Histone Lysine Methyltransferase SET7/9: Formation of a Water Channel Precedes Each Methyl Transfer[†]

Xiaodong Zhang and Thomas C. Bruice*

Department of Chemistry & Biochemistry, University of California at Santa Barbara, Santa Barbara, California 93106

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ABSTRACT: Molecular dynamics (MD) simulations and hybrid quantum mechanics/molecular mechanics (QM/MM) calculations have been carried out in an investigation of histone lysine methyltransferase (SET7/9). Proton dissociation (SET7/9·Lys4-NH_3^+·AdoMet → SET7/9·Lys4-NH_2·AdoMet + H^+) must be prior to the methylation by *S*-adenosylmethionine (AdoMet). We find that a water channel is formed to allow escape of the proton to solvent. The water channel appears in the presence of AdoMet, but is not present in the species SET7/9·Lys4-NH_3^+ or SET7/9·Lys4-N(Me)H_2^+·AdoHcy. A water channel is not formed in the ground state of SET7/9·Lys4-N(Me)H_2^+·AdoMet, and the second methyl transfer does not occur. The structure of SET7/9·Lys4-N(Me)H_2^+·AdoMet includes a greater distance (6.1 ± 0.3 Å) between Cγ(AdoMet) and N(MeLys4) than is present in SET7/9·Lys4-NH_3^+·AdoMet (5.7 ± 0.2 Å). The electrostatic interactions between the positive charges on AdoMet and SET7/9·Lys4-NH_3^+ 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·Lys4-N(Me)H_2^+·AdoMet species. The formation, or not, of a water channel, the distance between S$_\delta$(AdoMet) and N(Lys4), and the angle S$_\delta$(AdoMet)−Cγ(AdoMet)−N(Lys4) determine whether methyl transfer can occur. By QM/MM, the calculated free energy barrier of the methyl transfer reaction in the SET7/9 [Lys4-NH_2 + AdoMet → Lys4-N(Me)H_2^+ + AdoHcy] complex is $\Delta G^{\ddagger} = 19.0 \pm 1.6$ kcal/mol. This $\Delta G^{\ddagger}$ is in agreement with the value of 20.9 kcal/mol calculated from the experimental rate constant (0.24 min^{-1}).

S-Adenosylmethionine (AdoMet)[1] is the common methyl donor in the methyl transfer enzymes including M.*Hha*I DNA methyltransferase (*1*, *2*), guanidinoacetate methyltransferase (*3*), and protein lysine methyltransferases (PKMTs) (*4*−*17*). Most of the known PKMTs possess the conserved SET domain. The cofactor AdoMet and substrate bind at two adjacent sites of the conserved SET domain and are connected by a pore. The methylation by SET-domain enzymes is an important reaction to regulate chromatin structure, gene silencing, transcription activation, and other functions in eukaryotic genomes (*18*). The absence of the site-specific methyltransferase enzyme activity is the origin of human diseases, notably cancer (*19*).

Each SET-domain PKMT catalyzes mono-, di-, or trimethylations (known as product specificity) shown in Scheme 1. For a methylation reaction to take place, the protonated lysine substrate must be ionized for methylation by AdoMet (Scheme 1). One crucial question is how the positively charged protonated lysine substrate is, with a pK_a of ≥10.0, deprotonated at pH 8.0 so that methylation can occur. The electrostatic interactions of the positively protonated amine with adjacent positively charged AdoMet lower the pK_a of the protonated amine, allowing proton dissociation and escape of the proton via the water channel. We (*20*, *21*) have shown that the formation of a water channel is required for deprotonation of the positively charged (methylated or dimethylated) target lysine residue for each methyl transfer reaction to occur (Scheme 2). If a water channel (Scheme 2) is not present, methyl transfer does not occur. Xiao et al. (*16*) have hypothesized that the bulk solvent might play an important role in dissociation of the proton of the positively charged (methylated) lysine. We (*20*, *21*) have pointed out that the active site fits tightly to the reactants such that bulk water is not available prior to formation of a water channel. Dirk et al. (*22*) suggested that a water molecule could be the proton acceptor because it is found in the active site of many SET-domain protein lysine methyltransferases. A water molecule neighboring the positively charged lysine could not, by itself, be the proton acceptor because H_3O^+ is a much stronger acid than Lys-$CH_2NH_3^+$. This water becomes the starting point of a water channel. In addition to deprotonation, Dirk et al. (*22*) suggested that the reorganization of the target lysine ϵ-amine group is necessary for the subsequent methylation in protein lysine methyltransferases. Zhou and coworkers (*13*) employed the mutagensis technique and enzyme kinetics analysis to conclude that the general-base mechanism is unlikely for methylation by SET domain enzymes. Recently, Guo et al. (*23*) proposed that the conserved tyrosine 335 exists as a phenolate at the active site of SET7/9 and functions as a base to deprotonate methylated Lys4-N(Me)-H_2^+ in the SET7/9·Lys4-N(Me)H_2^+ complex (Scheme 3), and the free energy barrier for SET7/9·AdoMet·Lys4-

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* To whom correspondence should be addressed. E-mail: tcbruice@chem.ucsb.edu. Phone: (805) 893-2044. Fax: (805) 893-2229.

