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Substrate-differentiated Transition States of SET7/9-catalyzed Lysine Methylation

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Abstract

Transition state stabilization is essential for rate acceleration of enzymatic reactions. Despite extensive studies on various transition state structures of enzymes, an intriguing puzzle is whether an enzyme can accommodate multiple transition states (TS) to catalyze a chemical reaction. It is experimentally challenging to interrogate this proposition in terms of the choices of suitable enzymes and the feasibility to distinguish multiple TS. As a paradigm with the protein lysine methyltransferase (PKMT) SET7/9 paired with its physiological substrates H3 and p53, their TS were solved with experimental kinetic isotope effects as computational constraints. Remarkably, SET7/9 adopts two structurally distinct TS---a nearly symmetric S_N2 and an extremely early S_N2 ---for H3K4 and p53K372 methylation, respectively. The two TS are also different from those previously revealed for other PKMTs. The setting of multiple TS is expected to be essential for SET7/9 and likely other PKMTs to act on broad substrates with high efficiency.

Graphical Abstract

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Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website. Methods; Figure S1,S2; Table S1.

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Enzymes catalyze chemical reactions via stabilizing transition states (TS).^{1–2} The transition state (TS) theory argues that the rate acceleration by enzymatic catalysis arises from the stabilization energy differentially gained between reactants and their TS upon enzyme binding.³ To engage a TS with a transient lifetime of femtoseconds, an enzyme has to align many residues in ready conformations to match the TS geometry for catalysis.⁴ Given such stringent requirement of the conformational matching as well as certain specificity of an enzyme, it is intriguing to ask whether one enzyme can adopt multiple TS to catalyze a chemical reaction---the multi-transition-state theory. It has been known that homologous enzymes can adopt structurally different TS to catalyze a chemical reaction as exemplified by purine nucleoside phosphorylases^{5–7} and 5'-methylthioadenosine nucleosidases.^{8–10} However, it is not trivial to explore experimentally the multi-transition-state theory without the choice of proper enzymes with potential multiple TS and the methods to solve these TS structures.

The enzymes that catalyze posttranslational modifications such as ubiquitination, phosphorylation, acetylation and methylation as well as proteases are unique because of their abilities to act on broad substrates in an efficient manner.^{11–14} Protein lysine methyltransferases (PKMTs) are the posttranslationally-modifying enzymes that catalyze lysine methylation with S-adenosyl-L-methionine (SAM) as a cofactor.^{11, 15–16} The methods using experimental binding and kinetic isotope effects (BIE and KIEs) as geometrical constraints coupled with computational modeling allow to uncover atomistic details of the TS structures of PKMT-catalyzed methylation reactions.^{17–18} Similar to other homologous enzymes, closely related PKMTs can adopt distinct TS as revealed by a collection of characteristic KIEs.^{11, 17–18} SET7/9 has been characterized as a PKMT for its broad substrates such as histone H3, p53, FOXO3, DNMT1, and NF- κ B via diverse recognition motifs (Table S1).¹⁹ This observation inspired us to probe SET7/9 as a model enzyme to examine the multi-transition-state theory. Here we selected two substrates---histone H3K4 and p53K372---because of their biological relevance, homologous [R/K][S/T]K sequence, high and comparable activities, and available X-ray structures in complex with SET7/9.²⁰ With their experimental KIEs as computational constraints, we revealed that SET7/9 methylates the H3 and p53 peptides via nearly symmetrical and extremely early S_N2 TS, respectively. The two distinct TS are also different from those solved for other PKMTs.¹¹

To use KIEs as constraints to solve TS in an unsupervised manner, we implemented the density functional theory with the M062X functional and 6–31+G(d,p) basis set embedded in Gaussian09 for geometry optimization and KIE calculation.¹⁷ The S_N2 TS geometries of SAM-dependent methylation of a primary amine were optimized upon systemically altering C–S (1.9~2.6 Å) and C–N (1.6~2.5 Å) distances (Figure 1a). With the optimized TS candidates containing characteristic imaginary frequencies, we calculated three theoretical KIEs $^{CD}_3k$, $^{13}CH_3k$, $^{CT}_3/^{14}CH_3k$, $^{15}NH_2k$, and ^{34}Sk and generated KIE heat maps with the paired C–S and C–N distances as coordinates (Figure 1b–d, S1). These heat maps revealed two general principles: $^{CD}_3k$ values mainly report linear S–N distances with more inverse values for shorter S–N distances of S_N2 TS; $^{13}CH_3k$ values mainly reflect the S...Me...N center symmetry with smaller values for less symmetrical (early or late) S_N2 TS. The

