

VIBRATIONAL STUDIES OF THE DISULFIDE GROUP IN PROTEINS

Part III. A simplified ab initio force field for diethyl disulfide and SS and CS stretch frequency-conformation correlations for diisobutyl disulfide

WENYUN ZHAO*, JAGDEESH BANDEKAR and SAMUEL KRIMM

Biophysics Research Division and Department of Physics, University of Michigan, Ann Arbor, MI 48109 (U S A)

(Received 21 November 1989)

ABSTRACT

We have obtained a simplified ab initio force field for diethyl disulfide, based on our previous scaled ab initio force field, which can be used in normal coordinate analysis of proteins containing the disulfide bridge. A normal coordinate analysis has been performed for diisobutyl disulfide in all possible conformations. The correlations thus obtained between the SS and CS stretch frequencies and the conformation are useful in understanding similar correlations in proteins containing the disulfide bridge. A simple way is presented to identify local C_2 symmetry in a disulfide bridge through Raman polarization studies.

INTRODUCTION

The dependence of the SS stretch ($\nu(\text{SS})$) and CS stretch ($\nu(\text{CS})$) frequencies on the conformation of the disulfide bridge in proteins, $\text{H}-\overset{|}{\underset{|}{\text{C}}}-\text{CH}_2-\text{S}-\text{S}-\text{CH}_2-\overset{|}{\underset{|}{\text{C}}}-\text{H}$, has been the subject of several papers [1-6]. Since Van Wart and Scheraga [7] agreed with the assignments of Sugeta et al. [2], the controversial aspects of this subject seem to have been resolved. These correlations propose that $\nu(\text{SS})$ lies at about 510, 525 or 540 cm^{-1} when the conformation contains two, one, or no H atoms, respectively, *trans* to distal S atoms across the CS bond, while $\nu(\text{CS})$ lies at 630-670 or 700-745 cm^{-1} when the conformation contains an H or a C atom, respectively, *trans* to the S atom across the CC bond [2]. These simple rules indicate that $\nu(\text{SS})$ depends on $\tau(\text{CS})$, the CCSS dihedral angle, and $\nu(\text{CS})$ depends on $\tau(\text{CC})$, the HCCS

*Present address: Department of Chemical Engineering, Beijing Institute of Chemical Technology, Beijing 10013, China

dihedral angle However, the rules do not indicate if there is any correlation between $\nu(\text{SS})$ and $\tau(\text{CC})$ or between $\nu(\text{CS})$ and $\tau(\text{CS})$ Our previous study [8] showed that the latter dependence exists in diethyl disulfide, $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$, and thus a more detailed description of these correlations is necessary than had been indicated previously [2]

To investigate such correlations in a protein would require normal mode analysis on a peptide structure containing the disulfide bridge together with a force field that includes a reliable conformation dependence of the force constants In the latter respect, the scaled ab initio force field that was reported previously [8, 9] has proved to be much better than empirical force fields [6, 10] However, this force field, when transferred to other molecules, would require more than 1000 force constants for every conformation, which would be unwieldy We have therefore obtained a simplified force constant set based on the scaled ab initio force field

Even with a reliable simple force field, the systematic investigation of the vibrational modes of the disulfide bridge in proteins is very complicated For example, we have found from our calculation that $\nu(\text{SS})$ and $\nu(\text{CS})$ depend not only on $\tau(\text{SS})$ and the two $\tau(\text{CS})$, but also on the two χ' , two ϕ , two ψ and four ω dihedral angles of the peptide groups around the disulfide bridge The variation of any one of these angles may lead to about a 10 cm^{-1} change in $\nu(\text{SS})$ or $\nu(\text{CS})$ A simplified model calculation to obtain a general understanding of this subject seemed useful, and we have therefore analyzed the conformational dependence of the normal modes of diisobutyl disulfide, $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$