[1] Abbreviations: PKMT, protein lysine methyltransferase; SET7/9, histone lysine methyltransferase; AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosyl-L-homocysteine; MD, molecular dynamics; QM/MM, quantum mechanics/molecular mechanics; SCCDFTB, self-consistent-charge density-functional tight-binding; CPR, conjugate peak refinement; TS, transition state.

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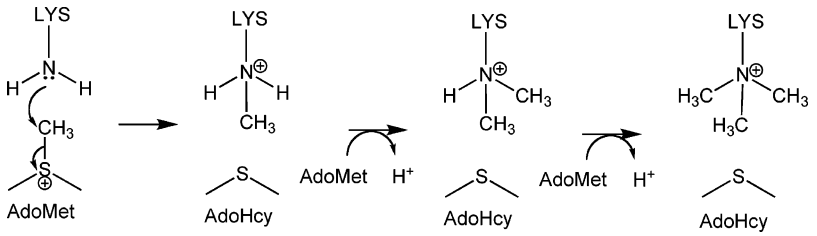
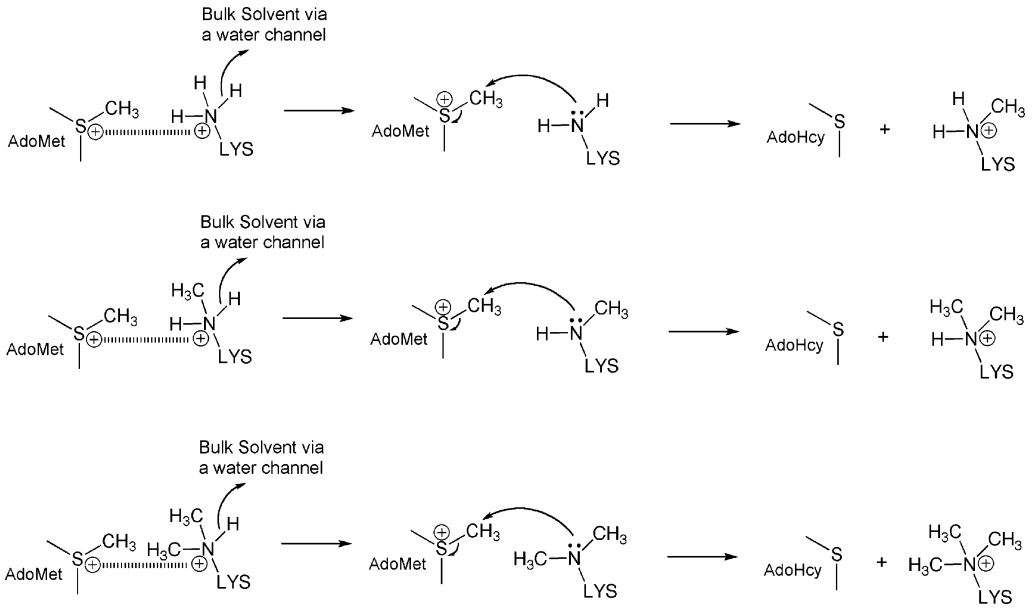
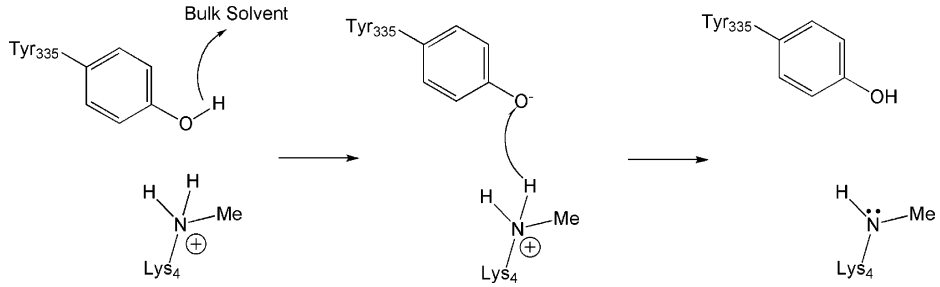
Scheme 1



Scheme 2



Scheme 3



N(Me)H → SET7/9·AdoMet·Lys4-N(Me)$_2$H$^+$ is calculated to be 22−23 kcal/mol. This allowed free energy barrier is in contrast with the fact that SET7/9 is a monomethyltransferase.

SET/9 only catalyzes the transfer of one methyl group to the amino acid lysine (Scheme 1), and the crystal structures of SET7/9 ternary complexes with peptide substrates and AdoHcy reveal that the lysine substrate is tightly bound in the active site. In the present study, we employ hybrid quantum mechanical/molecular mechanical (QM/MM) and molecular dynamics (MD) simulations to investigate the reaction mechanism and the nature of SET7/9 as a monomethyltransferase. Our calculations establish that the formation of a water channel is required for the lysine methylation by AdoMet and a further methylation step does not occur because there is the absence of formation of a water channel.

