

SIRT2 Reverses 4-Oxononanoyl Lysine Modification on Histones

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S Supporting Information

ABSTRACT: Post-translational modifications (PTMs) regulate numerous proteins and are important for many biological processes. Lysine 4-oxonanoylation (4-ONylation) is a newly discovered histone PTM that prevents nucleosome assembly under oxidative stress. Whether there are cellular enzymes that remove 4-ONyl from histones remains unknown, which hampers the further investigation of the cellular function of this PTM. Here, we report that mammalian SIRT2 can remove 4-ONyl from histones and other proteins in live cells. A crystal structure of SIRT2 in complex with a 4-ONyl peptide reveals a lone pair- π interaction between Phe119 and the ketone oxygen of the 4-ONyl group. This is the first time that a mechanism to reverse 4-ONyl lysine modification is reported and will help to understand the role of SIRT2 in oxidative stress responses and the function of 4-ONylation.

Protein post-translational modifications (PTMs) play crucial roles in regulating a wide range of biological processes, such as gene transcription, DNA replication, and chromosome segregation.¹ Histones can be modified by several reversible PTMs, such as acetylation, methylation, phosphorylation, and ubiquitination. Recently, a new acyl modification, 4-oxonanoyl (4-ONyl) lysine, by 4-oxo-2-nonenal (4-ONE), is reported to occur on histone H3 (Figure 1A).² The 4-ketoamide adduct has polar ketone oxygen that is different from other short chain fatty acyl groups. 4-ONE can be generated by the oxidation of cellular lipids upon stress and react with histone lysines.² This 4-ketoamide adduct at H3K27 and H3K23 can be stimulated by lipopolysaccharides, disrupt chromatin dynamics, and alter histone turnover.² However, no eraser has been known to remove this 4-ONyl lysine modification on histones.

Sirtuins, the class III histone deacetylases (HDACs), are enzymes with nicotinamide adenine dinucleotide (NAD)-dependent deacetylation activities.^{3–5} Mammalian sirtuins (SIRT1–7) display widespread subcellular localizations. SIRT1, SIRT6, and SIRT7 are predominantly nuclear; SIRT2 is mainly in cytoplasm whereas SIRT3–5 are mainly in mitochondria.⁶ Interestingly, SIRT2 was also found to deacetylate H3K18 upon bacterial infection or deacetylate H4K16 in the G2/M transition to ensure chromatin

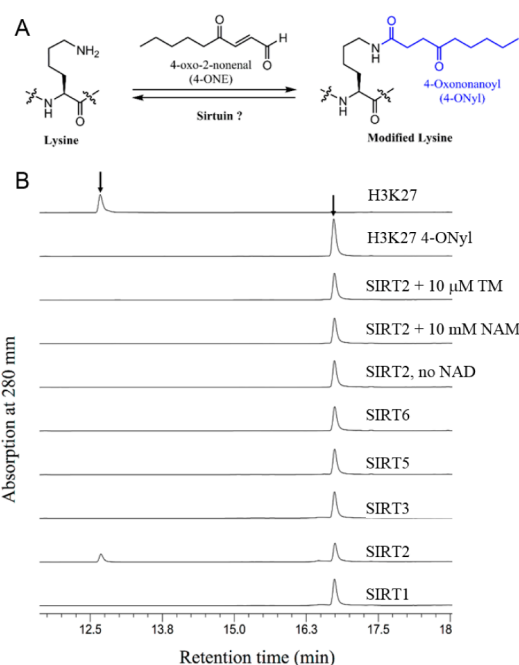


Figure 1. SIRT2 can hydrolyze 4-ONyl modification on lysine. (A) 4-ONyl modification on protein lysine residues. (B) HPLC traces showing 4-ONyl H3K27 can be hydrolyzed by SIRT2, but not other sirtuins.

condensation.^{7,8} Emerging evidence demonstrate sirtuins as deacylases that remove a variety of acyl groups on lysine. SIRT1–3 can depropionylate and debutyrylate.^{9,10} SIRT3 is reported to have decrotonylation activity and SIRT4 is reported to act as a lipoamidase regulating pyruvate dehydrogenase complex activity.^{11,12} SIRT5 can hydrolyze succinyl, malonyl and glutaryl lysines.^{13,14} SIRT6 can efficiently remove long chain fatty acyl groups on TNF α .¹⁵ Interestingly, biochemical studies also defined SIRT2 as demyristoylase.^{16,17} These findings broaden the substrates scope of sirtuins as deacylases.