SIMPLIFIED SCALED AB INITIO FORCE FIELD FOR DIETHYL DISULFIDE

As explained in our previous paper [8], for any one of six symmetric conformations, CGC, GGG, SGS, TGT, S'GS' and G'GG' of $\text{C}_2\text{H}_5\text{SSC}_2\text{C}_5$ ($\text{C} = \text{cis}$, $\text{G} = \text{gauche}$, $\text{S} = \text{skew}$, $\text{T} = \text{trans}$), a complete force constant matrix containing about 1080 elements (F_{ij} and F_{ji} are counted once) has been obtained by scaling the ab initio force field to fit the observed frequencies In order to simplify this force field, it is necessary to eliminate those force constants that are less important in the normal coordinate calculation. The vibrational eigenvalue λ_i is determined by

$$\lambda_i = \sum_{jk} L_{ji} L_{ki} F_{jk}$$

Letting F_{jk} be zero would affect λ_i by a relative difference $2L_{ji}L_{ki}F_{jk}/\lambda_i$, which is the potential energy distribution (PED) contribution to the i th normal vibration from this force constant, F_{jk} Therefore, a simple criterion can be used if

$$\sum_i (L_{ji} L_{ki} F_{jk} / \lambda_i)^2 < C$$

where C is a given constant, F_{jk} is considered to be negligible and is deleted from the force constant set

It was found that the number of force constants could be considerably reduced if the \mathbf{F} matrix in internal coordinates was transformed into the \mathbf{F}_s matrix in symmetry coordinates and the same criterion applied. Therefore, symmetry coordinates were used in the CH_2 group in the subsequent calculations. However, internal coordinates were retained in the CH_3 group so that these could be easily combined with the peptide force field, where C_{3v} symmetry no longer exists. The internal coordinates and symmetry coordinates are shown in Fig 1 and Table 1

The value of C was selected to be 0.001, which resulted in only five of 252 frequencies of the six symmetric conformers having errors of 0.6–0.7%, with none exceeding 0.7%, when this simplified force field was compared with the original complete scaled ab initio force field. The maximum error for $\nu(\text{SS})$ and $\nu(\text{CS})$ was 1.9 cm^{-1} . Only 20–25% of the force constants remained when C was set at 0.001. Increasing this value gives rise to larger errors and decreases

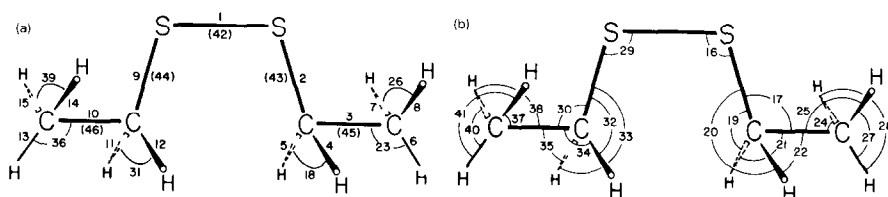


Fig 1 Numbering of internal coordinates for $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$

TABLE 1

Symmetry coordinates for $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5^a$

$$\begin{aligned} S_{17} &= 5R_{17} - R_{18} - R_{19} - R_{20} - R_{21} - R_{22} \\ S_{18} &= 4R_{18} - R_{19} - R_{20} - R_{21} - R_{22} \\ S_{19} &= R_{19} - R_{20} + R_{21} - R_{22} \\ S_{20} &= R_{19} + R_{20} - R_{21} - R_{22} \\ S_{21} &= R_{19} - R_{20} - R_{21} + R_{22} \\ S_{22} &= R_{17} + R_{18} + R_{19} + R_{20} + R_{21} + R_{22} \\ S_{30} &= 5R_{30} - R_{31} - R_{32} - R_{33} - R_{34} - R_{35} \\ S_{31} &= 4R_{31} - R_{32} - R_{33} - R_{34} - R_{35} \\ S_{32} &= R_{32} - R_{33} + R_{34} - R_{35} \\ S_{33} &= R_{32} + R_{33} - R_{34} - R_{35} \\ S_{34} &= R_{32} - R_{33} - R_{34} + R_{35} \\ S_{35} &= R_{30} + R_{31} + R_{32} + R_{33} + R_{34} + R_{35} \end{aligned}$$