MATERIALS AND METHODS

The initial structure of the SET7/9·Lys4·AdoMet complex was built from the X-ray structure of the SET7/9 enzyme with AdoHcy and the methylated Lys (MeLys4) peptide substrate (PDB entry 1O9S; *6*). The methyl group of enzyme-bound AdoMet was built on the basis of the SET7/9·MeLys4·AdoHcy structure. The crystal water molecules within the 3.0 Å around the protein, cofactor, and substrate have been kept and are denoted by Wat. The numbers of residues and crystal waters come from the crystal structure (PDB 1O9S). There are two SET7/9 molecules in the asymmetric unit of a cell (*6*), and a monomer SET7/9 exists in solution. The distance between the two active sites in the asymmetric unit is more than 45 Å. Thus, a single monomer was employed in this study.

We have described the MD procedure and QM/MM protocol in previous papers (*20*, *21*). Here, the exact same methods involving the criteria of the minimization, time-step (1 fs), SHAKE technique (*24*) in stochastic boundary molecular dynamics (SBMD) (*25*) simulation were applied on each complex, including SET7/9·Lys4-NH$_3^+$, SET7/9·Lys4-NH$_3^+$·AdoMet, SET7/9·Lys4-NH$_2$·AdoMet, SET7/9·Lys4-N(Me)H$_2^+$·AdoHcy, SET7/9·Lys4-N(Me)H$_2^+$, SET7/

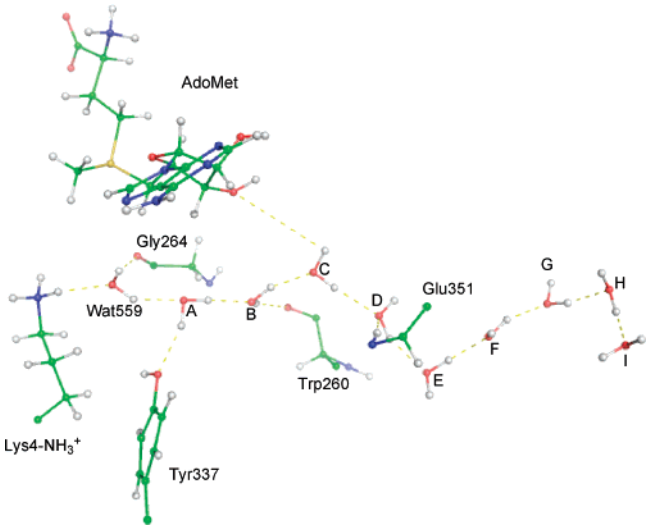


FIGURE 1: Hydrogen-bonding network around the water channel formed on the SET7/9·Lys4-NH_3^+·AdoMet complex. The solvent water molecules are designated by A−I, and I is on the surface of the water sphere with a 25 Å radius. The crystal water molecule is denoted Wat559.

9·Lys4-N(Me)H_2^+·AdoMet, and SET7/9·Lys4-N(Me)H·AdoMet during 3.0 ns. The same water channel conformation is used, i.e., a distance of 1.85 Å and the average densities of the water molecules. The same parameters were applied in computing the pK_a values of Lys4-NH_3^+ in SET7/9·Lys4-NH_3^+ and SET7/9·Lys4-NH_3^+·AdoMet as well as Lys4-N(Me)H_2^+ in both SET7/9·Lys4-N(Me)H_2^+·AdoMet and SET7/9[Y245F]·Lys4-N(Me)H_2^+·AdoMet complexes and of Tyr335 in SET7/9·Lys4-NH_3^+ and SET7/9·Lys4-NH_3^+·AdoMet as well as Lys4-N(Me)H_2^+ complexes.

The same QM/MM protocol, involving the extraction of the snapshots, defining of the reaction coordinates, QM formulism (SCCDFTB (*26*, *27*), which is validated for this type of methyl transfer reaction (*21*)), QM region, choice of link atoms, adiabatic mapping, refinement of the transitions state (*28*), characterization of the transition states, and calibration of the free energy barrier (*29*, *30*), is applied on the SET7/9·Lys4-NH_2·AdoMet and SET7/9·Lys4-N(Me)H·AdoMet complexes.

RESULTS AND DISCUSSION

Deprotonation of Lys4-NH_3^+. Throughout 3 ns of MD simulations of the SET7/9·Lys4-NH_3^+·AdoMet structure, a water channel is positioned to allow proton transfer from Lys4-NH_3^+ to the water solvent (Figure 1). The residues within 3.0 Å around the water channel are shown in stick−ball format in Figure 1. The hydrogen bonding between channel water (<1.85 Å) and the average densities (Table 1) in the channel water molecules confirm the formation of a channel of nine waters. Calculations (see the Materials and Methods) establish that the average pK_a of the Lys4-NH_3^+ in the complex SET7/9·Lys4-NH_3^+ is 10.9 ± 0.4. After the cofactor AdoMet enters, the average pK_a of the Lys4-NH_3^+ in the complex SET7/9·Lys4-NH_3^+·AdoMet decreases to 8.2 ± 0.6. While the calculated pK_a values of Tyr335 in SET7/9·Lys4-NH_3^+ and SET7/9·Lys4-NH_3^+·AdoMet are 14.3 ± 3.0 and 16.6 ± 4.2, respectively. The computations do not support the suggestion by Guo et al. (*23*) that Tyr335 is the general base in deprotonating the positively charged Lys4-NH_3^+. Thus, it is the charge repulsion between Lys4-NH_3^+ and the $-CH_2S^+(Me)CH_2-$ part of the AdoMet pair that is responsible for the decrease in the pK_a of the Lys4-NH_3^+ to 8.2 ± 0.6, and by some means, it creates a water channel to allow the proton dissociation from Lys4-NH_3^+ into solvent to form the neutral Lys4-NH_2 substrate for methylation.

