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The Structure of S-Adenosyl-L-homocysteine 2·5-Hydrate

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Abstract

S-Adenosyl-L-homocysteine (SAH), a potent inhibitor for S-adenosyl-L-methionine(SAM)-dependent trans-methylation reactions, crystallizes from aqueous solution: $C_{14}H_{20}N_6O_5S.2.5H_2O$, $M_r = 429.45$, $C2$, $a = 45.658$ (3), $b = 5.6790$ (4), $c = 15.592$ (1) Å, $\beta = 99.752$ (6)°, $V = 3984.42$ Å^3, $Z = 8$, $d_c = 1.43$ g cm^{-3}, μ(Cu $K\alpha$) = 18.0 cm^{-1}, $F(000) = 1816$. The crystal structure was solved by a combination of systematic searches of Patterson space and super-position. Its absolute configuration was established using hkl and $\overline{hkl}$ reflections. The structure was refined by a block-diagonal least-squares procedure to a discrepancy factor of 0.074 for 2329 reflections with $I \geq 2.33\sigma_I$. There are two independent SAH and five water molecules in one asymmetric unit. The two SAH molecules have different conformations with respect to both their amino acid and nucleoside portions. The crystal structure is held together by a hydrogen-bonding network that includes asymmetric base pairing, and interactions between the amino acid and ribose rings, adenine rings and symmetry-related amino acids.

Introduction

S-Adenosyl-L-homocysteine (SAH), the demethylated product of the S-adenosyl-L-methionine(SAM)-dependent transmethylation reactions, is also the natural inhibitor to these reactions. It was shown that SAH and SAM compete for the same enzymatic site (Baudry, Chast & Schwartz, 1973; Coward, Slisz & Wu, 1973; Borchardt, Huber & Wu, 1974). A model of the SAM–enzyme-binding complex was proposed by Zappia, Zydek-Cwick & Schlenk (1969). A similar binding site for SAH was proposed by Borchardt (1977) based on the activity studies of a series of SAH analogues. In the proposed model (Fig. 1) many parts of the SAH molecule are required for enzymatic binding. The amino acid moiety binds such that rigid spatial relationships may be maintained among the three functional groups, $-S-$, NH_3^+ and $-COO^-$. In the ribose moiety, the two $-OH$ groups are important although the exact nature of their roles has not been elucidated. It is known that in the base portion the 6-amino group is always required for transmethylation reactions while N(3) and N(7) participate in only a few such reactions.

The purpose of the present study was to determine the configuration and conformation of SAH. The structure of SAH has not been determined, although its absolute configuration at the sulfonium center was

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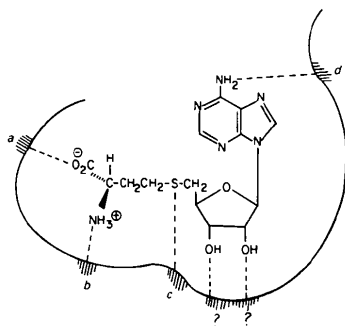
Fig. 1. SAH enzymatic binding site.

derived indirectly by Cornforth, Reichard, Talalay, Carrell & Glusker (1977). Knowledge of the absolute configuration of SAH also provides another indirect way to determine the configuration of the sulfonium center in SAM. A preliminary account of this SAH structure has been reported (Pett, Shieh & Berman, 1980).

Structure determination and refinement

Colorless needle crystals were grown from the saturated SAH aqueous solution diffused with 10% EtOH. A crystal $0.4 \times 0.15 \times 0.08$ mm was sealed in a capillary and used for single-crystal X-ray diffraction studies. Crystal data are presented in the *Abstract*. The reflections were measured on a Syntex $P\bar{1}$ diffractometer equipped with a graphite monochromator using Cu $K\alpha$ ($\lambda = 1.5418$ Å) radiation. A θ–2θ scan mode and variable scan rate were used. A total of 3996 unique reflections within $2\theta = 135°$ were obtained. Standard deviations, σ_I, were calculated to account for the counting statistics and instrumental instability (Stout & Jensen, 1968). 2329 reflections with intensity $I \geq 2.33\sigma_I$ were considered observed and used in the structure determination and refinement.

