Enzymatic mechanism and product specificity of SET-domain protein lysine methyltransferases

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Molecular dynamics and hybrid quantum mechanics/molecular mechanics have been used to investigate the mechanisms of ⁺AdoMet methylation of protein-Lys-NH₂ catalyzed by the lysine methyltransferase enzymes: histone lysine monomethyltransferase SET7/9, Rubisco large-subunit dimethyltransferase, viral histone lysine trimethyltransferase, and the Tyr245Phe mutation of SET7/9. At neutrality in aqueous solution, primary amines are protonated. The enzyme reacts with Lys-NH₃⁺ and ⁺AdoMet species to provide an Enz-Lys-NH₃⁺·⁺AdoMet complex. The close positioning of two positive charges lowers the *pK_a* of the Lys-NH₃⁺ entity, a water channel appears, and the proton escapes to the aqueous solvent; then the reaction Enz-Lys-NH₂·⁺AdoMet → Enz-Lys-N(Me)H₂·⁺AdoHcy occurs. Repeat of the sequence provides dimethylated lysine, and another repeat yields a trimethylated lysine. The sequence is halted at monomethylation when the conformation of the Enz-Lys-N(Me)H₂·⁺AdoMet has the methyl positioned to block formation of a water channel. The sequence of reactions stops at dimethylation if the conformation of Enz-Lys-N(Me)₂H⁺·⁺AdoMet has a methyl in position, which forbids the formation of the water channel.

molecular dynamics | QM/MM | SCCDFTB

The packaged structure of DNA in eukaryotic cells is called chromatin. Chromatin activity is mainly controlled by site-specific lysine methylation catalyzed by protein lysine methyltransferase enzymes (PKMT) (1). Absence of the methylation at the site-specific lysine by a PKMT is the origin of a number of human diseases, notably cancer (2).

All but one (3, 4) of the known PKMTs have a SET-domain structure (5, 6). These PKMTs include human histone methyltransferase SET7/9 (7–11), human SET8 (also known as PR-SET7) (12, 13), *Neurospora* DIM-5 (14), histone lysine methyltransferase Clr4 (15), viral histone lysine methyltransferase (vSET) (16, 17), and plant Rubisco large-subunit methyltransferase (LSMT) (18, 19). A SET domain, originally identified in three *Drosophila* genes involved in epigenetic processes, contains ≈130 aa residues. The cofactor *S*-adenosylmethionine (⁺AdoMet) and substrate bind at two adjacent sites of the conserved SET domain. The ⁺AdoMet methyl group approaches the target lysine amino group through a channel that passes through the middle of this SET domain to form Enz-Lys-NH₃⁺·⁺AdoMet.

PKMT enzymes transfer one, two, or three methyl groups to target lysine residues depending on the particular enzyme. This is called product specificity (shown in Scheme 1). For example, LSMT transfers two methyl groups to a single lysine (Lys), so that we refer to LSMT as a dimethyltransferase in the present study. Only a neutral Lys-NH₂, Lys-N(Me)H, or Lys-N(Me)₂ can be methylated by the ⁺AdoMet cofactor. Lys-NH₃⁺, Lys-N(Me)H₂⁺, or Lys-N(Me)₂H⁺ must be deprotonated before methylation (Scheme 1). Xiao *et al.* (13) proposed that the bulk solvent might play an important role in the dissociation of the proton of the positively charged lysine. Because the active site fits tightly to the reactants and does not allow the entrance of solvent molecules, this proposal is incomplete and insufficient. Dirk *et al.* (20) suggested that a

Scheme 1.

water molecule at the active site of SET-domain PKMTs functions as the proton acceptor. However, H₃O⁺ is a much stronger acid than Lys-CH₂-NH₃⁺, so that this water by itself could not deprotonate the charged substrate. Guo *et al.* (21) suggested that the conserved Tyr-335, as a phenolate, in SET7/9 acts as a base for the deprotonation. This proposal is unlikely because Tyr-335-OH has a calculated *pK_a* of >13.0 (22).