^aAll other symmetry coordinates have the same number as the internal coordinates

TABLE 2

Force constants for $C_2H_5SSC_2H_5^a$

(a) Conformer-independent force constant						
Coordinate ^b	Number	Value	Coordinate ^b	Number	Value	
CH s	(4,4) (5,5)	4 805	CH ₂ tw/CCH b	(21,24)	0 067	
CH s	(6,6)	4 738	CH ₂ tw/CCH b	(21,25)	-0 067	
CH s	(7,7) (8,8)	4 812	CH ₂ tw/CH ₂ tw	(21,34)	0 002	
CH s/CH s	(4,5) (6,7)	0 048	CCH b/CCH b	(23,24) (23,25)	-0 131	
	(6,8) (7,8)			(24,25)		
CH s/CH ₂ d	(4,18) (5,18)	0 089	CCH b/HCH b	(23,26)	-0 097	
CC s/HCH b	(3,26)	-0 141	CCH b/HCH b	(23,27) (23,28)	-0 086	
CC s/HCH b	(3,27)	-0 150	CCH b/HCH b	(24,26) (25,26)	-0 092	
SSC b/CH ₂ r	(16,32)	0 007	CCH b/HCH b	(24,27) (25,28)	-0 099	
CH ₂ r/CCH b	(19,24)	-0 059	CCH b/HCH b	(24,28) (25,27)	-0 096	
CH ₂ w/CCH b	(20,23)	0 031	CCH b	(25,25)	0 539	
CH ₂ w/CCH b	(20,24) (20,25)	-0 028	HCH b	(26,26)	0 447	
CH ₂ w/HCH b	(20,26)	0 012	HCH b	(27,27) (28,28)	0 450	
CH ₂ w/CH ₂ w	(20,33)	0 003	HCH b/HCH b	(26,27) (26,28)	-0 081	
			HCH b/HCH b	(27,28)	-0 077	

(b) Conformer-dependent force constants							
Coordinate ^b	Number	Value					
		CGC	GGG	SGS	TGT	S'GS'	G'GG'
SS s	(1,1)	2 610	2 636	2 626	2 650	2 622	2 541
SS s/SC s	(1,2)	0 122	0 095	0 092	0 075	0 096	0 110
SS s/SSC b	(1,16)	0 202	0 256	0 253	0 242	0 254	0 255
SS s/SCC d	(1,17)	0 000	-0 020	0 011	0 068	0 024	0 041
SS s/CH ₂ r	(1,19)	0 015	-0 036	-0 015	-0 003	0 008	0 038
SC s	(2,2)	2 746	2 852	2 780	2 841	2 781	2 837
SC s/CC s	(2,3)	0 185	0 197	0 185	0 180	0 185	0 161
SC s/CS s	(2,9)	0 022	0 015	0 016	0 017	0 017	0 048
SC s/SSC b	(2,16)	0 368	0 165	0 187	0 093	0 186	0 183
SC s/SCC d	(2,17)	0 408	0 294	0 204	0 125	0 192	0 302
SC s/CH ₂ d	(2,18)	-0 135	-0 143	-0 146	-0 139	-0 148	-0 150
SC s/CH ₂ r	(2,19)	-0 025	0 092	0 025	0 007	-0 021	-0 091
SC s/CH ₂ w	(2,20)	0 346	0 311	0 349	0 368	0 352	0 323
SC s/CCH b	(2,23)	0 024	0 057	0 052	0 028	0 048	0 053
SC s/SC t	(2,43)	0 002	0 032	-0 003	0 000	0 005	-0 022
CC s	(3,3)	4 139	4 153	4 113	4 074	4 099	4 174
CC s/SSC b	(3,16)	-0 013	-0 021	0 028	0 069	0 042	-0 061
CC s/SCC d	(3,17)	0 128	0 162	0 154	0 194	0 154	0 083
CC s/CH ₂ d	(3,18)	-0 110	-0 114	-0 122	-0 122	-0 122	-0 115
CC s/CH ₂ w	(3,20)	-0 284	-0 269	-0 272	-0 279	-0 271	-0 274
CC s/CCH b	(3,23)	0 128	0 102	0 099	0 125	0 101	0 111
CC s/CCH b	(3,24)	0 150	0 161	0 166	0 151	0 158	0 158
CC s/CCH b	(3,25)	0 158	0 165	0 159	0 149	0 162	0 150
CC s/HCH b	(3,28)	-0 151	-0 156	-0 144	-0 150	-0 153	-0 142