Lys4-NH_3^+ is hydrogen bonded to the first water molecule (Wat559) of the water channel (Figure 1). The crystal water molecule (Wat559) suggested by Dirk et al. (*22*) to be the proton acceptor takes this role at the formation of a water channel. When a hydroxide ion denoted by C is positioned at the water channel (Figure 1), there is no barrier for proton transfer from Lys4-NH_3^+ to HO^- at the QM/MM level (QM = both SCCDFTB and HF/6-31+G(d,p)). Because the concentration of HO^- at optimal pH 8.0 is 10^{-6} M, the activation energy barrier for proton dissociation would be 8.4 kcal/mol. Examination of the environment at the termination of the water channel shows that there is no general-base candidate to dissociate the proton of Lys4-NH_3^+ as suggested by Zhou and co-workers (*31*). Thus, only after formation of a water channel does the bulk solvent play an important role in dissociation of the proton of Lys4-NH_3^+ (*16*). Also, the 3 ns MD simulations on the SET7/9·Lys4-NH_3^+·AdoMet complex show that the distance of OH(Tyr335)···Nϵ(Lys4-NH_3^+) is 4.19 ± 0.31 Å, the average occupancy of the hydrogen bond between OH(Tyr335) and Hϵ(Lys4-NH_3^+) is less than 0.003, and the pK_a of Tyr335 in SET7/9·Lys4-NH_3^+·AdoMet is estimated to be 16.6 ± 4.2. Thus, Tyr335-O^- cannot play a role as a general base in deprotonating the positively charged Lys4-NH_3^+. The only interpretation allowed is that the formation of a water channel assisted by AdoMet controls the product specificity of protein lysine methyltransferases.

Free Energy Profile for the First Methyl Transfer Reaction. The reaction coordinates for the first methyl transfer reaction [SET7/9·Lys4-NH_2·AdoMet → SET7/9·Lys4-N(Me)-H_2^+·AdoHcy] are shown in Figure 2. The structure of the reactant state is shown in Figure 3A. The key geometric parameters (Scheme 4) are in reasonable agreement with

Table 1: Average Density (atoms/Å^3) of the Water Molecules at the Positions of the Water Channel (Shown in Figures 1 and 5) during the MD Simulations on Complexes SET7/9·Lys4-NH_3^+·AdoMet and SET7/9[Y245F]·Lys4-N(Me)H_2^+·AdoMet[a]

Complex SET7/9·Lys4-NH_3^+·AdoMet (Figure 1)										
position	Wat559	A	B	C	D	E	F	G	H	I
density	0.006	0.009	0.011	0.017	0.019	0.022	0.026	0.028	0.027	0.024

Mutated Complex SET7/9[Y245F]·Lys4-N(Me)H_2^+·AdoMet (Figure 5)										
position	Wat565	Wat660	Wat559	A	B	C	D	E	F	G	H
density	0.007	0.007	0.011	0.014	0.017	0.020	0.015	0.026	0.028	0.028	0.021

[a] 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.

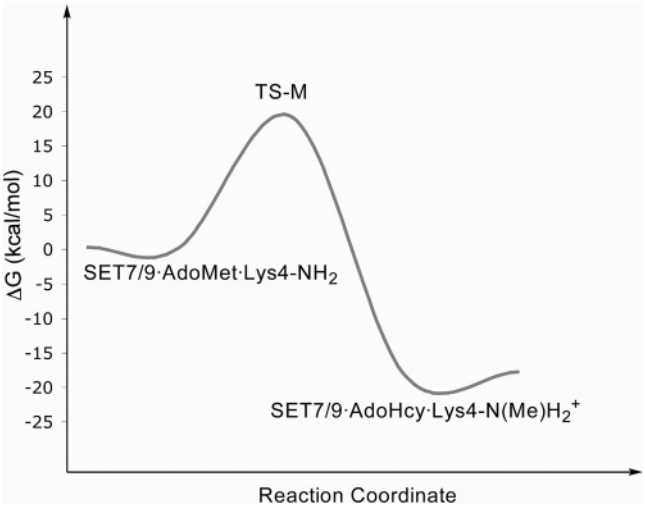
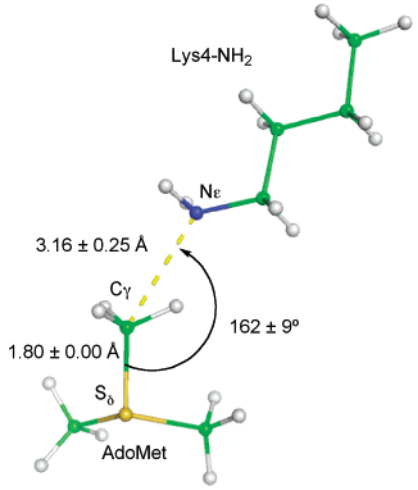


FIGURE 2: Schematic effective free energy surface for the methyl transfer reaction Lys4-NH_2 + AdoMet → Lys4-N(Me)H_2^+ + AdoHcy catalyzed by SET7/9.