Related are proposed mechanisms to explain the origin of product specificity by PKMTs. Hu *et al.* (23) proposed, on the basis of their molecular dynamics (MD) simulations, that the distributions of near-attack conformation at the ground state determined the product specificity by PKMTs. Xiao *et al.* (9) proposed that the mutation of Tyr-245 into Phe or Ala in SET7/9 alters the product specificity, and Cheng *et al.* (24) proposed that the Tyr/Phe switch controls the product by PKMTs based on their mutation experiments. However, these important experimental and computational results do not deal with the crucial questions: How do the charged substrates deprotonate, and what controls the specificity?

We report here the mechanisms of the catalysis by three SET-domain enzymes: histone lysine monomethyltransferase SET7/9 as well as its Tyr245Phe mutation, Rubisco LSMT, and vSET. Finally, a definitive mechanism is provided. The details of computations used were described in previous reports (22, 29, 30).

Results and Discussions

Processivity and Multiplicity of the Methyl Transfer Reactions. Histone lysine methyltransferase SET7/9 only catalyzes the transfer of one methyl group to the target lysine (Lys-4). LSMT transfers two methyl groups to a single lysine (Lys). vSET catalyzes the triple methylation of the target lysine Lys-27 (Scheme 1). There are three reaction steps in the ⁺AdoMet methylation of lysine-NH₂ catalyzed by a methyltransferase: (i) combination of enzyme with Lys-NH₃⁺ and ⁺AdoMet, (ii) proton dissociation to provide Enz-Lys-NH₂·⁺AdoMet, and (iii) methyl transfer providing Enz-Lys-N(Me)H₂·⁺AdoHcy and the release of AdoHcy.

***pK_a* Calculation of Lys-4-NH₃⁺ and Tyr-335-OH in SET7/9.** The *pK_a*s of Lys-4-NH₃⁺ and Tyr-335-OH are listed in Table 1. The calcu-

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Table 1. The calculated pK_a values of the target lysine Lys4 and the converted tyrosine Tyr335 in the various complexes of the SET7/9 monomethyltransferase

Complex	pK_a	
	Lys4-NH ₃ ⁺	Tyr335-OH
SET7/9-Lys4-NH ₃ ⁺	10.9 ± 0.4	14.3 ± 3.0
SET7/9 Lys4-NH ₃ ⁺ +AdoMet	8.2 ± 0.6	16.6 ± 4.2
SET7/9 Lys4-N(Me)H ₂ ⁺ +AdoMet	14.7 ± 4.9	13.7 ± 2.4

The pK_a is calculated by solving the Poisson–Boltzmann equation implemented in the PBEQ module of CHARMM suite at the MM level, with 80.0 and 4.0 as the dielectric constants of water and protein, respectively.

lated pK_a of the conserved Tyr-335-OH eliminates the presence of Tyr-335-O[−] as the base to deprotonate Enz·Lys-4-NH₃⁺+AdoMet or Enz·Lys-4-N(Me)H₂⁺+AdoMet. The calculated pK_a of Lys-4-NH₃⁺ in complex SET7/9·Lys-4-NH₃⁺+AdoMet is 8.2 ± 0.6. The decrease in pK_a from 10.9 to 8.2 on +AdoMet addition is due to the electrostatic interaction of the closely positively charged Lys-4-NH₃⁺ and +AdoMet species. Proton dissociation creates the reactive complex SET7/9·Lys-4-NH₂⁺+AdoMet. How does the proton dissociate from the active site?