Numerous attempts to use direct methods were unsuccessful. A computer program was written to search Patterson maps at several different resolutions and was used to locate the two S atoms. Fourier maps phased on the two S atoms did not reveal any structurally significant features. However, eightfold superpositions on the two S positions gave three promising C positions. A series of Fourier maps based on two S atoms and three C atoms revealed two complete SAH molecules. The three full and four half water positions were determined from subsequent difference Fourier maps. It is evident that $OW(5)A$ and $OW(5)B$ are positionally disordered.

The structure was refined by a block-diagonal-matrix least-squares procedure. A simple $1/\sigma_F^2$ weighting scheme was used. The positions of 26 H atoms that covalently bond to C atoms were calculated and

included in the structure factor calculations. Twelve H atoms that bond to hetero-atoms appeared in the last few difference Fourier maps and were included in structure factor calculations. Final refinement with 59 non-hydrogen atoms refined anisotropically converged at $R = 0.074$ and $R_w = 0.077$ for 2329 observed reflections. Structure factor calculations using all 3591 non-zero reflections resulted in values for R and R_w of 0.1105 and 0.0822 respectively. In the final cycle of refinement, the shifts of all parameters were less than their corresponding standard errors. The largest shift to e.s.d. ratio was 0.7. The final difference Fourier map had no electron density greater than 0.4 e Å^{-3} and did not reveal any significant structural features.

The absolute configuration of SAH was established by collecting all Friedel-related reflections up to $2\theta = 120°$. A quantity D, $D = (|F_{hkl}| - |F_{\bar{h}\bar{k}\bar{l}}|)/(|F_{hkl}| + |F_{\bar{h}\bar{k}\bar{l}}|)$, was calculated for each pair of reflections and 500 pairs of hkl, $\bar{h}\bar{k}\bar{l}$ observed reflections with the largest $|D|$ were then selected for use in refinement tests. With 38 H atoms included in the structure factor calculation, the refinement of 59 non-hydrogen atoms of the present structure converged to $R = 0.068$ and $R_w = 0.062$ for those 1000 reflections. Its enantiomer gave $R = 0.086$ and $R_w = 0.085$. Therefore, the former was confirmed to be the actual structure. The anomalous-scattering factors for C, N, O and S atoms were taken from *International Tables for X-ray Crystallography* (1974).

Results

The atomic positional and thermal parameters after the final cycle of refinement are shown in Table 1.* The covalent bond distances and angles of SAH molecules are shown in Fig. 2. Fig. 2 also illustrates the numbering scheme used in this paper. The two independent SAH molecules in the crystal are assigned as SAH-*A* and SAH-*B*.

The asymmetric C atoms of the amino acid portions of SAH-*A* and SAH-*B* are in the *S* configuration as shown in Fig. 3. If we assume the SAM molecule adopts conformations similar to either SAH-*A* or SAH-*B*, the position of the methyl group must be such that the configuration of the sulfur atom is *S*. An *R* configuration results in unfavorable intramolecular steric contacts. This finding is consistent with the result obtained by Cornforth *et al.* (1977) based on the absolute configuration of the diastereomeric *S*-carboxymethyl-(*S*)-methionine salts.

Table 1. *Fractional positional parameters* $(\times 10^4)$ *and equivalent isotropic temperature factors of the nonhydrogen atoms in SAH*

$$B_{eq} = \tfrac{1}{3} \sum_i \sum_j B_{ij} a_i^* a_j^* \mathbf{a}_i . \mathbf{a}_j.$$