magnitude of $^{CT_3}{}^{14}CH_3k$ arises from the combined effect and can be used as an additional constraint. The magnitudes of $^{15}NH_2k$ and, $^{34}S_k$ are 3-fold smaller than those of $^{CD_3}{}^{13}CH_3k$ and $^{CT_3}{}^{14}CH_3k$. Prior experimental and computational methods have revealed multiple S_N2 TS of PKMTs, including an early S_N2 TS for SETD8 (C–S distance: 2.1 Å; C–N distance: 2.5 Å) and a late S_N2 TS for NSD2 (C–S distance: 2.6 Å; C–N distance: 2.0 Å).^{11, 17–18} The KIE-constrained TS of SETD8 and NSD2 can be readily located within our KIE heat maps.

SET7/9 has been characterized to catalyze lysine methylation via an ordered bi-substrate sequential mechanism with the initial step of SAM binding followed by the substrate binding to form a ternary cofactor–enzyme–substrate complex.^{21–22} SET7/9 methylates H3K4 and p53K372 peptides with comparable efficiency ($k_{cat} = 1.1–1.2 \text{ min}^{-1}$; $K_{m, \text{substrate}} = 29 \text{ } \mu\text{M}$ and $69 \text{ } \mu\text{M}$, respectively).²⁰ Here we showed that SET7/9 has negligible forward commitment factor C_f of SAM for the two substrates (0.007 ± 0.005 and 0.013 ± 0.002 , Table 1, Supplementary Methods), arguing a rapid exchange of SAM between its free and SET7/9-bound form in the time scale of the lysine methylation. Therefore, the binding of SAM and then the substrate H3 or p53 won't significantly suppress the KIEs of the chemical step of lysine methylation. The H3 and p53 peptides are thus suitable SET7/9 substrates for KIE measurement. Mean-while, the BIEs of [*S*-CD₃]-SAM and [*S*-CT₃]-SAM were measured to be 1.06 ± 0.01 and 1.13 ± 0.02 , respectively (Table 1, Supplementary Method). In comparison, the small inverse BIEs of 1~4% were reported when [*S*-CD₃]-SAM and [*S*-CT₃]-SAM bind to SETD8;¹⁷ a small inverse BIE of 1% was reported when [*S*-CT₃]-SAM forms a ternary complex with NSD2 and H3K36M nucleosome; a large inverse BIE of 35% was reported when [*S*-CT₃]-SAM directly binds to NSD2.²³ Computational modeling revealed that these BIE values are extremely sensitive to noncanonical carbon–oxygen (C–H···O) hydrogen-binding interactions of SAM's methyl group with neighboring phenolic hydroxyl and amide oxygen residues as implicated in several PKMTs (Y1179, R1135, and F1117 of NSD2; Y336, R295, and C270 of SETD8; Y335, H293, and G264 of SET7/9).^{11, 23} These BIEs contribute to experimental KIEs and were corrected for KIE constraints of TS.

$^{CD_3}(V/K)$ of [*S*-CD₃]-SAM reports the changes of the binding environment of SAM's methyl group from a free ground state to the TS of lysine methylation. We thus measured α -2°-CD₃ KIE of SET7/9-catalyzed methylation with H3 and p53, respectively, through internal competition experiments with a mixture of [*S*-CH₃/CD₃]-SAM cofactors. The intrinsic α -2°-CD₃ KIE were derived from $^{CD_3}(V/K)$ (0.829 ± 0.007 and 0.88 ± 0.01) after correcting the C_f and the BIE of [*S*-CD₃]-SAM (eq. S19), resulting in the ^{CD_3}k values of 0.781 ± 0.007 and 0.82 ± 0.01 for H3 and p53, respectively (Table 1). These large inverse α -2° CD₃ KIEs indicate a compressed S–N distance and thus vibrational stiffness of the sulfonium-methyl group towards the formation of the S_N2 TS. The significant 4% difference of the two KIEs further argues a shorter S–N distance at the TS with the H3K4 peptide, and stands as the first piece of evidence that SET7/9 acts on H3 and p53 via different TS.