MD Simulations on Enzyme Michaelis Complexes. Inspections of the MD trajectories of the various enzyme complexes establish that a water channel for proton escape is formed only upon the establishment of the +AdoMet complexes: SET7/9·Lys-4-NH₃⁺+AdoMet, LSMT·Lys-NH₃⁺+AdoMet, LSMT·Lys-N(Me)H₂⁺+AdoMet, vSET·Lys-27-NH₃⁺+AdoMet, vSET·Lys-27-N(Me)H₂⁺+AdoMet, and vSET·Lys-27-N(Me)H₂⁺+AdoMet. The presence of a water channel is established by determining the distances between the hydrogen and oxygen atoms of the continuous chain of water molecules. A distance of 1.85 Å supports a water channel. Examination of the enzyme environment at the termination of the water channel shows that there is no general base candidate to dissociate the proton of the charged substrate as suggested by Zhou and coworkers (25). Thus, a water channel is positioned to allow proton transfer from the protonated substrate to the solvent. For instance, the presence of a water channel formed in SET7/9·Lys-4-NH₃⁺+AdoMet (Fig. 1) is supported

Fig. 1. A snapshot of a water channel observed during 3-ns MD simulation on SET7/9-Lys-4-NH₃⁺+AdoMet complex. The hydrogen bond distance is <1.85 Å.

Table 2. The average density (in atoms per Å³) of the water molecules at the positions of the water channel (shown in Figs. 1 and 5) during the MD simulations on complex SET7/9-Lys4-NH₃⁺+AdoMet and SET7/9[Y245F]·Lys4-N(Me)H₂⁺+AdoMet

Complex	Position	Density
SET7/9-Lys4-NH ₃ ⁺ +AdoMet (Fig. 1)	Wat559	0.006
	A	0.009
	B	0.011
	C	0.017
	D	0.019
	E	0.022
	F	0.026
	G	0.028
	H	0.027
	I	0.024
Mutated SET7/9[Y245F]·Lys4-N(Me)H ₂ ⁺ +AdoMet (Fig. 5)	Wat565	0.007
	Wat660	0.007
	Wat559	0.001
	A	0.014
	B	0.017
	C	0.020
	D	0.015
	E	0.026
	F	0.028
	G	0.028
	H	0.021

The solvent water molecules are designated by A–I, and I is on the surface of the water sphere with a 25-Å radius. The crystal water molecules are designated by Wat. The density values mean how many water molecules fill the given position during the molecular dynamics simulations. Nonzero values are expected to indicate that this position is filled by the water molecule.

by the average densities (Table 2). A crystal water becomes the starting point of this water channel, and solvent water molecules play a shuttle role in delivering a proton from the protonated substrate into solvent via this water channel.

Histone lysine monomethyltransferase SET7/9 does not transfer a second methyl because the methyl group of SET7/9-Lys-4-N(Me)H₂⁺+AdoMet interferes with the formation of a water channel to Lys-4. The same reason thus is a lack of a third methyl transfer with the LSMT enzyme. Deprotonation of LSMT·Lys-N(Me)₂H⁺+AdoMet does not occur because the water channel cannot reach Lys-4-N(Me)H₂⁺. The active site configurations of SET7/9-Lys-4-NH₃⁺+AdoMet and SET7/9-Lys-4-N(Me)H₂⁺+AdoMet are shown in Fig. 2. Inspections of Fig. 2 establish how the methyl group blocks what would be the entrance of the water channel to Lys-4-N(Me)H₂⁺. The active site configurations of LSMT·Lys-NH₃⁺+AdoMet,

Fig. 2. Schematic diagram of the position of the amine group at SET7/9-Lys-4-NH₃⁺+AdoMet (A) and SET7/9-Lys-4-N(Me)H₂⁺+AdoMet (B). Water molecules A and B are solvent. These pictures are based on the results from the MD simulations.

Fig. 3. Schematic diagram of positioning of the amine group at LSMT-Lys-NH₃⁺·AdoMet (A), LSMT-Lys-N(Me)H₂⁺·AdoMet (B), and LSMT-Lys-N(Me)₂H⁺·AdoMet (C). These pictures are based on the results from the MD simulations.