(b) Conformer-dependent force constants

Coordinate ^b	Number	Value					
		CGC	GGG	SGS	TGT	S'GS'	G'GG'
SSC b	(16,16)	1 517	1 205	1 212	1 082	1 214	1 221
SSC b/SCC d	(16,17)	0 286	0 005	0 068	0 215	0 090	0 077
SSC b/CH ₂ r	(16,19)	0 000	-0 067	-0 062	0 000	0 048	0 071
SSC b/CH ₂ w	(16,20)	0 083	0 058	0 018	-0 058	0 007	0 060
SSC b/CH ₂ tw	(16,21)	0 008	-0 131	-0 076	-0 011	0 074	0 119
SSC b/CCH b	(16,23)	-0 072	-0 019	0 016	0 036	0 018	-0 026
SSC b/CSS b	(16,29)	-0 005	-0 049	-0 030	-0 030	-0 033	0 181
SSC b/CCS d	(16,30)	0 027	0 014	0 033	-0 003	-0 023	0 116
SSC b/SS t	(16,42)	0 120	0 024	0 007	0 018	0 014	0 304
SSC b/SC t	(16,43)	0 003	0 067	-0 012	0 008	-0 003	-0 050
SSC b/CS t	(16,44)	0 050	0 014	-0 014	0 022	-0 021	0 008
SSC b/CH ₃ t	(16,45)	0 038	0 003	0 009	0 001	-0 010	0 013
SSC b/CH ₃ t	(16,46)	-0 001	-0 001	0 001	-0 003	0 002	0 005
SSC d	(17,17)	1 094	0 909	0 860	0 835	0 852	0 952
SCC d/CH ₂ r	(17,19)	-0 004	0 047	0 023	0 001	-0 030	-0 042
SCC d/CH ₂ w	(17,20)	0 040	0 034	0 026	0 020	0 027	0 037
SCC d/CCH b	(17,23)	0 056	0 096	0 101	0 094	0 100	0 092
SCC d/CCH b	(17,24)	-0 016	-0 033	-0 045	-0 035	-0 040	-0 043
SCC d/CCH b	(17,25)	-0 005	-0 046	-0 041	-0 036	-0 043	-0 042
SCC d/CCS d	(17,30)	0 040	0 001	0 009	0 004	0 001	0 142
SCC d/SS t	(17,42)	0 098	0 016	0 020	0 005	-0 015	0 213
SCC d/SC t	(17,43)	0 039	0 066	0 061	0 003	-0 075	-0 043
SCC d/CH ₃ t	(17,45)	0 021	0 003	-0 012	-0 001	0 014	0 006
CH ₂ d	(18,18)	0 563	0 539	0 533	0 533	0 536	0 545
CH ₂ d/CH ₂ w	(18,20)	-0 009	-0 012	-0 001	0 000	-0 002	-0 016
CH ₂ r	(19,19)	0 759	0 717	0 674	0 660	0 678	0 722
CH ₂ r/CH ₂ tw	(19,21)	-0 013	-0 027	-0 021	-0 020	-0 021	-0 017
CH ₂ r/CCH b	(19,25)	0 051	0 060	0 060	0 061	0 063	0 064
CH ₂ r/CH ₂ r	(19,32)	0 032	0 001	-0 001	0 001	0 004	-0 002
CH ₂ r/SS t	(19,42)	0 084	0 000	0 007	0 012	0 002	-0 023
CH ₂ r/SC t	(19,43)	-0 062	0 065	0 019	-0 002	0 014	0 025
CH ₂ r/CH ₃ t	(19,45)	-0 027	-0 017	-0 015	-0 015	-0 014	-0 016
CH ₂ w	(20,20)	0 590	0 595	0 610	0 618	0 607	0 594
CH ₂ w/CH ₂ tw	(20,21)	-0 003	-0 004	-0 004	-0 002	0 008	0 003
CH ₂ w/SS t	(20,42)	0 009	0 000	0 001	0 006	0 011	0 013
CH ₂ w/SC t	(20,43)	-0 007	-0 004	-0 020	-0 001	0 026	0 000
CH ₂ tw	(21,21)	0 589	0 585	0 589	0 598	0 588	0 588
CH ₂ tw/SC t	(21,43)	-0 059	0 005	0 031	-0 018	0 014	0 001
CCH b	(23,23)	0 533	0 525	0 522	0 521	0 521	0 527
CCH b	(24,24)	0 531	0 544	0 540	0 540	0 541	0 536
SS t	(42,42)	0 490	0 154	0 151	0 152	0 144	0 469
SS t/SC t	(42,43)	0 151	-0 001	0 004	-0 001	-0 002	-0 021
SS t/CH ₃ t	(42,45)	-0 022	0 007	0 000	-0 002	-0 001	0 040
SC t	(43,43)	-0 005	0 113	-0 053	0 059	-0 049	0 085
SC t/CS t	(43,44)	0 062	0 000	-0 001	-0 002	-0 006	0 007
SC t/CH ₃ t	(43,45)	-0 005	0 003	0 002	-0 008	0 000	0 026
CH ₃ t	(45,45)	0 098	0 096	0 092	0 081	0 090	0 114