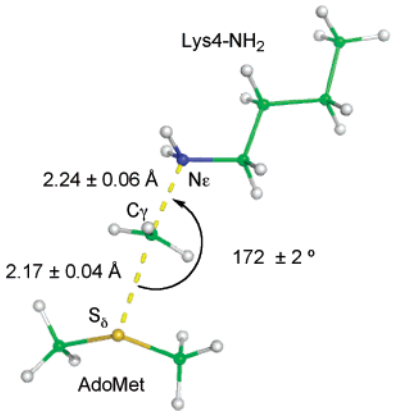
Scheme 4



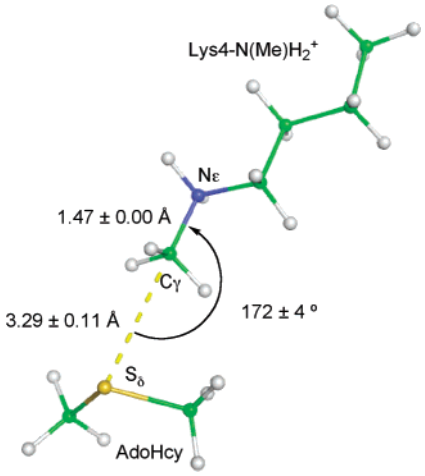
those obtained by Hu et al. at the B3LYP/6-31G*//MM level (*32*).

Normal-mode analysis characterizes the transition state of the first methylation step (TS-M) with only one imaginary frequency of 268 ± 97i cm^{-1}. The key geometric parameters of the TS-M are shown in Scheme 5, and the key hydrogen bonds around the reaction zone are shown in Figure 3B. Also, the linear S_δ(AdoMet)···C_γ(AdoMet)···N_ϵ(Lys4-NH_2) configuration (Scheme 5) in the transition state favors a concerted S_N2 mechanism (*33*). The average computed free energy barrier of the methyl transfer reaction is $\Delta G^\ddagger = \Delta E^\ddagger + \Delta(\mathrm{ZPE})^\ddagger - T\Delta S^\ddagger + \Delta E_{vib}{}^\ddagger = 19.0 - 0.58 + 0.82 - 0.22 = 19.0$ kcal/mol (Figure 2), which is in agreement with the free energy barrier (20.9 kcal/mol) calculated from the experimental rate constant (0.24 min^{-1}) (*34*). The deviations of $\Delta G^\ddagger$, $\Delta E^\ddagger$, $\Delta(\mathrm{ZPE})^\ddagger$, $T\Delta S^\ddagger$, and $\Delta E_{vib}{}^\ddagger$ are ±1.6, ±1.4, ±0.4, ±0.5, and ±0.2 kcal/mol, respectively. Comparison of $\Delta G^\ddagger$ for the methyl transfer step (∼19.0 kcal/mol) to that for proton transfer (∼8.4 kcal/mol) shows that the latter is not rate controlling. Also, compared with the calculated free energy (30.9 ± 0.2 kcal/mol) (*35*) of the corresponding methyl transfer reaction in aqueous solution, the enzyme reduces the barrier by 11.9 kcal/mol for this methyl transfer

Scheme 5



Scheme 6



step. This indicates that the enzyme enhances this methyl transfer reaction by ∼10^8.

The enzyme-catalyzed methyl transfer reaction is calculated to be exergonic overall: $\Delta G^\circ = \Delta E^\circ + \Delta(\mathrm{ZPE})^\circ - T\Delta S^\circ + \Delta E_{vib}{}^\circ = -20.3 + 2.5 - 0.8 + 0.1 = -18.5$ kcal/mol (Figure 2). The deviations in ΔG°, ΔE°, $\Delta(\mathrm{ZPE})^\circ$, $T\Delta S^\circ$, and $\Delta E_{vib}{}^\circ$ are ±3.2, ±3.1, ±0.3, ±0.6, and ±0.1 kcal/mol, respectively. The structure of the product is shown in Figure 3C, and the key geometric parameters of the product are listed in Scheme 6. On comparison with the immediate product of the first methyl transfer reaction by LSMT (153 ± 6°) (*20*) and vSET (153 ± 12°) (*21*), the linear N(Lys4-N(Me)H_2^+)···C_γ(Lys4-N(Me)H_2^+)···S_δ(AdoHcy) configuration (Scheme 6) is not favorable for a future methylation reaction. As seen in the crystal structure of SET7/9·Lys4-N(Me)H_2^+·AdoHcy, the linear N(Lys4-N(Me)H_2^+)···C_γ-(Lys4-N(Me)H_2^+)···S_δ(AdoHcy) configuration and the hydrogen bonds between two tyrosine residues (Tyr245 and Tyr305) and Lys4-N(Me)H_2^+ are unfavorable for formation of a water channel in the complex SET7/9·Lys4-N(Me)H_2^+·AdoMet due to a lack of rearrangement of the active site.