LSMT-Lys-N(Me)H₂⁺·AdoMet, and LSMT-Lys-N(Me)₂H⁺·AdoMet are shown in Fig. 3. Comparison of Fig. 3 A and B reveals that an alternative water channel appears upon formation of Enz-Lys-N(Me)H₂⁺·AdoMet, although the first methyl group of Lys-N(Me)H₂⁺ takes the position of the proton of Lys-NH₃⁺ that forms a water channel. Proton dissociation from Lys-N(Me)₂H⁺ does not occur because of the two methyl substituents preventing the formation of a complete water channel (Fig. 3C). Because the neutral Lys-4-N(Me)H and Lys-N(Me)₂ are not available, SET7/9 is a monomethyltransferase, and LSMT is a dimethyltransferase. In addition, the neutral Lys-27-NH₂, Lys-27-N(Me)H, and Lys-27-N(Me)₂ are created by a water channel in vSET-Lys-27-NH₃⁺·AdoMet, vSET-Lys-27-N(Me)H₂⁺·AdoMet, and vSET-Lys-27-N(Me)₂H⁺·AdoMet, respectively. vSET is a trimethyltransferase (Fig. 4). These findings afford a definitive explanation to product specificity by PKMTs (Scheme 2). Methyl transfer does not occur if a water channel is not present. Each allowed methyl transfer step includes (i) the formation of a water channel to allow proton dissociation from protonated charged lysine into solvent, (ii) methylation of a neutral lysine by ⁺AdoMet, and (iii) the product formed and released. The formation of a water channel explains the processivity and multiplicity of the methyl transfer steps by PKMTs.

Mutation of Tyr245Phe in SET7/9 Provides Additional Evidence for This Definitive Mechanism. Mutation of Tyr245Phe in SET7/9 has been reported to convert the enzyme from a mono- to a dimethyltransferase (9, 24). Our MD simulation examination of this mutated SET7/9[Y245F]·Lys-4-N(Me)H₂⁺·AdoMet complex reveals the presence of a water channel (Fig. 5), as indicated by the average densities (Table 2) of the crystal waters (Wat565, Wat660, and Wat559 in Fig. 5) and solvent water molecules (A–H in Fig. 5).

The configuration of active site-bound Lys-4-N(Me)H₂⁺ and ⁺AdoMet in this mutated SET7/9[Y245F] is depicted in Fig. 6. Comparing Fig. 2B and Fig. 6 shows that the mutation of Tyr-245 into Phe-245 in SET7/9 positions the Lys-4-N⁺(Me)H-H proton at the beginning of a water channel, whereas the Tyr-245 in the

wild-type enzyme does not allow water to contact Lys-4. In this mutated complex, the proton from Lys-4-N(Me)H₂⁺ is delivered into the solvent via the formed water channel (Figs. 5 and 6), and the resultant neutral Lys-4-N(Me)H is primed for the second methyl transfer reaction. These observations are in agreement with the experimental observation (9, 24) that the mutation of Tyr245Phe converts a mono- to a dimethyltransferase. Meanwhile, the presence of a water channel in this mutation verifies that the formation of a water channel determines the product specificity by PKMTs.

Dissociation Barrier for Proton From Charged Substrates into Solvent.

When a hydroxide ion (HO[−]) is positioned on the enzyme surface next to the water channel (denoted by C in Fig. 1), there is no energy barrier for proton transfer from Lys-4-NH₃⁺ to HO[−] determined by QM/MM [QM = both self-consistent charge density functional tight binding (SCCDFTB) (26, 27) and HF/6-31+G(d,p) (Gamess-U.S. version June 22, 2002) (28)]. The same results are found in both LSMT and vSET. Because the concentration of HO[−] at optimal pH = 8.0 is 10^{−6}, the activation energy barrier for proton dissociation would be 8.4 kcal/mol.

Comparable Mechanisms of the Methyl Transfer Steps. The SCCDFTB/MM protocol is validated by B3LYP/6-31G*//MM (31). The calculated free energy barriers for methyl transfer steps by SET7/9, LSMT, and vSET are listed in Table 3. The calculated free energies barriers are in reasonable agreement with the values obtained from the experimental rate constants (Table 3). The average deviation of the differences is ±0.9 kcal/mol.

