S-Adenosylmethionine Conformations in Solution and in Protein Complexes: Conformational Influences of the Sulfonium Group†

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Received February 14, 2002; Revised Manuscript Received April 23, 2002

ABSTRACT: *S*-Adenosylmethionine (AdoMet) and other sulfonium ions play central roles in the metabolism of all organisms. The conformational preferences of AdoMet and two other biologically important sulfonium ions, *S*-methylmethionine and dimethylsulfoniopropionic acid, have been investigated by NMR and computational studies. Molecular mechanics parameters for the sulfonium center have been developed for the AMBER force field to permit analysis of NMR results and to enable comparison of the relative energies of the different conformations of AdoMet that have been found in crystal structures of complexes with proteins. *S*-Methylmethionine and *S*-dimethylsulfoniopropionate adopt a variety of conformations in aqueous solution; a conformation with an electrostatic interaction between the sulfonium sulfur and the carboxylate group is not noticeably favored, in contrast to the preferred conformation found by in vacuo calculations. Nuclear Overhauser effect measurements and computational results for AdoMet indicate a predominantly anti conformation about the glycosidic bond with a variety of conformations about the methionyl $C_\alpha-C_\beta$ and $C_\beta-C_\gamma$ bonds. An AdoMet conformation in which the positively charged sulfonium sulfur is near an electronegative oxygen in the ribose ring is common. Comparisons of NMR results for AdoMet with those for the uncharged *S*-adenosylhomocysteine and 5′-methylthioadenosine, and the anionic ATP, indicate that the solution conformations are not dictated mainly by molecular charge. In 20 reported structures of AdoMet·protein complexes, both anti and syn glycosidic torsional angles are found. The methionyl group typically adopts an extended conformation in complexes with enzymes that transfer the methyl group from the sulfonium center, but is more folded in complexes with proteins that do not catalyze reactions involving the sulfur and which can use the sulfonium sulfur solely as a binding site. The conformational energies of AdoMet in these crystal structures are comparable to those found for AdoMet in solution. The sulfonium sulfur is in van der Waals contact with a protein heteroatom in the structures of four proteins, which reflects an energetically favorable contact. Interactions of the sulfonium with aromatic rings are rarely observed.

Sulfonium ions play central roles in the metabolism of all known organisms. Since sulfonium compounds were discovered in marine algae (*1*), the diversity of their functions throughout biological systems increasingly has been recognized. The most common naturally occurring sulfonium ion is *S*-adenosylmethionine (AdoMet),[1] which is the primary in vivo alkylating agent (*2*, *3*) (Figure 1). Methyl transfer from the sulfonium group of AdoMet is involved in the biosynthesis of numerous compounds, including other sulfonium ions such as the osmoregulatory agents *S*-methylmethionine (SMM), dimethylsulfoniopropionic acid [$(CH_3)_2S^+CH_2CH_2CO_2^-$ (DMSP)], and dimethylsulfonioactetate [$(CH_3)_2S^+CH_2CO_2^-$ (DMAT)] [see Figure 1 (*4*−*6*)]. Algal production of dimethyl sulfide from DMSP provides a major component of atmospheric sulfur with substantial influence on the global sulfur cycle (*7*). Methylation of nucleic acids by AdoMet has important effects on DNA replication and transcription, restriction of DNA transfer between microorganisms, and RNA function (*8*−*10*). Protein methylation is involved in the regulation of metabolic processes, including chromatin dynamics, chemotaxis, and the repair of isopeptide bonds formed by polypeptide rearrangements in aging proteins (*11*−*13*). Decarboxylation of AdoMet generates the sulfonium compound that donates the propylamine group used in the biosynthesis of the polyamines, spermidine and spermine, which are involved in the regulation of cellular proliferation (*14*). Homolytic cleavage of a $C-S^+$ bond of AdoMet yields a 5′-deoxyadenosyl free radical that is utilized in enzymatic reactions in a fashion analogous to that of the

† This work was supported by National Institutes of Health Grants GM31186 and CA06927, by the Danish Natural Science Research Council, and by an appropriation from the Commonwealth of Pennsylvania.

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[1] Abbreviations: AdoMet, *S*-adenosylmethionine; AdoHcys, *S*-adenosylhomocysteine; DMAT, dimethylthioacetic acid; DMSP, dimethylthioproprionic acid or dimethylsulfoniopropionic acid; MTA, 5′-methylthioadenosine; NOE, nuclear Overhauser effect; NOESY, two-dimensional nuclear Overhauser effect spectroscopy; ROESY, two-dimensional rotating frame nuclear Overhauser effect spectroscopy; SMM, *S*-methylmethionine.

10.1021/bi025682c CCC: $22.00
Published on Web 05/23/2002

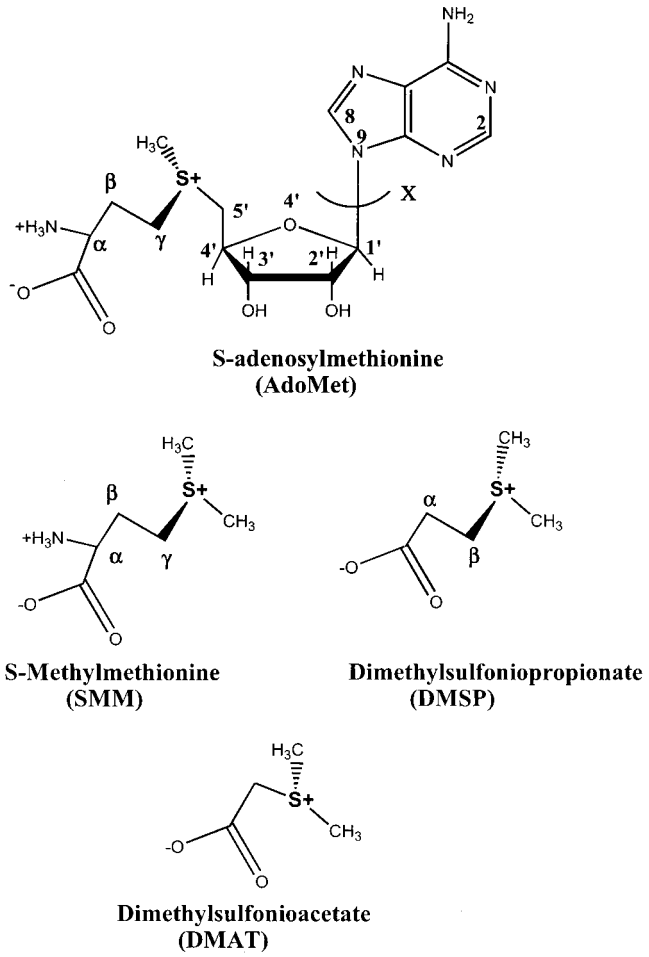

FIGURE 1: Structures and labeling of *S*-adenosylmethionine (AdoMet), dimethylsulfonioacetate (DMAT), dimethylsulfonioproprionate (DMSP), and *S*-methylmethionine (SMM). Some hydrogen atoms have been omitted for clarity.

radical formed by cleavage of the carbon−cobalt bond of coenzyme B_{12} (*15*, *16*). AdoMet is a precursor of the acylhomoserine lactones used by some bacteria to sense cell density (quorum sensing) (*17*) and is also a predecessor of the plant growth hormone ethylene (*6*). Recently, some natural products containing sulfonium ions have been reported to act as potent inhibitors of enzyme-catalyzed hydrolysis of carbohydrates, and this finding has generated additional pharmacological interest (*18*). Notably, the antitumor natural product bleomycin A2 contains a sulfonium moiety (*19*).