Ease of Water Channel Formation Relates to the Number of Transferred Methyl Group. MD simulations (3 ns) on the product SET7/9·Lys4-N(Me)H_2^+·AdoHcy of the first methyl transfer reaction fail to show a water channel to allow proton dissociation from Lys4-N(Me)H_2^+ when AdoHcy is present. In addition, a water channel does not form in either SET7/9·Lys4-N(Me)H_2^+ or, most importantly, SET7/9·Lys4-N(Me)-H_2^+·AdoMet. Also, the pK_a of Tyr335 in SET7/9·Lys4-

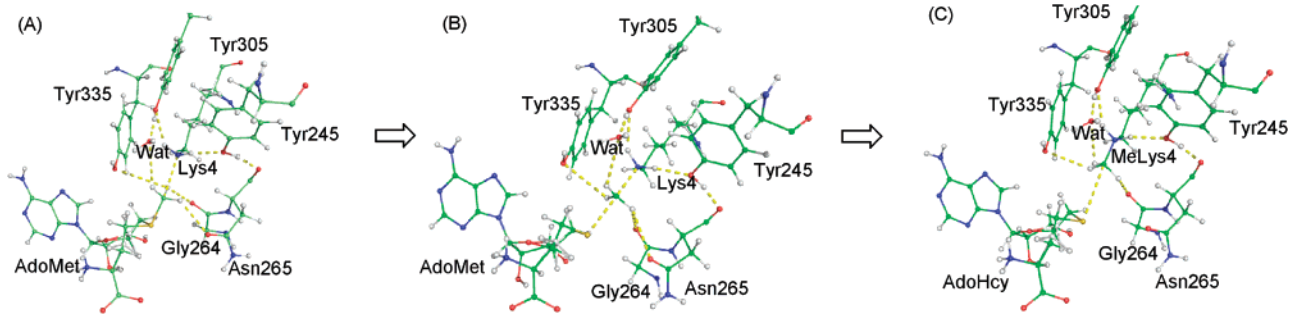


FIGURE 3: Enzyme structure and the hydrogen-bonding networks of (A) the ground state SET7/9·Lys4-NH_2·AdoMet, (B) the transition state (TS-M), and (C) the product SET7/9·Lys4-N(Me)H_2^+·AdoHcyin of the methyl transfer reaction SET7/9·Lys4-NH_2·AdoMet → SET7/9·Lys4-N(Me)H_2^+·AdoHcy.

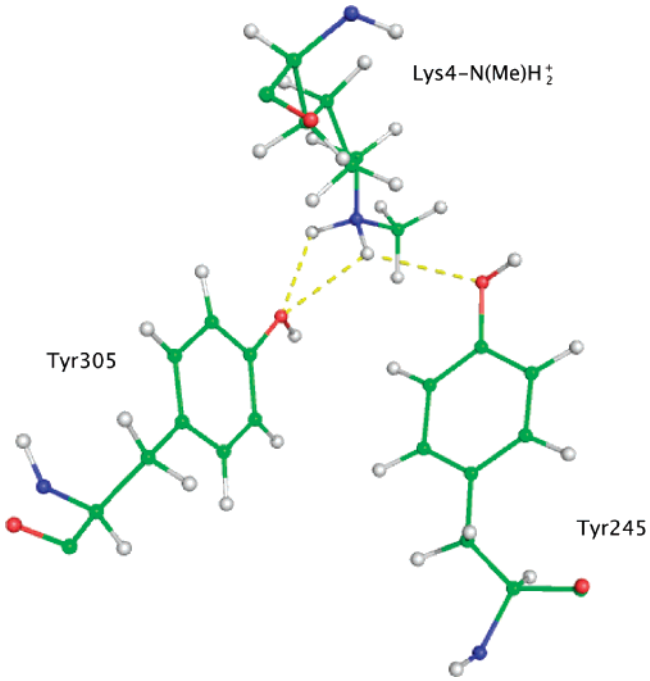


FIGURE 4: Hydrogen bonds between methylated lysine and the tyrosine residues at the active site of the complex SET7/9·Lys4-N(Me)H_2^+·AdoMet. The hydrogen-bonding distances between Hϵ^1-(MeLys4)-OH(Tyr245) and Hϵ^2(MeLys4)-OH(Tyr305) are 1.87 ± 0.11 and 1.92 ± 0.13 Å, respectively.

N(Me)H_2^+ is calculated to be 13.7 ± 2.4. This does not support the suggestion by Guo et al. (*23*) that Tyr335-O^- could be the general base in deprotonating the positively charged Lys4-NH_3^+ after release of AdoHcy and before binding of AdoMet. The calculated pK_a (14.7 ± 4.9) of Lys4-N(Me)H_2^+ in SET7/9·Lys4-N(Me)H_2^+·AdoMet is comparable to that in the absence of AdoMet. This is due to the substrate and cofactor being more separated than those in SET7/9·Lys4-NH_3^+·AdoMet (6.1 ± 0.3 Å vs 5.7 ± 0.2 Å). In the latter case the pK_a of the substrate is lowered by electrostatic interaction of the positive charges of the cofactor and substrate.