Because the 4-ONyl modification is similar to fatty acyl groups and sirtuins can act as defatty-acylase,¹⁸ we wonder whether sirtuins are capable of hydrolyzing 4-ONyl lysine and

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Table 1. Kinetic Parameters on 4-ONyl Lysine Peptides

SIRT2	acyl lysine peptide	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($\text{s}^{-1} \text{M}^{-1}$)
WT	H3K27 4-ONyl ^a	0.0248 ± 0.0013	48 ± 9	522
	H3K27 acetyl ^a	0.0538 ± 0.0024	38 ± 7	1400
	H3K23 4-ONyl ^b	0.0121 ± 0.0010	12 ± 6	1010
	H3K23 acetyl ^b	0.0644 ± 0.0026	21 ± 4	3130
F119A	H3K27 4-ONyl ^a	0.0133 ± 0.0019	104 ± 22	127
	H3K27 (acetyl) ^a	0.0089 ± 0.0003	55 ± 9	162
	H3K23 4-ONyl ^b	0.0134 ± 0.0019	124 ± 5	108
	H3K23 (acetyl) ^b	0.0249 ± 0.0011	55 ± 8	452

^aPeptide sequence: TKAARK*SAPATWW. ^bPeptide sequence: KQLATK*AARKSWW.

regulate the change of the 4-ONylation level on endogenous histones. To investigate the hypothesis, we screened the activities of several sirtuins on H3K27 4-ONyl peptide. The results demonstrated that with SIRT2, but not other sirtuins, can hydrolyze H3K27 4-ONyl (Figure 1B). The activity of SIRT2 on H3K27 4-ONyl was NAD-dependent (Figure 1B). Nicotinamide (NAM, a pan-sirtuin inhibitor) or the TM molecule¹⁹ (a SIRT2-specific inhibitor), inhibited the hydrolysis of H3K27 4-ONyl (Figure 1B). We also measured the kinetics of SIRT2 on H3K27 4-ONyl and acetyl peptides. The catalytic efficiency of SIRT2 on H3K27 4-ONyl was comparable to that of deacetylation (Table 1). Similarly, SIRT2 could catalyze the hydrolysis of H3K23 4-ONyl and acetyl with similar efficiencies (Table 1). SIRT2 also shows the similar K_d values for 4-ONyl and acetyl H3K27 and H3K23 peptides (Figure S3).

To find out more about how SIRT2 recognizes the 4-ONyl group, we obtained the crystals of SIRT2 with both H3K27 4-ONyl and H3K23 4-ONyl peptides. A crystal of SIRT2 in complexes with the H3K23 (4-ONyl) and carba-NAD diffracted to 2.0 Å resolution after optimization. Crystallographic data collection statistics are shown in Table S1. The asymmetric unit consisted of 2 molecules, each containing one SIRT2-H3K23 4-ONyl in complex with carba-NAD (Figure 2A). The two globular domains of SIRT2 composed of an NAD-binding Rossmann fold and a zinc-binding motif are similar to other sirtuins (Figure 2A).^{13,15,20} The H3K23 4-ONyl bound to SIRT2 in a way similar to that of H3K9 myristoyl binding to SIRT2 (PDB 4R8M) (Figure S2).¹⁶ The 4-ONyl group was accommodated in a pocket of SIRT2 formed by hydrophobic residues Phe96, Phe119, Phe131, Thr134, Tyr139, Phe190, and Ile232 (Figure 2B). The catalytic His187 interacted with the oxygen of amide bond (Figure 2B). Interestingly, the benzene ring of Phe119 interacted with the ketone oxygen of 4-ONyl group. The distance d between the carbonyl oxygen atom and the phenyl ring-centroid was 3.4 Å and the dihedral angle ω between the planes defined by the O=CRR' moiety and the aromatic ring was 60.5°, indicating a lone pair- π interaction with a standard distance and angle constraints (Figure 2C,D).^{21,22} Mutation of this Phe to Ala (F119A) significantly decreased the activity of SIRT2 on 4-ONyl and acetyl peptides (Table 1).