Despite the myriad roles of sulfonium ions in cell growth, and interest in designing analogues with potential chemotherapeutic function (*18*, *20*, *21*), remarkably little is known about the conformational preferences of AdoMet or other biological sulfonium ions. Data from ultraviolet absorption and optical rotation spectroscopy have been interpreted as indicating an anti conformation about the $N_9-C_{1'}$ glycosidic bond (i.e., the $C_8-N_9-C_{1'}-O_{4'}$ torsional angle χ as illustrated in Figure 1) (*22*−*24*). An NMR conformational analysis based on the three-bond $^1H-^1H$ couplings within the ribose moiety and the methionyl side chain of AdoMet and SMM was reported (*25*); the data were interpreted in terms of restricted rotation about the $O_{4'}-C_{4'}-C_{5'}-S$ torsional angle in AdoMet, with a preferred conformation that places the S^+ in the proximity of $O_{4'}$ (a gauche-trans relationship to $O_{4'}$ and $C_{3'}$) (*25*). This conformation is in contrast to that preferred by nucleosides and nucleotides which have an oxygen atom at the 5′ position and which prefer structures that place $O_{5'}$ above the ribose ring (in a gauche−gauche relationship to $O_{4'}$ and $C_{3'}$) (*26*−*28*). However, the NMR studies were conducted at pH 3, resulting in nonphysiological protonation states of the adenine base and the carboxyl group (*25*). In AdoMet, as well as in SMM and DMSP, a favorable electrostatic interaction could occur between the positively charged sulfonium sulfur and electron rich heteroatoms, either intramolecularly, with solvent, or within protein binding sites. Close nonbonded contacts between positively charged sulfur atoms and either oxygen or nitrogen atoms have been found in many crystal structures of small molecules (*29*, *30*), and the favorable energetics of these interactions have been substantiated by molecular orbital (MO) and density functional theory (DFT) calculations (*31*−*35*).

Three-dimensional structures of complexes of AdoMet (or a derivative) with 12 different proteins have been determined by X-ray crystallography or NMR (*36*−*53*, *81*, *83*). The present study of the relationship between the conformations of AdoMet in solution and those found in protein binding sites is complementary to ongoing investigations of other adenosine-containing cofactors such as ATP and NAD (*27*, *28*, *54*, *55*). To evaluate the energetic consequences of the conformational restrictions imposed by ligand interactions with macromolecules, a molecular mechanics approach is desirable for computational efficiency. However, common molecular mechanics force fields (such as AMBER, Charmm, MM2, MMFF, and OPLS) have not been explicitly parametrized for the sulfonium moiety. Recent molecular dynamics simulations of AdoMet complexes with catechol *O*-methyltransferase have used charge and nonbonded parameters for the sulfonium as obtained from a semiempirical calculation on AdoMet or ab initio calculations on $(CH_3)S^+$, rather than extensive parametrization (*56*, *57*).

In the study presented here, we have utilized the recently developed methodology for the automated combination of crystallographic data with MO and or DFT results (*58*) to develop parameters for the sulfonium group in the context of the widely used AMBER force field (*59*, *60*). We have employed this extended force field to compare the relative conformational energies of AdoMet bound to a variety of proteins. The results of these force field calculations are compared to the experimental aqueous solution conformational preferences of AdoMet, DMSP, and SMM as assessed by NMR using nuclear Overhauser effect (NOE) spectroscopy.

MATERIALS AND METHODS

Sulfonium Ion Crystallographic Data. Crystal structures of small sulfonium ions were extracted from the Cambridge Structural Database [CSD, 1998 version (*61*)]. The database was searched for structures of trivalent sulfur compounds in which the *R* factor was better than 10% and in which there was no disorder. The resulting structures were screened manually to remove ylids and sulfur atoms at ring bridgehead positions. Refcodes for the remaining 26 structures are provided in Table 1S of the Supporting Information.

Crystallographically determined structures of AdoMet bound to proteins were obtained from the Protein Databank

Table 1: Protein X-ray Structures with Bound *S*-Adenosylmethionine

protein	PDB entry	resolution (Å)	other ligands	no. of AdoMets
catechol-O-MTase (*40*)	1vid	2.0	dinitrocatecho1	l
*Hha*I DNA cytosine N4 MTase (*37*, *38*, *51*)	1hmy	2.5	−	1
	2hmy	2.6	DNA	1
	6mht	2.05	DNA	1
*Dpn*II DNA N6-adenine MTase (*50*)	2dpm	1.8	−	1
TaqI DNA N6-adenine MTase (*39*, *46*, *47*, *49*)	2adm	2.4	−	2
ErmC RNA N6-adenine MTase (*52*)	1qao	2.2	RNA	1
FtsJ RNA Mtase (*46*)	1eiz	1.70	−	1
	1ejo	1.50	−	1
VP39 RNA MTase (*42*)	1vpt	1.85	−	1
glycine-N-Mtase (*81*, *83*)	1xva	2.2	acetate	2
methionine synthase (*41*)	1msk	1.8	acetate	1
MetJ repressor (*36*)	1cma	2.8	DNA	2
	1cmc	1.8	DNA	2
mutant	1mjl	2.1	−	2
mutant	1mj2	2.4	DNA	4
mutant	1mjo	2.1	DNA	4
mutant	1mjq	2.4	DNA	8
AdoMet decarboxylase (*53*)	1i7b	1.9	1,4-diaminobutane	1[a]
AdoMet synthetase (*63*)	−	NMR	−	1

[a] This structure has the substrate analogue, the carboxymethyl ester of AdoMet, bound as a Schiff base to the pyruvate group of the protein.

(PDB) (*62*). The structures used, their resolutions, and other bound ligands are listed in Table 1; values for various torsional angles and selected nonbonded distances are listed in Table 2S of the Supporting Information. The analogous data for the single NMR-determined conformation of a protein-bound AdoMet (*63*) are also included.

Density Functional Theory Calculations and Force Field Parameter Development. Optimized structures, analytical vibrational frequencies, and energy dependencies on dihedral angles were calculated for $(CH_3)_3S^+$, CH_3-S^+-$(CH_2CH_3)_2$, $(CH_3)_2$-S^+-CH_2CO_2H, $(CH_3)_2$-S^+-$CH_2CO_2^-$, and $(CH_3)_2$-S^+-$CH_2CH_2CH_3$ using the GAUSSIAN-98 series of programs (*64*); the B3LYP hybrid DFT method was employed in conjunction with the 6-31G* basis set (*65*, *66*). The dihedral angles in these structures were varied in 30° increments to derive the torsional force constants.

The AMBER force field parameters were optimized using standard methods (*58*, *67*−*69*). The penalty function that was minimized consisted of a weighted sum of the squared deviations of all data points. The following weight factors were employed: 100 $Å^{-1}$ for bond lengths, 2 deg^{-1} for bond angles, 1 deg^{-1} for dihedral angles, 43 $Å^2$ for squared inverse interatomic distances, 7 kJ/mol for energy variations in rigid rotation profiles, and 0.002 kJ mol^{-1} $Å^{-2}$ for Hessian elements (75 times this value for atoms in a 1−4 relationship). In addition, all charges (computed internally from the bond moment parameter) were tethered to CHELPG charges from the DFT calculations. Parameters that had a low influence on the reference data were also subjected to a weak tethering. The AMBER parameters derived from these calculations are collected in part A of Table 3S of the Supporting Information.