MD simulations on SET7/9·Lys4-N(Me)H_2^+·AdoMet show that the hydrogen bonds between Lys4-N(Me)H_2^+ and tyrosine residues (Tyr245 and Tyr305) (Figure 4) are very stable. The hydrogen-bonding analysis shows that the average occupancies are 0.99 and 0.98, respectively. However, the corresponding hydrogen bonds in SET7/9·Lys4-NH_3^+·AdoMet are not very stable, and the corresponding average occupancies are 0.33 and 0.47, respectively. The much higher occupancy of hydrogen bonds between two tyrosine residues (Tyr245 and Tyr305) and the target substrate (Lys4-N(Me)H_2^+) in the complex SET7/9·Lys4-N(Me)H_2^+·AdoMet is unfavorable for optimal orientation of the active site for the second methyl transfer step as compared with the lower occupancy in SET7/9·Lys4-NH_3^+·AdoMet.

Mutation of Tyr245 into Phe provides SET7/9[Y245F]·Lys4-N(Me)H_2^+·AdoMet, and MD simulation establishes the presence of a water channel (Figure 5), as indicated by the average densities (Table 1). The crystal water molecules (Wat565, Wat660, and Wat559) are only in the starting points of a water channel. The residues within 3.0 Å around the water channel are shown in Figure 5 in a stick−ball representation. Also in this mutated complex SET7/9[Y245F]·Lys4-N(Me)H_2^+·AdoMet, the calculated pK_a (5.7 ± 1.5) of Lys4-N(Me)H_2^+ is significantly depressed. There is no general base, the proton from Lys4-N(Me)$_2$H$^+$ in this mutated complex is delivered into the solvent via the formed water channel, and the neutral substrate for the second methyl transfer reaction is created. These observations are in agreement with the experimental observation by Cheng et al. (*36*) that the second methyl transfer reaction occurs when either Tyr245 or Tyr305 is mutated into a phenylalanine. The results of the computational simulations of the mutant Y245F essentially validate the definitive conclusion that the formation of the water channel is required for methylation and product specificity of SET7/9.

Free Energy Profile for the "Supposed" Second Methyl Transfer Reaction. Assuming a second methyl transfer reaction, the calculated average free energy by QM/MM would be $\Delta G^{\ddagger} = \Delta E^{\ddagger} + \Delta(\mathrm{ZPE})^{\ddagger} - T\Delta S^{\ddagger} + \Delta E_{vib}{}^{\ddagger} = 19.8 + 0.4 + 1.4 - 0.5 = 21.1$ kcal/mol. The deviations of $\Delta G^{\ddagger}$, $\Delta E^{\ddagger}$, $\Delta(\mathrm{ZPE})^{\ddagger}$, $T\Delta S^{\ddagger}$, and $\Delta E_{vib}{}^{\ddagger}$ are ±5.1, ±5.4, ±0.5, ±1.2, and ±0.3 kcal/mol, respectively. Because a water channel does not form to allow proton dissociation of Lys4-N(Me)H_2^+, the second methylation SET7/9·Lys4-N(Me)H·AdoMet → SET7/9·Lys4-N(Me)$_2$H$^+$·AdoHcy (Scheme 1) does not occur, although this step has an allowed free energy barrier (21.1 ± 5.1 kcal/mol).

Comparison with the Previous Theoretical Investigations. The calculated free energy barrier ($\Delta G^{\ddagger} = 19.0 \pm 1.6$ kcal/mol) for the methyl transfer reaction by SET7/9 is in reasonable accord with the value of 20.9 kcal/mol calculated from the experimental rate constant ($k_{cat} = 0.24$ min^{-1}), also in agreement with 20.4 ± 1.1 kcal/mol by the QM/MM free energy perturbation method (*32*) and 17−18 kcal/mol by the potential of mean force technique (*23*). However, the calculated average pK_a values (>13.0) of the conserved residue Tyr335 in both SET7/9·Lys4-NH_3^+ and SET7/9·

