employ nonconserved N- and C-terminal segments to modulate catalytic activity (16). It is therefore possible that the N- and C-terminal segments of other sirtuins might play a particularly important and specifying role in modulating sirtuin-specific function and an understanding of the molecular basis for this in other sirtuin proteins might lead to other sirtuin-specific modulators that might have therapeutic applications.

**FIGURE 7.** Model for SIRT1 potentiation of deacetylation activity by the N- and C-terminal segments. The SIRT1-CC and N- and C-terminal segments are color-coded as indicated with additional SIRT1 regions colored in gray. The model illustrates that the N- and C-terminal segments are integral components of the SIRT1 holoenzyme with the N-terminal segment contributing significantly to $k_{cat}$ and the C-terminal segment contributing significantly to $K_m$ for NAD$^+$. 

**Deacetylase Potentiating Regions of SIRT1**

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