We then determined the intrinsic 1° - ^{13}C KIE $^{13}\text{CH}_3k$ as additional constraints. Here $^{13}\text{CD}_3(V/K)$ of 0.883 ± 0.002 for H3 and 0.92 ± 0.02 for p53 were obtained with the [*S*- $\text{CH}_3/^{13}\text{CD}_3$]-SAM cofactor pair (Table 1). The intrinsic 1° - ^{13}C KIE was derived after correcting the BIE and $^{13}\text{CH}_3k$: $^{13}\text{CH}_3k$ of 1.07 ± 0.01 and 1.05 ± 0.03 for the H3 and p53 substrates, respectively (Table 1, eqs. S19~S20). The two-large normal 1° - ^{13}C KIEs report a significant decrease of the overall bond order of SAM's methyl carbon from its ground state to TS. The 2% larger KIE with the H3 substrate further argues a more symmetrical $\text{S}_\text{N}2$ TS, and stands as the second piece of evidence for substrate-differentiated TS of SET7/9.

For further validation, we measured $^{14}\text{CH}_3k$ with the [*S*- $\text{CT}_3/^{14}\text{CH}_3$]-SAM cofactor pair. The $^{14}\text{CH}_3k$ values of 0.605 ± 0.004 for H3 and 0.66 ± 0.01 for p53 are in good agreement with their respective $^{13}\text{CH}_3k$ and $^{13}\text{CD}_3k$ values (Table 1). Such consistency further strengthens that SET7/9 acts on H3 and p53 via two different TS.

With $^{13}\text{CH}_3k$, $^{13}\text{CD}_3k$ and $^{14}\text{CH}_3k$ as collective constraints, two structurally distinct TS can be located by the three KIE heat maps (Figure 2). Here SET7/9-catalyzed H3K4 methylation adopts a nearly symmetric $\text{S}_\text{N}2$ TS with the 2.1~2.2 Å C-S distance and the 2.0~2.1 Å C-N distance (the S-N distance around 4.2 Å, Figure 2). In contrast, SET7/9-catalyzed p53K372 methylation adopts an extremely early $\text{S}_\text{N}2$ TS with the short 2.0 Å C-S distance and the long 2.3 Å C-N distance (the S-N distance around 4.3 Å, Figure 2). For structure candidates with C-S distances longer than 2.6 Å, no TS-characteristic imaginary frequency along the $\text{S}_\text{N}2$ TS coordination could be identified when C-N distances are shorter than 2.0 Å. Given that the KIEs of the nitrogen nucleophile and sulfonium leaving group were included as constraints upon solving the TS of NSD2,¹⁸ we evaluated the potential contribution of ^{15}N and ^{34}S KIEs, two KIEs that could be obtained with our current MS method, upon modeling TS. Based on their KIE heat maps (Figure S1), H3K4 with the nearly symmetric $\text{S}_\text{N}2$ TS and p53K372 with the extremely early $\text{S}_\text{N}2$ TS are expected to show $^{15}\text{NH}_2k$ of 1.019~1.022 and 1.025~1.026; $^{34}\text{S}k$ of 1.008~1.009 and 1.006~1.007, respectively. The small KIE difference between the two TS, which are within KIE errors, thus prevented us from pursuing $^{15}\text{NH}_2k$ and $^{34}\text{S}k$ as additional constraints.

In contrast to other experimentally solved TS of PKMTs, it is striking to uncover the two new TS--- nearly symmetric and extremely early $\text{S}_\text{N}2$ ---adopted by a single PKMT. In this context, an intriguing hypothesis is whether SET7/9 could methylate a substrate via multiple distinct TS and these experimental KIEs were the weighted averages of individual TS. We argue that this effect is limited in our case because the solved TS are associated with extreme KIEs such as the nearly maximal $^{13}\text{CH}_3k$ for H3K4 or extreme structures such as the