Next, we investigated whether SIRT2 could remove 4-ONyl group from histones in cells. Mouse embryonic fibroblast (MEF) cells were used for all the cellular experiments. SIRT2 was detected on extracted histones (Figure 3B), indicating that SIRT2 interacts with histones. Interestingly, treating cells with increasing concentrations of 4-ONE, the end product of lipid peroxidation,^{2,23} led to increased SIRT2 on histones (Figure 3B). To monitor the changes of protein 4-ONylation levels in

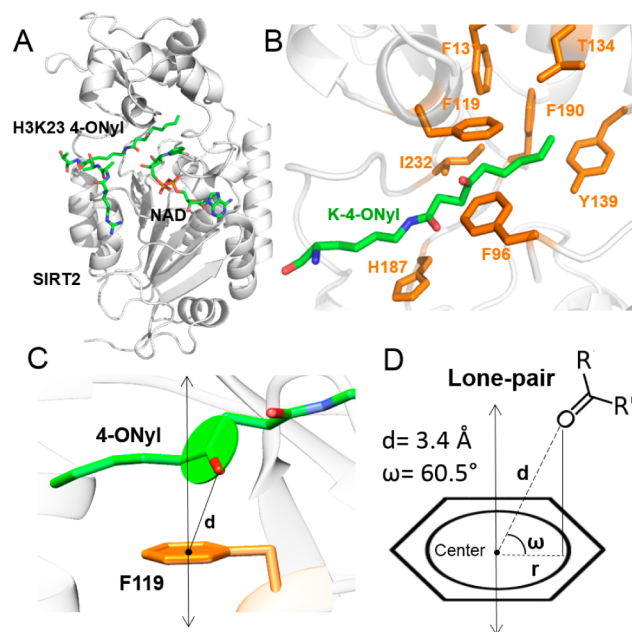


Figure 2. Structural basis for recognition of 4-ONyl lysine by SIRT2. (A) Overall structure of SIRT2 (gray) in complex with H3K23 4-ONyl peptide and carba-NAD (green). (B) The binding pocket formed by hydrophobic residues (orange). (C) A side view of the lone pair- π interaction between Phe119 and the 4-ONyl group. (D) Scheme showing the distance (d) and dihedral angle ω between the ketone oxygen and the phenyl ring of Phe119.

cells, a chemical reporter, alkynyl-4-ONE (a4-ONE), was used to label the Lys-4-ONylated proteins.² Histones were then extracted and subjected to an azide-alkyne click chemistry to conjugate the alkynyl-4-ONE labeled proteins with a rhodamine dye (Figure 3A and Figure S4). The alkynyl-4-ONE could label histones in a dose dependent manner (Figure S1). In contrast, histone acetylation level showed no obvious changes upon 4-ONE treatment (Figure 3B). Treating cells with nicotinamide and TM led to the increase of alkynyl-4-ONE labeling on histones (Figure 3C). The potent and SIRT2-specific inhibitor, TM, led to much stronger labeling, suggesting that SIRT2 regulates lysine 4-ONylation in cells. To confirm this further, we carried out the labeling experiments in control and SIRT2 knockdown (KD) MEF cells. As shown in Figure 3D, the labeling in SIRT2 KD cells was stronger than that in control cells, suggesting the reduction of SIRT2 resulted in the accumulation of the 4-ONylation on histones and nonhistone proteins. To make sure that SIRT2 can remove directly the 4-ONylation on these proteins, we added purified recombinant SIRT2 into histones extracted from SIRT2 KD cells that were