The transition states in the SET7/9, LSMT, and vSET reactions were obtained by the conjugate peak refinement (CPR) (31) technique and characterized by only one imaginary frequency from normal mode analysis. The key geometrical parameters are schematically shown in Fig. 7. All calculated transition states have a near linear S₈(⁺AdoMet)⋯Cγ(⁺AdoMet)⋯N(Sub⁺) configuration and equal bond breaking and formation as required for linear concerted S_N2 displace-

Fig. 4. Schematic diagram of position of the amine group at vSET-Lys-27-NH₃⁺·AdoMet (A), vSET-Lys-27-N(Me)H₂⁺·AdoMet (B), and vSET-Lys-27-N(Me)₂H⁺·AdoMet (C). These pictures are based on the results from the MD simulations.

Scheme 2.

Fig. 5. A snapshot of a water channel during 3-ns MD simulation on SET7/9[Y245F]-Lys-4-NH₃⁺·AdoMet complex. The hydrogen bond distance is <1.85 Å.

ment (32). Also, all methyl transfer steps by the same enzyme have the identical transition state geometries.

Conclusion

Rubisco LSMT, vSET, and histone lysine methyltransferase SET7/9 as well as the SET7/9[Y245F] mutant have now been used in the study of the product specificity by PKMTs. The stepwise methylations are shown in Scheme 2. Methyl transfer occurs when a critical water channel appears. This is so for SET7/9·Lys-4-NH₃⁺·AdoMet, SET7/9[Tyr245Phe]·Lys-4-N(Me)H₂⁺·AdoMet, LSMT·Lys-NH₃⁺·AdoMet, LSMT·Lys-N(Me)H₂⁺·AdoMet, vSET·Lys-27-NH₃⁺·AdoMet, vSET·Lys-27-N(Me)H₂⁺·AdoMet, and vSET·Lys-27-N(Me)₂H⁺·AdoMet. The electrostatic interactions between the positive charges on ⁺AdoMet and SET7/9·Lys-4-NH₃⁺ decrease the *pK_a* of the latter from 10.9 ± 0.4 to 8.2 ± 0.6, and this is not seen in the SET7/9·Lys-4-N(Me)H₂⁺·AdoMet complex (Table 1). The dissociation of the Lys-CH₂-NH₃⁺, Lys-CH₂-N(Me)H₂⁺, and Lys-CH₂-N(Me)₂H⁺ proton into solvent via this water channel is associated with the energy barrier of ≈8.4 kcal/mol (at pH 8.0). Most important, a water channel does not form in SET7/9·Lys-4-N(Me)H₂⁺·AdoMet or LSMT·Lys-N(Me)₂H⁺·AdoMet, such that methyl transfer does not occur. This explains the inabilities of SET7/9 and LSMT to transfer second and third methyl groups to the target lysine, respectively. The configuration of the nitrogen substituent of Lys-4-N(Me)H₂⁺ or Lys-N(Me)₂H⁺ determines whether a water channel can be formed, such that a proton dissociation creates Lys-4-NH₂ or Lys-N(Me)H (Figs. 2*B* and 3*C*). Methyl transfer does not occur when the lysine-N⁺-H is not at the beginning of the water channel. The dependence of methyl transfer on the formation of a water channel establishes a definitive mechanism for the deprotonation of the charged substrate (Scheme 2). Our

Fig. 6. Schematic diagram of position of the amine group at Lys-4-N(Me)H₂⁺ in SET7/9[Y245F]-Lys-4-N(Me)H₂⁺·AdoMet. This picture is based on the results from the MD simulations.