extremely early S_N2 TS for p53. Given that SET7/9 catalyzes H3K4 and p53K372 methylation with comparable k_{cat} ($1.1\text{--}1.2\text{ min}^{-1}$),²⁰ the activation energy of the two TS from the SAM-SET7/9-substrate Michaelis complexes are expected to be similar. Interestingly, while the KIE-constrained TS of the SET7/9-catalyzed H3K4 methylation is in modest agreement with the symmetrical TS via quantum mechanics/molecular mechanics (QM/MM) simulation ($2.2\text{--}2.3\text{ \AA}$ C–S and C–N distances),²⁷ the KIE-constrained TS of the SET7/9-catalyzed p53 methylation is significantly different from the late S_N2 TS solely determined by QM/MM (C–S distance: $2.6\text{ \AA}$; C–N distance: $2.0\text{ \AA}$).²⁸ In addition, the KIE-constrained S–N distances of $4.2\text{--}4.3\text{ \AA}$ are $0.3\text{ \AA}$ shorter than those solely simulated with QM/MM. Collectively, we uncover the two characteristic, substrate-differentiated TS of SET7/9-catalyzed lysine methylation.

While the SET7/9 methylation sites of H3K4 and the K372 of p53 are embedded within a homologous sequence [R/K][S/T]K, the X-ray structures of SET7/9 in complex with the two methylated product peptides are different in terms of how SET7/9 engages its residues and water molecules for peptide recognition (Figure S2).²⁰ It is likely that these different peptide-recognition patterns pre-determine the path of assembling respective TS. We recently showed that PKMTs exist as a dynamic ensemble of numerous conformations.²⁹ Multiple transition-state-like conformational states, readily located in the conformational landscape of a PKMT, can be functionally relevant for catalysis in the context of its broad substrate profile.²⁹ With the potential contribution of conformational dynamics of the SAM-SET7/9-substrate ternary complex, we envision that multiple conformations can facilitate the formation of corresponding structurally-matched TS via activating the Lys nucleophile upon deprotonation, aligning the methyl group along a linear S_N2 trajectory, or promoting the coordinated motion of the Lys nucleophile and sulfonium leaving group, alone or in combinations.¹¹

In summary, with SET7/9 as a model enzyme, we measured the commitment factors, BIEs and three KIEs ($^{CD}_3k$, $^{13}CH_3k$ and $^{CT}_3/^{14}CH_3k$) of the SAM cofactor with two biologically relevant substrates H3 and p53. In the context of the KIE heat maps with possible TS geometries as coordinates, we revealed that SET7/9 adopts two distinct S_N2 TS---a nearly symmetrical one for the H3 substrate and an extremely early one for the p53 substrate. The two TS are also distinct from those solved for SETD8 and NSD2. The feature of the substrate-dependent TS can be essential for SET7/9 to process structurally diverse substrates in a biological context. It will be interesting to examine whether it is a general mechanism for enzymes to achieve broad substrate scopes via multiple TS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Computational modeling and theoretical KIEs of TS candidates of lysine methylation. (a) TS candidates of lysine methylation and their isotopic reporters. (b-d) Heat maps of theoretical KIEs of ¹³CH₃, CD₃ and CT₃/¹⁴C with C-S and C-N distances as the coordinates.

Figure 2.

Transition state geometries of SET7/9-catalyzed lysine methylation. (a,c) Constraints of experimental KIEs ($^{13}\text{CH}_3$, CD_3 , $\text{CT}_3/^{14}\text{CH}_3$) of H3 (upper) and p53 (bottom) in the coordinates of C-S and C-N distances. TS geometries matching $^{13}\text{CH}_3$, CD_3 and $\text{CT}_3/^{14}\text{CH}_3$ KIEs are shown in light red, blue and grey, respectively. The overlaid TS geometries matching the three experimental KIEs are shown in black. (b,d) Schematic TS structures of SET7/9-catalyzed lysine methylation on H3K4 (upper) and p53K372 (bottom).

Table 1.

Forward commitment factors, BIEs and KIEs of SET7/9-catalyzed lysine methylation with H3 and p53 substrates.

Parameters	H3	p53
C_f	0.007±0.005	0.013±0.002
CD ₃ BIE	1.06±0.01	
CT ₃ BIE	1.13±0.02	
$CD_3(V/K)$	0.829±0.007	0.88±0.01
CD_{3_k}	0.781±0.007	0.82±0.01
$^{13}CD_3(V/K)$	0.883±0.002	0.92±0.02
$^{13}CD_{3_k}$	0.832±0.002	0.87±0.02
$^{13}CH_{3_k}$	1.07±0.01	1.05±0.03
$CT_3/^{14}CH_3(V/K)$	0.686±0.005	0.75±0.01
$CT_3/^{14}CH_{3_k}$	0.605±0.004	0.66±0.01