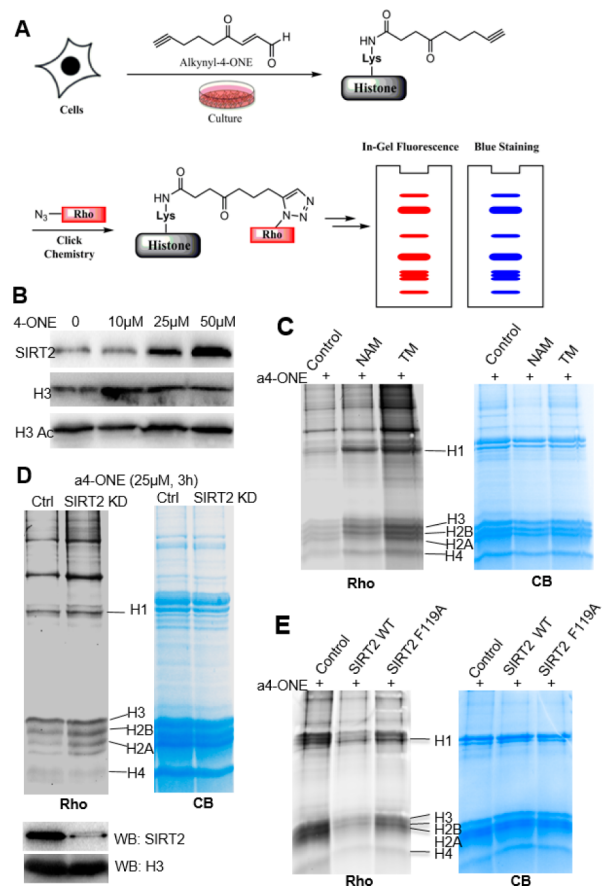


Figure 3. SIRT2 regulates alkynyl-4-ONyl level on histones. (A) A scheme of showing the use of a chemical reporter, alkynyl-4-ONE, to detect histone 4-ONylation. (B) Western blot (WB) showing increased SIRT2 and the similar acetyl level on H3 (H3 Ac) in extracted histones upon 4-ONE treatment (10, 25, and 50 μ M for 3 h). H3 was used as a loading control. (C) Modification of histones by alkynyl-4-ONyl in the absence and presence of SIRT2 inhibitors. (D) Analysis of alkynyl-4-ONE labeling on histones from control and SIRT2 knockdown cells. SIRT2 knockdown was confirmed by Western blot and H3 was used as loading control. MEF cells were labeled with alkynyl-4-ONE for 3 h. Histones were extracted and click chemistry was carried out to attach rhodamine. The modification level was readout by rhodamine fluorescence (Rho) after SDS-PAGE. (E) The ability of SIRT2 WT and F119A mutant to remove alkynyl-4-ONyl from proteins isolated from SIRT2 knockdown cells. The Coomassie blue (CB) stained gel was used to show equal loading in different lanes.

treated with a4-ONE. SIRT2 treatment led to dramatic reduction of the alkynyl-4-ONE labeling on histones, supporting SIRT2 directly remove the 4-ONylation on these proteins (Figure 3E). The SIRT2 F119A mutant with diminished activity on 4-ONyl peptide also had much weaker activity on the extracted histones (Figure 3E). Taken together, we conclude that SIRT2 can interact with histones and hydrolyze 4-ONyl lysine in living cells.

To make sure the endogenous 4-ONyl modification on histones (modification without adding exogenous 4-ONE) could also be regulated by SIRT2, we examined the endogenous 4-ONyl modification in SIRT2 WT and knockout (KO) MEF cells by LC-MS/MS. Lipopolysaccharide was used to treat cells and then histones were extracted for LC-MS/MS analysis. We consistently detected the 4-ONyl modification on histone H3K23 as reported (Figure S5A,B).² The quantification

result showed that the 4-ONyl H3K23 level in SIRT2 KO MEFs was 1.61-fold higher than that in WT MEFs (Figure 4). The LC-MS/MS analysis demonstrates SIRT2 is able to regulate endogenous 4-ONylation on histones.

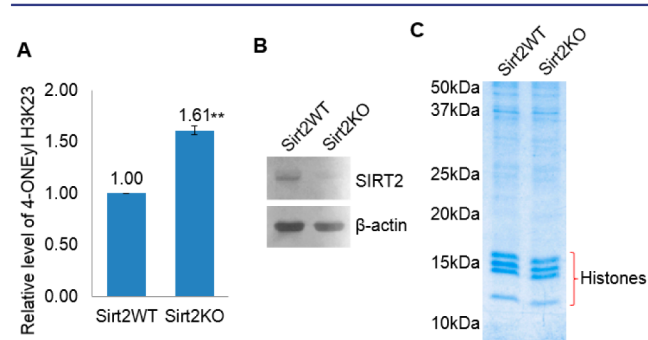


Figure 4. SIRT2 regulates endogenous 4-ONyl level on histones in mouse embryonic fibroblasts (MEFs). (A) MS quantification of 4-ONyl H3K23 levels in SIRT2 WT and KO MEFs based. The levels of 4-ONyl H3K23 is normalized by unmodified H3 Y41-R49 peptide. The results were from two independent biological replicates. (B) Western blot analysis showing the levels of SIRT2 in WT and KO MEFs. (C) Coomassie blue-stained gel showing histones extracted from SIRT2 WT and KO MEFs that were used for the MS quantification.

Upon stress, the 4-ketoamide adduct on histones disrupts the interaction between the histone H3 and DNA, therefore preventing the nucleosome assembly.² Thus, the ability of SIRT2 to remove the 4-ONyl groups on histones serves to mitigate the negative impact of protein 4-ONylation caused by oxidative stress. Our studies demonstrate that SIRT2 is able to hydrolyze 4-ONyl lysine on endogenous histones in living cells and reveal a lone pair- π interaction for the substrates recognition. This is first time a mechanism to reverse protein lysine 4-ONylation is reported. It broadens the activity scope of sirtuins and more importantly provides a new research direction to understand the role of SIRT2 in oxidative stress.

■ ASSOCIATED CONTENT

📄 Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b04977.

Experimental details (PDF)

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📄 Notes

The authors declare no competing financial interest.

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