|	x	y	z	B_{eq} (Å^2)
N(1)A	734 (2)	5087 (20)	−603 (4)	3·7 (5)
C(2)A	575 (2)	6870 (21)	−378 (5)	3·3 (6)
N(3)A	501 (2)	7419 (16)	386 (5)	3·5 (5)
C(4)A	616 (2)	5785 (17)	969 (5)	2·5 (6)
C(5)A	773 (2)	3843 (20)	839 (6)	3·2 (6)
C(6)A	841 (2)	3506 (20)	−12 (5)	3·0 (6)
N(6)A	994 (2)	1697 (19)	−236 (5)	4·0 (5)
N(7)A	863 (2)	2583 (17)	1588 (5)	3·9 (6)
C(8)A	753 (2)	3784 (21)	2189 (6)	3·8 (6)
N(9)A	596 (2)	5743 (15)	1837 (5)	3·3 (4)
C(1')A	458 (2)	7464 (20)	2326 (5)	3·5 (6)
O(1')A	676 (1)	8201 (14)	3052 (4)	3·5 (3)
C(2')A	193 (2)	6562 (21)	2695 (6)	3·2 (6)
O(2')A	−71 (1)	6619 (15)	2133 (4)	4·2 (3)
C(3')A	199 (2)	8178 (22)	3505 (6)	3·5 (6)
O(3')A	16 (1)	10180 (16)	3286 (4)	4·6 (4)
C(4')A	520 (2)	8985 (20)	3739 (5)	3·5 (6)
C(5')A	696 (2)	8166 (21)	4576 (6)	3·9 (6)
SA	736 (1)	5000	4577 (2)	3·8 (2)
C(6')A	826 (2)	4351 (20)	5731 (6)	3·4 (6)
C(7')A	552 (2)	4243 (20)	6166 (7)	4·2 (6)
C(8')A	615 (2)	3682 (19)	7127 (6)	3·2 (6)
N(8')A	822 (2)	5437 (17)	7607 (5)	4·6 (6)
C(9')A	739 (2)	1164 (22)	7293 (6)	4·1 (6)
O(9')A	973 (2)	954 (14)	7825 (5)	5·5 (6)
O(10')A	590 (2)	−439 (13)	6907 (4)	4·2 (4)
N(1)B	1351 (2)	−2227 (21)	790 (6)	5·5 (7)
C(2)B	1499 (3)	−3545 (30)	266 (7)	6·3 (8)
N(3)B	1684 (2)	−5348 (23)	464 (6)	6·3 (7)
C(4)B	1704 (2)	−5808 (22)	1339 (7)	4·4 (7)
C(5)B	1567 (2)	−4716 (25)	1913 (6)	4·6 (7)
C(6)B	1393 (2)	−2719 (22)	1658 (7)	4·1 (7)
N(6)B	1258 (2)	−1429 (21)	2192 (6)	5·4 (6)
N(7)B	1639 (2)	−5709 (19)	2735 (6)	5·2 (6)
C(8)B	1818 (2)	−7480 (27)	2602 (8)	5·9 (8)
N(9)B	1868 (2)	−7642 (19)	1757 (5)	4·9 (6)
C(1')B	2036 (2)	−9383 (28)	1373 (8)	6·4 (9)
O(1')B	2126 (2)	−11171 (16)	2004 (6)	6·2 (5)
C(2')B	2333 (3)	−8354 (31)	1093 (8)	7·0 (8)
O(2')B	2412 (2)	−9630 (27)	443 (6)	10·7 (8)
C(3')B	2548 (3)	−8959 (29)	1957 (9)	7·4 (9)
O(3')B	2858 (2)	−8927 (26)	1852 (7)	10·7 (9)
C(4')B	2463 (2)	−11316 (33)	2213 (9)	7·6 (1·0)
C(5')B	2531 (3)	−12034 (39)	3187 (9)	6·9 (9)
SB	2474 (1)	−9903 (10)	3958 (3)	7·8 (3)
C(6')B	2845 (3)	−8538 (32)	4172 (10)	7·9 (9)
C(7')B	3048 (3)	−9814 (28)	4947 (8)	6·2 (8)
C(8')B	3371 (2)	−9196 (22)	4949 (7)	4·9 (7)
N(8')B	3542 (2)	−10834 (17)	5588 (6)	4·6 (6)
C(9')B	3443 (2)	−6681 (27)	5205 (8)	6·0 (8)
O(9')B	3565 (2)	−6256 (18)	5923 (6)	8·1 (6)
O(10')B	3392 (2)	−5113 (22)	4610 (7)	9·8 (7)
OW(1)†	0000	10281 (22)	0000	4·8 (6)
OW(2)	−93 (2)	12241 (17)	1558 (5)	6·7 (6)
OW(3)	1456 (2)	5226 (30)	7368 (7)	11·7 (8)
OW(4)	1948 (2)	−7535 (39)	−874 (7)	16·3 (1·2)
OW(5)A†	3358 (8)	−5129 (62)	2788 (16)	19·8 (2·4)
OW(5)B†	3272 (10)	−4095 (80)	3020 (26)	19·1 (2·7)
OW(6)†	1581 (7)	−853 (74)	8371 (20)	25·5 (3·8)

† Occupancies for these atoms are 0·5.