Computational Studies of Conformational Preferences. The conformational preferences of AdoMet, SMM, and DMSP were investigated using a Monte Carlo simulation method. In each case, six thousand conformations were generated and all conformations within 20 kJ/mol of the lowest-energy form were examined further. The simulations were performed both in vacuo and in the presence of the generalized Born surface area (GB/SA) aqueous solvent model; this model attenuates electrostatic interactions and reduces the propensity for the formation of potentially extraneous hydrogen bonds (*70*). In each case, the lowest-energy conformer was found more than 500 times. Since explicit solvation values for the sulfonium sulfur were not available in the context of the GB/SA model, and since the dominant interaction of S^+ with the solvent will be electrostatic, the default surface parameters for sulfur were used for the calculations. There appear to be no reported experimental solvation energies for a sulfonium ion. However, comparison of calculated solvation energies for a few test cases showed good agreement with those obtained from the self-consistent reaction field (SCRF) continuum solvation method implemented in the program Jaguar, version 4.1 (*71*); the geometries were optimized at the B3LYP/6-31G* computational level in vacuo and in the water model (part B of Table 3S of the Supporting Information). This SCRF method determines the reaction field by numerical solution of the Poisson−Boltzmann equations; the solvent is represented as a layer of charges at the molecular surface.

Analysis of AdoMet Binding Sites in Protein Crystal Structures. Protein crystal structural data were analyzed with the program MacroModel, version 7.0 (*72*, *73*); this program was also employed for the molecular mechanics studies. For calculations of the relative energies of protein-bound conformers, the AdoMet ligands were extracted and modified manually to add hydrogen atoms. In all cases, the zwitterionic form of the methionyl group ($-NH_3^+$, $-CO_2^-$) was employed. The relative conformational energies of protein-bound AdoMet conformers were obtained by energy minimization using the truncated Newton conjugate gradient (TNCG) method. Since protein crystal structures have significant experimental uncertainty, during each energy minimization the relative heavy atom positions were allowed to deviate from their crystallographic values by as much as 0.3 Å with no energy penalty (*74*). This procedure has been shown to be suitable for analysis of flexible ligands in protein structures (*74*). An alternate approach in which the non-hydrogen atom dihedral angles were constrained to their observed values (with the exception of the necessarily planar adenine ring which was allowed to relax) resulted in relative energies as much as

200 kJ/mol above those obtained after relaxation of the heavy atom positions; values from this method were not used further.

NMR Studies. *S*-Adenosylmethionine was synthesized as described previously (*63*). Dimethylsulfoniopropionic acid was purchased from TCI America. Other compounds were obtained from Sigma. NMR spectra were recorded at 600.13 and 300.13 MHz on Bruker Avance DMX spectrometers. Solutions contained 4−10 mM nucleoside, DMSP or SMM, in 0.1 M d_{11}-Tris·HCl, in D_2O, at pH 7.0 to provide physiological ionization states. Spectra were obtained at 25 °C. Two-dimensional rotating frame nuclear Overhauser effect spectra (ROESY) were collected in the States−TPPI mode at a spin lock time of 300 ms. A spectral width of 10 ppm was used in both dimensions. For each spectrum, 256 t_1 increments were recorded after 16 dummy scans. For each FID, 1024 complex data points were acquired, and 16 scans were accumulated. There was a relaxation delay of 3 s between each scan. In view of the small size of DMSP and SMM, laboratory frame NOE data were also obtained from NOESY spectra at 300.13 MHz; the mixing time was 350 ms. Two-dimensional NMR data were processed on a Silicon Graphics computer using the program *Felix 2000* (MSI Inc.). A 90°-shifted sine function was applied in each dimension before Fourier transformation, and the data were zero-filled to yield a final 512 × 1024 matrix. Subsequently, NOE cross-peak volumes were measured.

In analysis of nucleoside conformations, the rate of cross relaxation between the $H_{1'}$ and $H_{2'}$ protons is commonly used as an internal reference because the distance ($r_{H1'-H2'}$) is 2.90 ± 0.2 Å regardless of the ribose conformation or the glycosidic torsional angle (*55*, *75*). The rate of cross relaxation between a pair of protons A and B (R_{A-B}) was referenced to the $H_{1'}-H_{2'}$ cross-relaxation rate ($R_{1'-2'}$):

$$r_{A-B} = r_{1'-2'}(R_{1'-2'}/R_{A-B})^{1/6} \quad (1)$$

to obtain a measure of the distance between protons, r_{A-B}. In cases of groups with multiple degenerate protons (e.g., $-CH_3$), the observed relaxation rates were normalized for the number of interacting protons before distance calculations. These distances was then used as constraints in a 6000-step Monte Carlo conformational search with our modified AMBER* force field; an up to 10% deviation of interatomic distances from the NOE calculated distances was allowed without energy penalty, beyond which an energy penalty of 100 kJ/Å was applied. The resulting collection of conformers that were within 20 kJ/mol of the minimum was taken to be representative of the population of solution conformers.

Spin−spin coupling constants were obtained by numerical fitting of the spin system to one-dimensional NMR spectral data (digitized at 0.2 Hz/point) using the program Spinworks, version 1.2 (*76*). It is important to note that ^3J values reflect the proportion of conformers present, averaged over the millisecond time scale, whereas the NOE data reflect the distribution of conformers weighted by the r^6 distance dependence and hence emphasize the closest approach of two nuclei when a distribution of distances is present.

The glycosidic torsional angle for nucleosides, χ, is defined as the $C_8-N_9-C_{1'}-O_{4'}$ angle. The pseudorotation angle, P, which describes the puckering of the ribose ring is given by

$$P = \tan^{-1}\{[(\nu_{-4} + \nu_{-1}) - (\nu_{-3} + \nu_{-0})]/[\nu_{-2}(\sin 36° + \sin 72°)]\}$$

where ν_{-0}, ν_{-1}, ν_{-2}, ν_{-3}, and ν_{-4} are the $C_{4'}-O_{4'}-C_{1'}-C_{2'}$, $O_{4'}-C_{1'}-C_{2'}-C_{3'}$, $C_{1'}-C_{2'}-C_{3'}-C_{4'}$, $C_{2'}-C_{3'}-C_{4'}-O_{4'}$, and $C_{3'}-C_{4'}-O_{4'}-C_{1'}$ torsional angles, respectively (*26*).

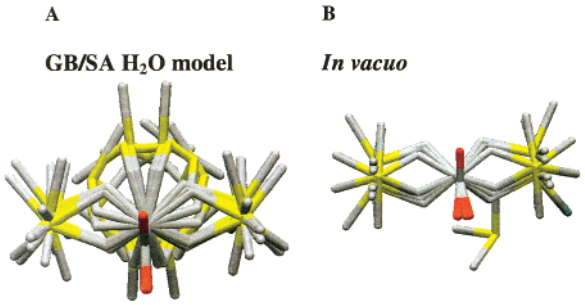

FIGURE 2: Overlay of conformers found by conformational searching for dimethylsulfoniopropionate [(A) GB/SA water model and (B) vacuum]. In each case, the carboxylate group was treated as an anion. The structures are superimposed on the $C_1-C_2-C_3$ fragment. Hydrogen atoms are not shown.