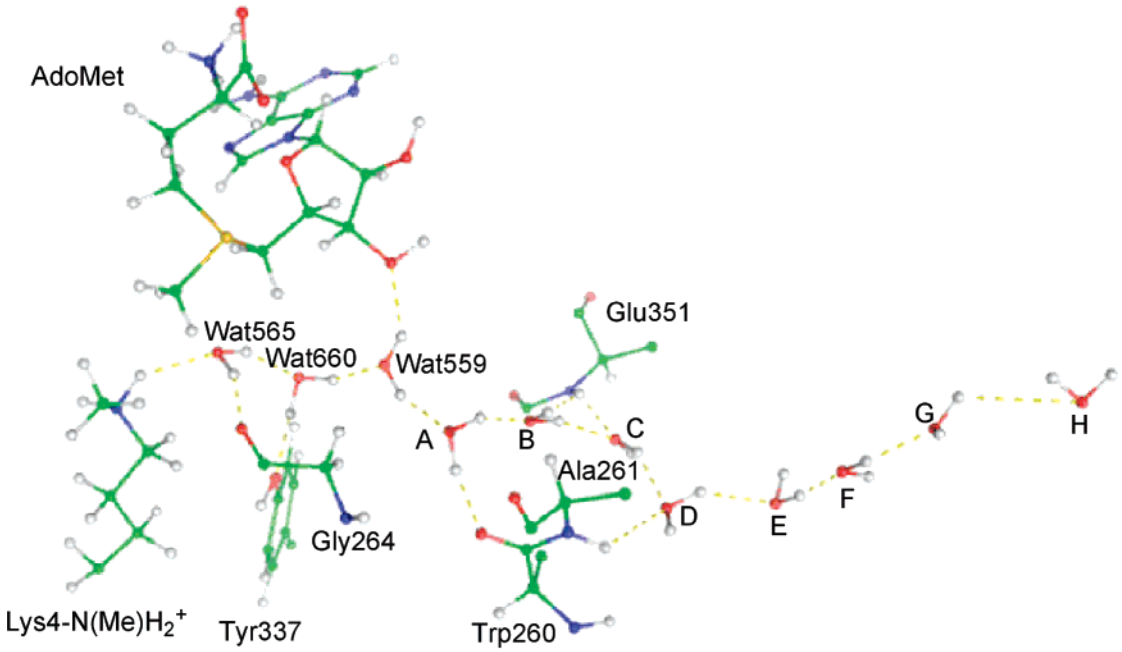


FIGURE 5: Hydrogen-bonding network around the water channel created on formation of the SET7/9[Y245F]·Lys4-NH_3^+·AdoMet complex. The solvent water molecules are designated by A−H, and H is on the surface of the water sphere with a 25 Å radius. The crystal water molecules are denoted Wat565, Wat660, and Wat559.

Lys4-$N(Me)H_2^+$ do not support the general-base mechanism proposed by Guo et al. (*23*). The calculated $\Delta G^{\ddagger}$ (21.1 ± 5.1 kcal/mol (this study) and 22−23 kcal/mol (*23*)) is allowed for the supposed second methyl transfer reaction (SET7/9·Lys4-N(Me)H·AdoMet → SET7/9·Lys4-$N(Me)_2H^+$·AdoHcy). However, experiments confirm that the second methyl transfer reaction does not occur. The only interpretation allowed is the absence of a water channel.

CONCLUSIONS

This and previous studies from our laboratory (*20*, *21*) establish that the presence of a water channel is required for proton dissociation of protonated lysine substrate prior to its methylation by AdoMet in the protein lysine methyltransferases. The calculated pK_a (>13.0) of Tyr335 indicates that Tyr335-O^- does not exist in the optimum pH environment. The deprotonation step (SET7/9·Lys-NH_3^+·AdoMet → SET7/9·Lys-NH_2·AdoMet + H^+) is preceded by formation of a water channel that allows passing of the proton to solvent water. Thus, the formation of a water channel determines whether methylation can occur. The water channel appears in the presence of AdoMet (SET7/9·Lys4-NH_3^+·AdoMet), but is not present in the product (SET7/9·Lys4-$N(Me)H_2^+$·AdoHcy). Our QM/MM-calculated free energy barrier for the first methyl transfer reaction (SET7/9·Lys4-NH_2·AdoMet → SET7/9·Lys4-$N(Me)H_2^+$·AdoHcy) is 19.0 ± 1.6 kcal/mol. This is in excellent agreement with the free energy barrier of 20.9 kcal/mol calculated from the experimental rate constant (0.24 min^{-1}) and those from previous theoretical investigations (20.4 ± 1.1 kcal/mol (*32*) and 17−18 kcal/mol (*23*)). The second methyl transfer reaction does not occur due to the lack of the possibility of proton dissociation from SET7/9·Lys4-$N(Me)H_2^+$·AdoMet. This is due to the absence of a water channel. Compared with the average pK_a (8.2 ± 0.6) of Lys4-NH_3^+ in the complex SET7/9·Lys4-NH_3^+·AdoMet, the calculated pK_a (14.7 ± 4.9) is higher for Lys4-$N(Me)H_2^+$ in the SET7/9·Lys4-$N(Me)H_2^+$·AdoMet complex. This is due to the different electrostatic interaction of the positive charges on AdoMet and the protonated lysine species. The average distance between S_δ(AdoMet) and N(Lys4) in SET7/9·Lys4-$N(Me)H_2^+$·AdoMet (6.1 ± 0.3 Å) is greater that that in SET7/9·Lys4-NH_3^+·AdoMet (5.7 ± 0.2 Å). Also, the angle S_δ(AdoMet)−C_γ(AdoMet)−N(Lys4) in SET7/9·Lys4-N(Me)-H_2^+·AdoMet (112 ± 17°) is greater than that in SET7/9·Lys4-NH_3^+·AdoMet (90 ± 10°). The electrostatic interaction between the positive charges of the cofactor and substrate, the pK_a of the substrate, the formation of a water channel or not, and the hydrogen bonds between the tyrosine residues at the active site (Tyr245 and Tyr305) and the substrate control the product specificity such that the second methyl transfer reaction does not occur.

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