(a)

(b)
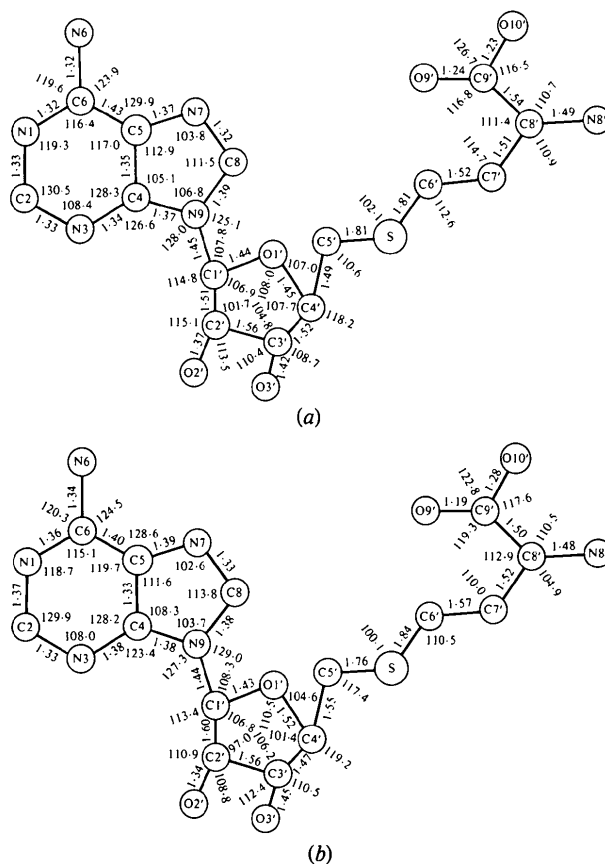
Fig. 2. Bond distances (Å) and angles (°) in (a) SAH-A and (b) SAH-B. The average e.s.d. for the distances is 0·01 Å and for the angles 0·8°.
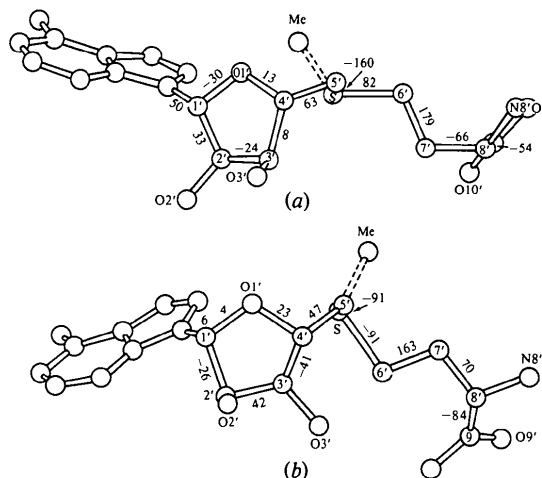

(a)

(b)

Fig. 3. The conformations of (a) SAH-A [C(2')-endo] and (b) SAH-B [C(3')-endo]. The torsion angles (°) in the ribose rings and along the main chain are indicated; their average e.s.d. is 1°. The likely position for the methyl group in the parent SAM molecule is also indicated.
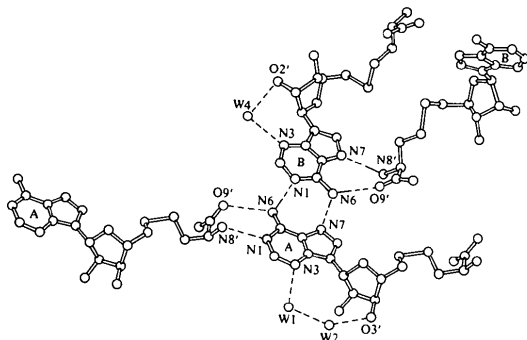
Fig. 4. The hydrogen bonding in the crystal of SAH. The base–base bonding, the cyclic base–amino acid bonding and the water bridges are indicated.