RESULTS

Force Field Parameters for Sulfonium Sulfur. Force field parameters for sulfonium sulfur were determined in the context of the AMBER force field as implemented in MacroModel [AMBER* (*59*, *60*, *73*)]. The standard geometrical values for bond lengths, bond angles, and torsional angles and their corresponding force constants were determined from a combined analysis of small molecule crystallographic data and B3LYP/6-31G* DFT calculations as described in Materials and Methods. The resultant values are listed in Table 3S of the Supporting Information.

Conformational Studies of SMM and DMSP. The conformations of DMSP and SMM in aqueous solution were examined experimentally using NOE methods in both the laboratory reference frame and the rotating frame. In both DMSP and SMM, NOEs were only observed between protons separated by at most four bonds (H−C−S−C−H or H−C−C−C−H); thus, there was no evidence for a preferred conformation with the sulfur in the proximity of an oxygen, wherein the methyl group protons would be near the protons on C_2 (i.e., the α carbon; see Figure 1). Free rotation about the C−C bonds of SMM in acidic aqueous solutions was proposed previously on the basis of the equivalence of ^3J values between the α and β, and β and γ, protons (*25*); the ^3J values under current conditions are indistinguishable from those reported previously (Table 3). The sole coupling constant observed in DSMP is the ^3J of 7.0 Hz between the protons of the methylene groups, providing no indication for a preferred conformation.

Monte Carlo simulations of DMSP using AMBER* and the GB/SA aqueous solvent model were consistent with the NMR data, yielding only conformations with extended distances between the sulfur and carboxylate oxygens (>3.8 Å) (Figure 2A). Simulations without the solvent model (i.e., in vacuo) produced more compact (folded) forms with 30% of the conformers having S···O distances of <3.3 Å (Figure 2B); these conformations would be expected to result in

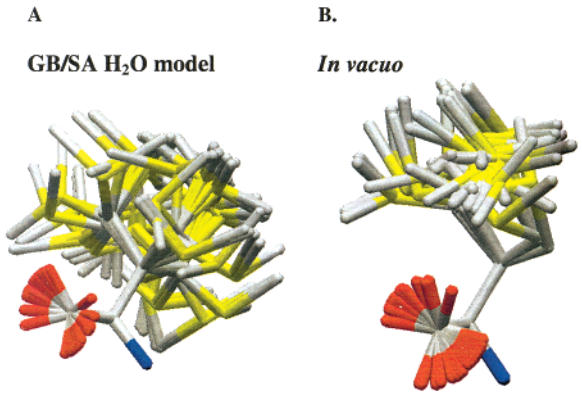

FIGURE 3: Overlay of structures found by conformational searching for *S*-methylmethionine [(A) GB/SA water model and (B) vacuum]. In each case, the carboxylate group was treated as an anion and the amino group was treated as the ammonium cation to represent physiological ionization states. The conformers are superimposed on the $C_1-C_2-C_3$ fragment. Hydrogen atoms are not shown.

NOEs between the methyl protons and other C−H protons, but these NOEs are not observed. In the absence of solvent, the minimum energy conformation found for DMSP is folded; it is 29 kJ/mol *lower* in energy than the extended conformation that is the energy minimum found using the GB/SA water model. Conversely, when the GB/SA water model is applied, the extended conformer is 28 kJ/mol *lower* in energy than the conformation corresponding to the folded gas phase conformation, because the computed solvation energy is 57 kJ/mol greater for the extended form. When the gas phase and solvation energies of these conformations were compared using DFT calculations at the B3LYP/6-31+G* level, with the torsional angles constrained at those obtained from the force field calculations, the folded gas phase conformation (Figure 2B) is found to be 18 kJ/mol lower in energy than the extended conformation (Figure 2A). However, the solvation energy obtained using the SCRF water model in Jaguar is 80 kJ/mol larger for the extended conformer, predicting that the extended form is lower in total energy in aqueous solution. Thus, although the values of the energy differences vary between the molecular mechanics and DFT methods, the ordering of the energies of the conformers is the same for both methods.

In the case of SMM, molecular mechanics simulations in the absence of a solvent model gave folded conformations as the lowest-energy forms, whereas in the presence of the GB/SA solvation model, extended conformations were lowest in energy (Figure 3A,B). The presence of extended conformations is consistent with the NMR data. In the absence of solvation, the energy of the minimum energy gas phase conformation found for SMM is 69 kJ/mol lower than that of the extended conformation found using the GB/SA water model. Conversely, in the GB/SA water model, the extended conformer is 38 kJ/mol lower in energy than the minimum-energy gas phase conformer due to a 107 kJ/mol larger solvation energy. It was not possible to compare the gas phase and solution energies of these conformations using DFT calculations because the zwitterionic amino acid form ($-CO_2^-$, $-NH_3^+$) isomerizes to the $-CO_2H$, $-NH_2$ form in the gas phase.

Thus, the molecular mechanics force field parameters that we have developed for the sulfonium ion, in conjunction with those in AMBER*, provide a realistic representation of the conformational preferences observed for both SMM and DMSP.

Table 2: NMR-Determined Cross-Relaxation Rates (R) and Intramolecular Distances (r) for AdoMet, ATP, *S*-Adenosylhomocysteine, and 5′-Methylthioadenosine[a]

proton pair (A−B)	$R_{A-B}/R_{H1'-H2'}$	r_{NOE} (Å)	r_{em} (Å)	r_{avg} (Å)
(A) AdoMet				
8−1′	1.82	2.6	3.4	3.4
8−2′	0.68	3.1	3.5	3.5
8−3′	0.98	2.9	3.2	3.3
1′−2′	1.00	2.9	2.9	2.9
1′−3′	1.69	2.7	3.8	3.8
2′−4′	1.60	2.7	3.6	3.8
3′-methyl	0.79	3.7	3.9	4.2
4′-methyl	0.62	3.8	4.4	4.5
β-methyl	1.44	3.7	3.7	3.7
(B) ATP				
8−1′	0.46	3.4	3.8	3.8
8−2′	1.80	2.6	2.2	2.2
8−3′	1.14	2.8	2.7	2.7
1′−2′	1.00	2.9	3.0	3.0
1′−3′	0.91	3.0	3.7	2.8
1′−4′	0.98	2.9	2.7	2.7
2′−5′ (5″)	0.89	3.0	3.2	3.2
(C) 5′-methylthioadenosine				
8−1′	0.61	3.2	3.8	3.7
8−2′	0.44	3.3	2.9	3.1
8−3′	0.37	3.3	3.7	3.7
1′−2′	1.00	2.9	2.9	2.9
1′−3′	0.85	3.0	3.8	3.8
2′−5′ (5″)	0.98	3.3	4.2	4.1
3′-CH_3	0.40	4.0	4.3	4.0
4′-CH_3	0.41	4.0	4.2	4.4
(D) *S*-adenosylhomocysteine				
8−1′	1.30	2.8	3.7	3.6
8−2′	1.28	2.8	2.5	2.6
8−3′	0.66	3.1	3.6	3.6
8−4′	0.29	3.6	4.2	4.2
1′−2′	1.00	2.9	3.0	3.0
1′−3′	0.15	4.0	3.9	4.0
1′−4′	1.08	2.9	3.2	3.2
2′−5′ (5″)	0.95	2.9	3.8	3.8
3′−γ	0.31	4.0	4.6	4.6
4′−β	1.41	3.1	5.1	4.7
4′−γ	1.21	3.2	3.3	3.4
$\alpha-\gamma$	0.75	3.4	3.4	3.3