Table 3. Comparison of the experimental free-energy barriers ($\Delta G_E^\ddagger$ in kilocalories per mole) and the calculated average $\Delta G_C^\ddagger$ by the MP2/6-31 + G(d,p)//MM single-point computations based on the structures determined by SCCDFTB/MM for methyl transfer reactions in PKMTs

Enzyme	$\Delta G_E^\ddagger$	$\Delta G_C^\ddagger$	$\Delta G_E^\ddagger - \Delta G_C^\ddagger$	Step	Ref.
Viral histone lysine methyltransferase	21.7	22.5	-0.8	First methyl transfer	30
	22.4	22.6	-0.2	Second methyl transfer	30
	23.0	23.1	-0.1	Third methyl transfer	30
Rubisco LSMT	23.9	22.8	1.1	First methyl transfer	29
	20.5	22.0	-1.5	Second methyl transfer	29
Histone lysine methyltransferase SET7/9	20.9	19.0	1.9	First methyl transfer	22

The average of the differences is 0.6 ± 0.9 . The $\Delta G_E^\ddagger$ values were determined from the experimental k_{cat} by use of the equation $k_{cat} = (k_B T/h) \exp(-\Delta G_E^\ddagger/RT)$. The $\Delta G_C^\ddagger$ is obtained by using the equation $\Delta G_C^\ddagger = \Delta E_C^\ddagger + \Delta(ZPE)_C^\ddagger - T\Delta S_C^\ddagger + \Delta E_{vib,C}^\ddagger$. $\Delta E_C^\ddagger$ is from the MP2/6-31 + G(d, p)//MM single calculations; the vibrational contributions ($\Delta(ZPE)_C^\ddagger$, $T\Delta S_C^\ddagger$, $\Delta E_{vib,C}^\ddagger$) are provided by normal mode analysis. The details of the computational methods are described in refs. 22, 29, and 30.

QM/MM calculated free-energy barrier for methyl transfer reactions (Scheme 1) are in reasonable agreement with the values determined from the experimental rate constants. The transition state structures for the methyl transfer step catalyzed by SET-domain enzymes are in accord with a linear S_N2 mechanism.

Materials and Methods

The initial structure is built based on the available crystal structure. Molecular dynamics simulations are carried out with the default parameter implemented in CHARMM. Snapshots extracted from the MD trajectory are used as initial QM/MM structure. The SCCDFTB is used. The bond $S_6(\text{AdoMet})\text{-C}\gamma(\text{AdoMet})$ and $\text{C}\gamma(\text{AdoMet})\text{-N(Lys)}$ are the two-dimensional reaction coordinates. The transi-

Fig. 7. Schematic pictures of the transition state for the first methyl transfer step Lys-NH₂ + ⁺AdoMet → Lys-N(Me)H₂⁺ + AdoHcy (A), the second methyl transfer step Lys-N(Me)H + ⁺AdoMet → Lys-N(Me)₂H⁺ + AdoHcy (B), and the third methyl transfer step Lys-N(Me)₂ + ⁺AdoMet → Lys-N(Me)₃⁺ + AdoHcy (C).

tion state is refined by the CPR technique and characterized by only one imaginary frequency from normal mode analysis. The single-point computations at the MP2/6-31+G(d,p)/MM level were carried out based on the structure by SCCD-FTB/MM to obtain the more accurate potential barrier. The vibrational contributions [$\Delta(\text{ZPE})$, ΔE_{vib} , and $-\Delta\Delta S$] were determined with harmonic approximation at 298 K by normal mode analysis. Thus, the calculated free-energy barrier is obtained by using the equation $\Delta G^\ddagger_c = \Delta E^\ddagger_c + \Delta E_{\text{vib}}^\ddagger_c + \Delta(\text{ZPE})^\ddagger_c - T\Delta S^\ddagger_c$. The average calculated free-energy barriers are listed in Table 3. The experimental free-energy barrier $\Delta G^\ddagger_E$ for the enzymatic reaction is determined from the

experimental rate constant by use of the equation $k_{\text{cat}} = (k_B T/h) e^{-\Delta G^\ddagger/RT}$, in which k_{cat} is the experimental rate constant, k_B is the Boltzman constant, h is Planck's constant, T is the temperature, and R is the gas constant. $\Delta G^\ddagger_E$ is the experimental free-energy barrier. The details of the computational methods are described in refs. 22, 29, and 30.

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