As shown in Fig. 3, molecules SAH-A and SAH-B have very different conformations. In both molecules the glycosidic angles [χ, defined by the torsion angle C(8)–N(9)–C(1')–O(1')] are *anti* but in SAH-A this angle has a much higher value than in SAH-B. The sugar puckering of SAH-A is C(2')-*endo* while in SAH-B it is C(3')-*endo*. Surrounding both molecules are water molecules that bridge the N(3) atoms on the bases to the hydroxy groups in the sugars; in SAH-A N(3) and O(3') are bridged by $W(1)$ and $W(2)$ and in SAH-B $W(4)$ bridges N(3) and O(2') (Fig. 4). These different water structures may help to stabilize the different molecular conformations. Fig. 3 also shows that the amino acid portions of both SAH molecules exhibit conformational flexibility.

The molecular charge distribution in this structure was determined from examination of the hydrogen bonding. In both molecules, SAH-A and SAH-B, the amino nitrogen [N(8')] atoms bind to three hydrogen-bond acceptors and the carboxyl oxygen atoms [O(9') and O(10')] each bind to two hydrogen-bond donors (Table 2). Therefore, both SAH-A and SAH-B are zwitterions in this crystal with the amino group N(8') as $-NH_3^+$ and the carboxyl group C(9') as $-COO^-$, which is the active form in biological systems.

The crystal structure is held together by an elegant network of hydrogen bonds with all hydrogen-bonding donors and acceptors being utilized (Figs. 4 and 5, and Table 2). The bases are involved in asymmetric base pairing such that the N(6) and N(7) atoms of SAH-A hydrogen bond to the N(1) and N(6) atoms of the adenine in SAH-B. This type of base pair was observed in the structure of deoxyadenosine monohydrate (Watson, Sutor & Tollin, 1965). SAH-A also binds to a translationally related molecule and SAH-B to a twofold-symmetry-related molecule through cyclic base amino acid hydrogen bonds: N(6)→O(9'), N(8')→N(1) for SAH-A and N(6)→O(9'), N(8')→N(7) for SAH-B (Fig. 4). Translationally related molecules of both SAH-A and SAH-B are held together *via* N(8')→O(10') hydrogen bonds (Fig. 5). A single ribose

Table 2. *Hydrogen-bond distances* (Å) *in SAH*

The average e.s.d. is 0·01 Å, except for distances involving OW(5)A, OW(5)B, and OW(6) in which case the e.s.d.'s are 0·04 Å.