[a] R_{A-B} is the observed (unscaled) rate of cross relaxation between protons A and B. $R_{H1'-H2'}$ is the cross-relaxation rate of the $H_{1'}-H_{2'}$ proton pair. r_{NOE} is the interproton distance determined from the relative NOE intensity referenced to the $H_{1'}-H_{2'}$ distance of 2.90 Å. r_{em} is the interproton distance determined from the energy-minimized model. Cross-relaxation rates for the indicated proton pairs are shown as ratios of the rates observed for the $H_{1'}-H_{2'}$ proton pair to the observed rates of the indicated proton pairs. r_{NOE} values for the proton pairs involving the $H_{5'(5'')}$, CH_3, and $H_{\beta,\beta'}$ protons reflect an r^6-weighted average of their positions. The r_{em} values are those of the lowest-energy member of the family found in a constrained energy minimization, and r_{avg} is the average for the conformers within 20 kJ/mol of the energy minimum.

Conformation of AdoMet in Solution. AdoMet is clearly a more complex molecule than DSMP or SMM. Rotating frame NOE methods (ROESY) are efficacious for determination of the conformational preferences of nucleosides in solution since compounds of this size typically yield very small laboratory frame NOEs (cf. refs *77* and *78*). The ROESY results for AdoMet illustrate the proximity of H_8 of the adenine ring to the ribose 1′, 2′, and 3′ protons, and of the methyl protons to the 3′ and 4′ protons of the ribose (Table 2A). No cross-peaks were observed between methionyl and adenine protons, or between the adenine H_2 proton

Table 3: $^1H-^1H$ Coupling Constants for AdoMet, AdoHcys, MTA, SMM, and ATP[a]

	J (Hz)				
proton pair	AdoMet	AdoHcys	MTA	SMM	ATP
1′−2	4.4	4.6	5.3		6.3
2′−3	5.1	5.4	5.0		5.0
3′−4	4.2	4.8	3.7		3.9
4′−5	10.8	6.5	5.3		4.1
4′−5	4.5	4.8	6.5		2.2
5′−5	−14.0	−14.3	−13.7		−12.4
α−*β*	6.2	6.8		6.5	
α−*β*′	6.2	6.6		6.5	
β−*β*	0	−14.6		0	
β−*γ*	7.9	6.7		8.4	
β−*γ*	7.7	7.5		8.4	
γ−*γ*	−13.2	0		−12.6	

[a] Uncertainties in coupling constants are ∼0.4 Hz.

and the ribose. The cross-relaxation data were analyzed using an $H_{1'}-H_{2'}$ distance of 2.9 Å as a reference because it is independent of ribose conformation (*75*). Interproton distances were calculated from the ratios of the observed NOE values for various proton pairs to the NOE value measured for $H_{1'}-H_{2'}$. The calculated distances were imposed as constraints in a Monte Carlo search for conformations within 20 kJ/mol of the energy minimum; the family of conformers consistent with these constraints is illustrated in Figure 4A. The results indicate that the solution conformation predominantly has an anti configuration about the glycosidic bond, with a torsional angle of ∼20° (Table 2S of the Supporting Information). Deviations between distances calculated directly from the NOE data and those obtained from the constrained energy minimization model may reflect the variety of conformers that are interconverting in solution.

Further insight into the solution conformation was obtained by analysis of the three-bond scalar coupling constants, ^3J. The conformation of the ribose ring appears to be variable as indicated by the nearly equivalent ^3J coupling constants ($^3J_{1'2'}$ and $^3J_{3'4'}$ = 4.4 and 4.2 Hz, respectively; Table 3), the ratio of which provides an estimate of the equilibrium ratio between the $C_{3'}$-endo and $C_{2'}$-endo forms (*79*). The NOE data, which are weighted toward the closest approach distances by the dependence on the inverse sixth power of the interproton distances, reflect a substantial population of the $C_{3'}$-endo conformer. We observed similar $^3J_{1'2'}$ and $^3J_{3'4'}$ values for AdoMet and its thioether metabolites *S*-adenosylhomocysteine (4.6 and 4.8 Hz, respectively) and 5-methylthioadenosine (5.3 and 3.7 Hz, respectively), and slightly different values for ATP (6.3 and 3.9 Hz, respectively), indicating that the population of ribose ring conformers is not dramatically affected by the presence of oxygen or sulfur as the 5′ substituent, or by the charge on the sulfur.

The ^3J couplings between $H_{4'}$ and $H_{5'}$ and between $H_{4'}$ and $H_{5''}$ in AdoMet are unequal (10.8 and 4.5 Hz, respectively) and indicate a preference for one rotamer about the $C_{4'}-C_{5'}$ bond, apparently with one nearly trans H−C−C−H torsional angle. The lack of stereospecific assignments of the $H_{5'}$ and $H_{5''}$ resonances precludes a definitive conformational determination based on the coupling constant values. Either assignment places the sulfur near an oxygen, either $O_{3'}$ or $O_{4'}$. In the presence of the GB/SA solvent model, the NOE-constrained conformational search only yielded conformers with distances greater than the sum of the van der Waals radii (3.3 Å) between S^+ and oxygens in the ribose and carboxylate, indicating the absence of predominant close contacts in the majority of conformers. In the absence of the solvent model, however, the computed lowest-energy conformer had close contacts between the S^+ and both $O_{4'}$ (3.08 Å) and a carboxylate oxygen (2.82 Å).

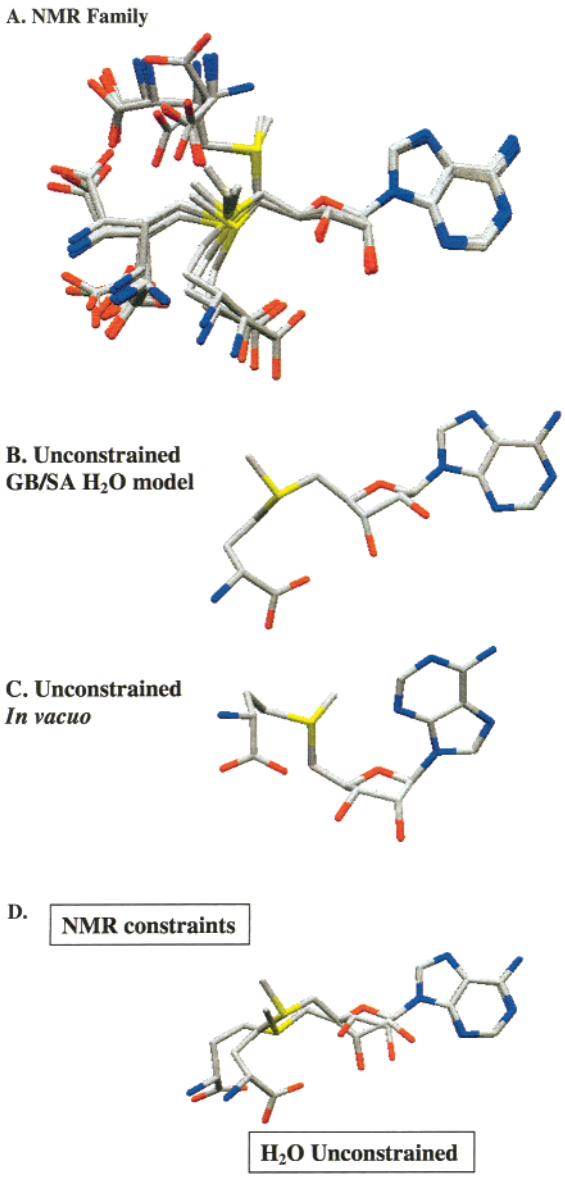

FIGURE 4: Lowest-energy conformations found for *S*-adenosylmethionine. Part A illustrates the family of conformers obtained in a conformational search using NOE constraints and the GB/SA water model. The structures are superimposed on the $C_{1'}-O_{4'}-C_{4'}$ atoms. Part B shows the lowest-energy conformer obtained using the GB/SA solvent model without constraints. Part C shows the lowest-energy conformation found in vacuo. Part D is a superposition of the lowest-energy conformer obtained using the GB/SA solvent model with the NOE constraints (extended methionyl group) and without (folded methionyl group) the NOE constraints. The carboxylate group was treated as an anion, and the amino group of AdoMet was taken to be the ammonium cation to represent physiological ionization states. Hydrogen atoms are not shown.

A variety of conformations of the methionyl group are compatible with the NMR data. Analysis of the three-bond ^1H coupling constants in the methionyl group suggests free rotation around both the $C_\alpha-C_\beta$ bond and the $C_\beta-C_\gamma$ bond (i.e., ^3J is the same between the α proton and two *β* protons,

and there is a single value of ^3J for the β and β', and γ and γ', protons), as reported at pH 3 (*25*); the coupling constants observed for the methionyl group at pH 7 in the study presented here are all indistinguishable from those reported at pH 3. NOEs were not observed from the α proton to other protons, suggesting an extended conformation of the amino acid moiety rather than the predominance of a form with a close interaction between the S^+ and the carboxylate. This finding is consistent with the absence of NOEs between the α and γ or methyl protons in SMM and the near identity of the ^3J values in SMM and AdoMet.

Unconstrained conformational searches using AMBER* were performed for comparison with the NOE constrained results. The minimum energy conformation found in these searches, which were conducted using the GB/SA solvation model, has an anti conformation ($\chi = 38°$), a $C_{2'}$-endo sugar pucker, and a conformation of the methionyl group slightly more folded than that found when the NOE constraints are enforced (Figure 4B and superimposed in Figure 4D). This conformation of the methionyl chain is consistent with the NMR data. The steric energy of this conformer is 87 kJ/mol lower than that of the NMR minimum energy conformer (calculated in the presence of the solvent model, but corrected for solvation energy). The unconstrained conformation is slightly more compact than the NMR form (the solvent accessible surfaces areas are 603 and 627 Å^2, respectively), and the computed solvation energy of the NMR conformer is 18 kJ/mol more favorable. The closest $S^+\cdots O_C$ distance is 3.28 Å in the unconstrained form compared to 3.95 Å in the extended (NMR) form. Because syn glycosidic conformations of AdoMet have been found in some protein complexes (see below), the relative energy of a syn conformer was examined. The lowest-energy conformation with a syn glycosidic bond ($\chi = -128°$) found in this search had a steric energy that is 18 kJ/mol above the energy minimum, and the solvation energy was 16 kJ/mol less favorable.

In contrast, the lowest-energy conformation found by molecular mechanics in the absence of a solvent model does have a syn conformation about the glycosidic bond ($\chi = -128°$); there are three close nonbonded interactions with the sulfonium sulfur: between the S^+ and $O_{4'}$ (3.18 Å), a carboxylate oxygen (2.84 Å), and N_3 of the purine (3.29 Å) (Figure 4C). This is a relatively compact conformation (the solvent accessible surface area is 560 Å^2, 67 Å^2 less than that for the extended NMR conformation). A hydrogen bond is present between a carboxylate oxygen and $HO_{3'}$. In vacuo, the energy of the conformer in Figure 4C is 68 kJ/mol lower than that of the minimum energy solution conformer (126 kJ/mol less than that of the NMR conformation); the lowest-energy anti form in vacuo ($\chi = 38°$) is 13 kJ/mol higher in energy than the syn form. The calculated solvation energy for this syn form is 156 kJ/mol less than that for the extended NMR conformation.

In general, when the solvation model is employed, the calculations are consistent with the NMR data in the predominance of anti conformers and an extended overall disposition. The range of energies of the conformers may be somewhat exaggerated, consistent with the general difficulty in computing conformational energies of strongly polar molecules (*74*).

Influence of the Charge of the 5′ Substituents on Solution Conformation. Conformational Preferences of the Anion ATP. ROESY spectra for the well-studied ATP were obtained for comparison of the conformational effects of an anionic substituent at the 5′ position. ATP showed purine−ribose NOEs from H_8 to $H_{1'}$, $H_{2'}$, and $H_{3'}$ with relative intensities of 14, 53, and 33, respectively (Table 2B), in good agreement with published results [15, 52, and 33 (*78*) and 20, 65, and 15 (*77*), respectively]. Comparison of the NOE ratios for H_8 to $H_{1'}$, $H_{2'}$, and $H_{3'}$ with those for AdoMet (52, 20, and 28, respectively, Table 2A) indicates a difference in average glycosidic conformation. Modeling of ATP with these NOE constraints yielded a glycosidic bond angle of ∼65°. NMR studies of a variety of ATP-utilizing enzymes have found glycosidic torsional angles of ∼50° (45−60° range), while a broader distribution has been found in crystallographic studies (*27*, *28*, *55*).

The scalar coupling constant between $H_{1'}$ and $H_{2'}$ is 6.3 Hz for ATP, compared to 4.4 Hz for AdoMet, showing a difference in the average ribose conformation. In ATP, the values of the ^3J couplings between $H_{4'}$ and the two nonequivalent $H_{5'}$ and $H_{5''}$ protons (4.1 and 2.2 Hz, respectively) are both smaller and more nearly equivalent than in AdoMet, consistent with the generally accepted view that nucleotides in solution have a preferred orientation around the $C_{4'}-C_{5'}$ bond that places $O_{5'}$ above the ribose ring, yielding two gauche H−C−C−H torsional angles (*26*). This conformation is distinct from that adopted by AdoMet.

Conformational Preferences of the Neutral S-Adenosylhomocysteine (AdoHcys) and 5′-Methylthioadenosine (MTA). To evaluate the contribution of the positive charge and steric requirements of the trivalent sulfur to the conformation of AdoMet, ROESY spectra and ^3J coupling constants were measured for the thioethers AdoHcys and MTA, which are the two major metabolites of AdoMet (parts C and D of Table 2 and Table 3). The AdoHcys and MTA models, obtained using the NOE values as constraints, reflect a predominance of an anti conformation about the glycosidic bond, with χ near 45° in both cases. In both AdoHcys and MTA, the coupling constants between $H_{4'}$ and $H_{5'(5'')}$ are more nearly equivalent than in AdoMet (Table 3), suggesting a diminished contribution of a trans-gauche conformer around the $C_{4'}-C_{5'}$ bond, particularly for MTA. In AdoHcys, as in AdoMet, coupling constants within the methionyl group indicate that a variety of conformers are present. However, for AdoHcys, NOEs are observed between the methionyl γ-methylene protons and the α proton, as well as the ribose 3′ and 4′ protons. These NOEs and the inequivalence of the chemical shifts of the β protons (rather than inequivalence of the γ protons in AdoMet and SMM) suggest a different distribution of methionyl conformers.