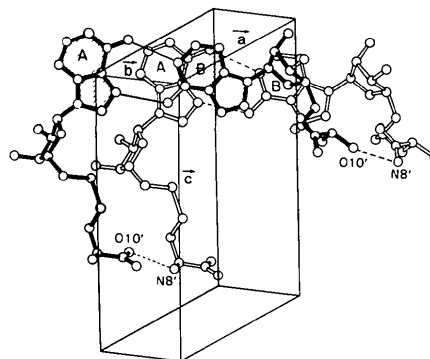| i	j		Symmetry (j)
Base–base			
N(6)A	N(1)B	3·05	x,y,z
N(6)B	N(7)A	2·96	x,y,z
Base–amino acid			
N(6)B	O(9)B	2·92	$\frac{1}{2}-x, y, 1-z$
N(8')B	N(7)B	2·87	$\frac{1}{2}-x, -\frac{1}{2}+y, 1-z$
N(6)A	O(9')A	3·04	$x, y, -1+z$
N(8')A	N(1)A	2·89	$x, y, 1+z$
Amino acid–amino acid			
N(8')A	O(10')A	2·72	$x, 1+y, z$
N(8')B	O(10')B	2·89	$x, -1+y, z$
N(8')B	O(9')B	3·12	$x, -1+y, z$
Amino acid–sugar			
O(3')A	O(10')A	2·75	$-x, 1+y, 1-z$
Water contacts			
OW(1)	N(3)A	2·79	x,y,z
	OW(2)	2·77	x,y,z
OW(2)	O(2')A	2·64	$x, 1+y, z$
	O(3')A	2·90	x,y,z
OW(3)	N(8')A	2·99	x,y,z
	O(3')B	3·20	$-\frac{1}{2}-x, \frac{1}{2}+y, 1-z$
	OW(5)A	3·18	$-\frac{1}{2}-x, \frac{1}{2}+y, 1-z$
	OW(5)A	2·80	$\frac{1}{2}-x, \frac{1}{2}+y, 1-z$
	OW(5)B	2·86	$+\frac{1}{2}-x, \frac{1}{2}+y, 1-z$
	OW(6)	2·73	$x, 1+y, z$
OW(4)	N(3)B	2·86	x,y,z
	O(2')B	2·94	x,y,z
	O(3')B	2·79	$\frac{1}{2}-x, \frac{1}{2}+y, -z$
	OW(6)	2·66	$x, -1+y, -1+z$
OW(5)A	O(10')B	2·82	x,y,z
	OW(6)	1·92	$\frac{1}{2}-x, -\frac{1}{2}+y, 1-z$
	OW(5)B	0·82	x,y,z
OW(5)B	O(10')B	2·51	x,y,z
OW(6)	O(9')A	2·95	x,y,z
	O(3')B	3·17	$\frac{1}{2}-x, \frac{1}{2}+y, 1-z$
	OW(5)B	2·58	$\frac{1}{2}-x, \frac{1}{2}+y, 1-z$

Fig. 5. The stacking of the adenine base pairs and the amino acid–amino acid hydrogen bonding in SAH.

amino acid contact [O(3')A→O(10')A] exists. Finally, in addition to the N(3)A···W(1)···W(2)···O(3')A and N(3)B···W(4)···O(2')B intramolecular water bridges, there are two intermolecular bridges O(10')B··· W(5)A···W(3)···N(8')A and O(10')B···W(5)B··· W(6)···O(9')A.

Discussion

Borchardt & Wu (1974) have pointed out that SAH–enzyme bindings involve three points of attachment in the amino acid terminal of the SAH molecule, i.e. –S–, –NH$_3^+$ and –COO$^-$ as shown in Fig. 1. A rigid spatial arrangement of these three groupings must be required in SAH–enzyme complex formation. Our studies show that the spatial relationships of –S–, –NH$_3^+$ and –COO$^-$ are roughly the same in the SAH-A and SAH-B molecules.

	S···N(8')	S···O(9')	S···O(10')
SAH-A	4·68 Å	5·50 Å	4·90 Å
SAH-B	5·13	5·77	4·96

	N(8')–S–O(9')	N(8')–S–O(10')	O(9')–S–O(10')
SAH-A	29°	44°	24°
SAH-B	27	42	22

It is very interesting to see this kind of structure invariance in the two different conformers. Whether this is significant in biological systems is unclear. The two groups, –NH$_3^+$ and –COO$^-$, interact with other groups extensively as described before. However, the whole structure reveals only a single possible –S– involved interaction, i.e. SA···N(8')B(0·5 − x, 1·5 + y, 1 − z) 3·38 Å. Furthermore, the role of –S– in direct binding to enzyme is questionable. Recent reports from Borchardt, Eiden, Wu & Rutledge (1979) show that the analogue of SAH replacing –S– by –C(–NH₂)– still retains activity in some SAM-dependent methyltransferases.

Finally, it should be noted that the two conformers also have quite different thermal motions in the crystal. The SAH-A molecule has an average equivalent isotropic temperature factor of 3·7 Å² while the value for SAH-B is 6·6 Å². Since both molecules are involved in a similar number of hydrogen bonds, the different

thermal motions may indicate that SAH-A has a more stable conformation than SAH-B.

Note: After submission of this manuscript an independent determination of this structure was reported by Ishida, Morimoto, Inoue, Fujiwara & Tomita (1981). That structure is almost identical to ours except that there are three water molecules in the asymmetric unit rather than 2·5.

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