AdoMet Conformations in Protein Complexes. Conformations of 38 crystallographically independent AdoMet molecules in 20 protein crystal structures were examined, and selected geometrical parameters are illustrated in Figure 5 and listed, along with additional values, in Table 2S. The relative conformational energies were assessed by restrained geometry optimization of the crystallographic conformations using the enhanced AMBER* force field (see Materials and Methods), and these energies are used as the vertical axis in displaying the conformational variations. The overall range of energies is 251 kJ/mol in vacuo, but is reduced to 117 kJ/mol upon inclusion of the solvation energy (which also reduces the standard deviation from the mean from 59 to 18

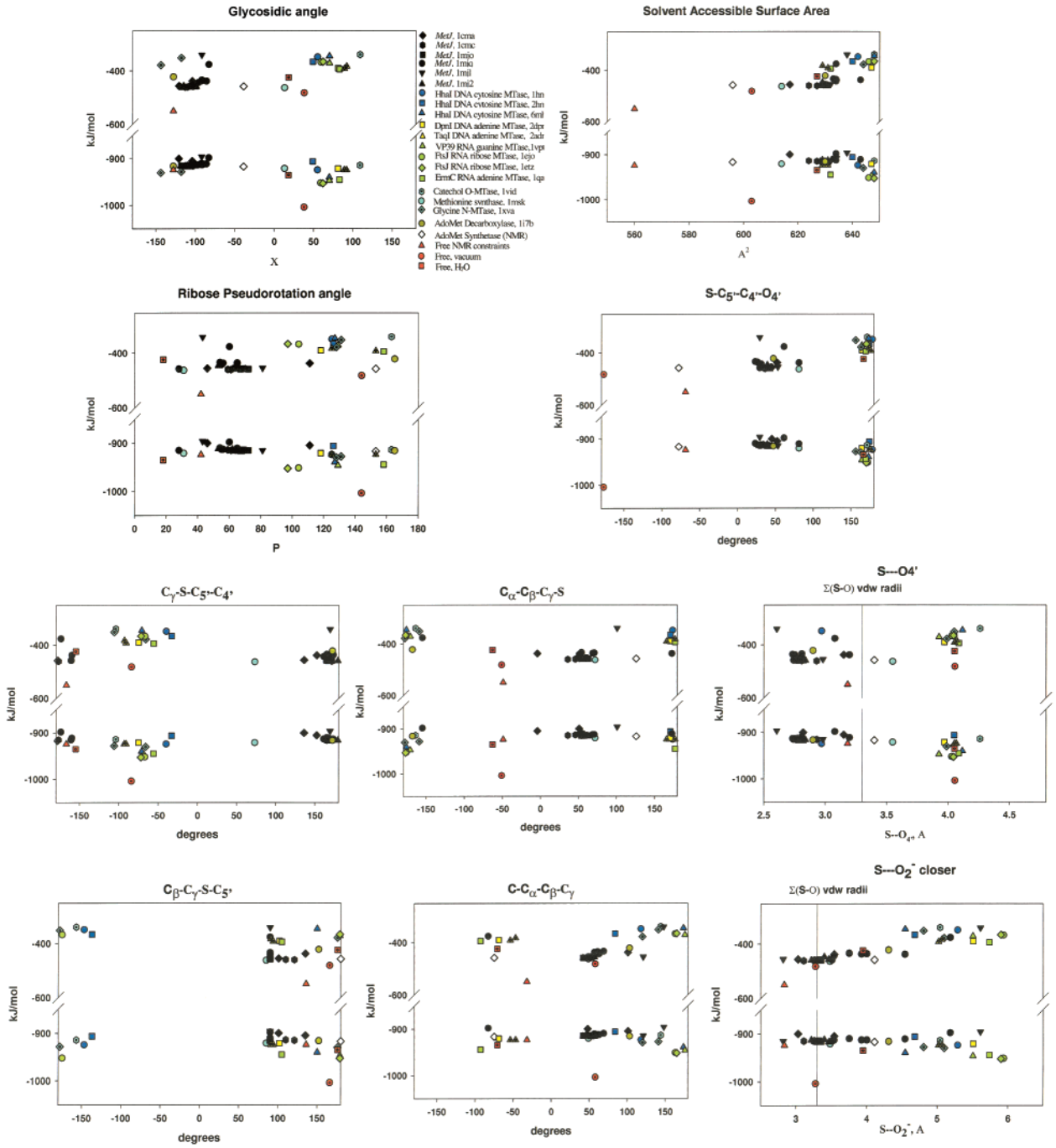

FIGURE 5: Comparison of geometrical parameters for AdoMet alone and in protein complexes. The glycosidic bond angle, ribose pseudorotation angle (*P*; see Materials and Methods), and solvent accessible surface area, in addition to other selected torsional angles and intramolecular nonbonded distances, are plotted vs the molecular mechanics energy. Values for the conformation for uncomplexed AdoMet constrained by NMR data and those for the unconstrained minimum energy conformation in a vacuum and the GB/SA continuum solvation model for water are shown along with the crystallographically observed conformations. The energy values calculated for each conformer are shown in two ways: including solvation energy (lower energy range) and with the solvation energy contribution removed (upper energy range). Both the color and symbol are the same for each independent occurrence of AdoMet from a particular protein structure; a different combination of symbol and color is used for each structure. When the same protein is present in more than one PDB entry, the same color is used for each representative, but the symbol differs. Symbols for AdoMet conformations taken from *MetJ* repressor structures are black. Those for small molecule methylases are cyan. Those for the HhaI cytosine DNA methylase are blue. Those for DNA adenine methylases are yellow. Those for RNA methylases are green. Those for AdoMet decarboxylase are orange. Those for AdoMet synthetase are white. Conformations computed in the absence of protein, with or without NMR restraints, are in red. The correspondence of symbol shapes and colors with structures is listed in the figure.

kJ/mol). When the singularly low energy of the computed unconstrained conformation is excluded, the range is reduced to 66 kJ/mol in the presence of solvation, with a standard deviation of 14 kJ/mol. Thus, when the limitations of both the continuum solvation model and force field calculations are considered, it appears that the conformations of protein-bound AdoMet are energetically consistent with the population of solution conformers, rather than representing unusual states.

Figure 5 relates the conformational energy and selected torsional angles and interatomic distances observed in the protein complexes and in solution. The structures show a

wide variation in glycosidic bond angles, in ribose pucker (as reflected in the pseudorotation angle *P*; see Materials and Methods), and in the orientation of the methionyl chain with respect to the adenine ring. It is clear that the variations in energy in vacuo (illustrated in the top part of each panel) are greatly attenuated when the solvation energies are included (shown in the lower part of each panel). There is not a monotonic variation in energy with any single geometrical parameter. All of the energies of bound AdoMet are higher than that computed for the unconstrained solution conformation, but the energies are comparable to that of the potentially more realistic NOE-constrained form. The low energy of the unconstrained form in part reflects a favorable electrostatic interaction between the S^+ and a carboxylate oxygen at a distance of 3.28 Å, which is not required by the NMR data. Most nucleic acid methyltransferases have similar glycosidic angles χ, sugar pseudorotation angles *P*, and $S-C_{5'}-C_{4'}-O_{4'}$ angles (reflecting the relative orientation of methionyl and ribose moieties) as might be expected from their evolutionary relationships (*49*). However, in one small molecule methylase, glycine *N*-methyltransferase, the glycosidic torsional angle is distinctly syn (−119° and −144° in two crystallographically independent molecules). The conformation of AdoMet bound to the noncatalytic *Escherichia coli MetJ* methionine repressor protein is also syn with a χ value of approximately −100° (range of −83 to −120° in 22 examples) (*36*). A syn conformation of the glycosidic bond ($\chi = -128°$) was reported in a complex of the methyl ester of AdoMet with AdoMet decarboxylase, which also does not catalyze a chemical reaction at the sulfonium center (*53*). The majority of the AdoMet conformations in methyltransferase complexes are extended with a $S-C_{5'}-C_{4'}-O_{4'}$ torsional angle near 180°; the exception is methionine synthase in which AdoMet has a $S-C_{5'}-C_{4'}-O_{4'}$ torsional angle of 82° (*48*). The conformations of AdoMet complexes with *MetJ* and AdoMet decarboxylase are also compact with $S-C_{5'}-C_{4'}-O_{4'}$ torsional angles of 30−80°; these conformations place the sulfur in the proximity of $O_{4'}$. There is a paucity of $C_{\gamma}-S-C_{5'}-O_{4'}$ torsional angles near 60°; the only observed case is the complex with AdoMet synthetase, which is unique in catalyzing a chemical reaction at $C_{5'}$ (*63*). The $C_{\beta}-C_{\gamma}-S-C_{5'}$ torsional angle has a preference for the 90−180° range which directs the methionyl moiety away from the ribose, giving an extended conformation.

In the *MetJ* repressor and AdoMet decarboxylase complexes, the AdoMet molecules have close contact distances between $O_{4'}$ of the ribose and the sulfur, with distances of 2.6−3.2 Å, i.e., less than the sum of the van der Waals radii of 3.3 Å. In addition, the intramolecular distance between the S^+ and a carboxylate oxygen equals 2.8−3.3 Å in 11 of the *MetJ* complexes. In both the *MetJ* repressor and AdoMet decarboxylase, the sulfonium center does not have a reactive role, and thus, the S^+ can be used as a conformational motif rather than as an electrophilic activator for transfer of an alkyl substituent. No conformations were found with close interactions between the sulfur and either $O_{3'}$ of the ribose or N_3 of the adenine.

Whether close contacts between the S^+ of AdoMet and protein heteroatoms were present in the complexes was investigated by evaluating all distances of <4 Å from the sulfur in each of the AdoMet·protein complexes [the sum of the van der Waals radii of two sulfur atoms is 3.6 Å (*80*)]. Close contacts are relatively rare in the available structures. There is close, nonbonded, interaction between the sulfonium sulfur and a carboxylate oxygen in one subunit of the AdoMet:glycine *N*-methyltransferase in which the S^+ is 3.1−3.2 Å from an oxygen of the glutamate-15 carboxylate; in the second subunit, the sulfur is 4.0 Å from both oxygens [PDB entry 1xva (*81*)]. A similar interaction was seen in the *Hha*I DNA methylase structure, in the absence of DNA, where the S^+ is 2.8 Å from the carboxylate oxygens of aspartate 60 (PDB entry 1hmy). In the catechol *O*-methyltransferase structure, the sulfur of methionine 40 is within van der Waals contact of the S^+ at 3.4 Å (PDB entry 1vid). The *MetJ* repressor binding site is represented in 18 independent AdoMet molecules in six different structures; in five cases, the main chain carbonyl of alanine 64 is in the proximity of the S^+ (3.1−3.3 Å). In general, however, the conformations of AdoMet bound to proteins do not reveal common contacts between the S^+ and a particular type of protein residue.

Interactions between cations and the aromatic rings of tyrosine, phenylalanine, and tryptophan have been suggested as a binding mode (*82*). In the majority of the available protein structures, the AdoMet binding sites do not have close contacts between the sulfonium center and an aromatic ring. The closest approach is 4.1 Å from the S^+ and the center of the phenyl ring of phenylanine 223 in AdoMet decarboxylase (the S^+ is located above the ring plane with an angle of 74° with respect to the ring plane). A cation−π interaction at 3.1−3.5 Å with the face of tryptophan 41 was observed for AdoMet bound at one site in the *Hha*I methylase [PDB entry 1hmy (*37*)]. Thus, the cation−π interaction seen in some protein complexes with metal ions and alkylammonium ions (*82*) does not appear to be a recurring theme in sulfonium ion recognition.

CONCLUSIONS

NMR and computational results indicate that AdoMet has conformations in solution where an anti orientation about the glycosidic bond predominates, but otherwise, it is rather flexible and generally extended. Apparently, interactions between the S^+ and the anionic carboxylate oxygens are attenuated in solution, as they are in the simpler compounds SMM and DMSP. In AdoMet, interactions between the S^+ and either $O_{4'}$ or $O_{3'}$ of the ribose are retained in solution, presumably facilitated by bonded geometrical constraints. In the absence of solvent, and presumably in other less polar environments, electrostatic interactions involving the S^+ are more important in determining conformational preferences of each these molecules.

A comparison of the AdoMet conformations in AdoMet·protein complexes reveals that the nucleoside is usually extended in cases where AdoMet acts as a donor of a methyl group from the S^+ atom, maximizing the accessibility of the methyl group. Open conformations also enhance the accessible surface area for interactions of the ligand with the protein, and can optimize affinity through van der Waals interactions, as well as by making available more specific interactions. In the case of AdoMet, however, the presence of potentially favorable intramolecular interactions between the S^+ and the ribose oxygens or the carboxylate can mitigate the benefits of interactions with the protein groups, and such

interactions are common when the S^+ is not involved in the reaction catalyzed by the protein complex. Studies of binding sites in proteins for anionic adenine mononucleotides, such as ATP, have indicated that they bind in low-energy conformations similar to those in the "free" compounds, with the notable exception being that the conformations tended to be more open due to rotation about the $C_{4'}-C_{5'}$ bond (*27*, *28*). Similarly, open conformations are widely observed in protein complexes with nicotinamide adenine dinucleotide, NAD (*54*). The conformational energies of AdoMet in the protein complexes are comparable to one another and not greatly above those for AdoMet in solution. In particular, the differences in steric energy among the protein-bound conformers are attenuated by the presence of solvent, indicating that the bound conformations are comparable to one another in energy in solution. This is consistent with the observation that assessment of the steric energetic effects of binding must consider the effects of the aqueous environment from which the ligand is extracted (*74*). The available protein complex structures reflect only a few of the classes of catalytic sites to which AdoMet and other sulfonium ions bind. It will be interesting to learn what conformations of AdoMet are present in protein complexes with alternate types of catalytic activities, such as acting as a free radical progenitor.

ACKNOWLEDGMENT

We thank Dr. H. Cheng of the Fox Chase Spectroscopy Support Facility for assistance with the NMR measurements.

SUPPORTING INFORMATION AVAILABLE

CSD structure files used in force field development, conformations of AdoMet in protein complexes and solution, AMBER parameters for sulfonium sulfur, and computed solvation energies for sulfonium compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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BI025682C