TABLE 2 (continued)

(c) Special force constants					
CGC conformer only			G'GG' conformer only		
Coordinate ^b	Number	Value	Coordinate ^b	Number	Value
SC _s /CCS d	(2,30)	0 023	SC _s /CH ₂ tw	(2,21)	-0 039
SC _s /SS t	(2,42)	0 066	SC _s /CSS b	(2,29)	0 090
CH _s /SS t	(7,42)	-0 074	SC _s /CCS d	(2,30)	0 074
SSC b/CH ₂ d	(16,18)	-0 044	SC _s /SS t	(2,42)	0 122
SSC b/CCH b	(16,25)	0 041	SCC d/CH ₂ r	(17,32)	-0 023
SCC d/CS t	(17,44)	0 061	SCC d/CCH b	(17,36)	-0 013
CH ₂ r/CH ₃ t	(19,46)	-0 005	SCC d/SC t	(17,44)	-0 017
CH ₂ tw/SS t	(21,42)	-0 042	SCC d/CH ₃ t	(17,46)	0 020
CH ₂ tw/CH ₃ t	(21,45)	-0 006	CH ₂ tw/CH ₃ t	(21,45)	-0 011
CCH b/SS t	(24,42)	0 028	CCH b/SS t	(23,42)	-0 020
CCH b/CH ₃ t	(24,45)	-0 014	HCH b/HCH b	(26,39)	0 004
SC t/CH ₃ t	(43,46)	-0 008	SC t/CH ₃ t	(43,46)	0 004

^aForce constants that can be obtained by the C_2 symmetry operation are not listed in the table
^bs = stretch, b = bend, w = wag, tw = twist, r = rock, t = torsion, d = deformation (see symmetry coordinates of Table 1)

ing it leads to the retention of more force constants. The force constants of this simplified force field for $C_2H_5SSC_2H_5$ are listed in Table 2, the force constants which can be obtained by the C_2 symmetry operation are not listed. Because of the stronger intramolecular interactions in the CGC and G'GG' conformers than in the other four symmetric conformers, the interaction force constants between the two ethyl groups were larger in these two conformers and more force constants were retained for $C=0.001$, these are listed separately. For convenience, if a force constant remained in any one of the other four symmetric conformers by this criterion, we tabulated it for all six conformers.

SOME COMMENTS ON THE APPLICATION OF THIS FORCE FIELD

It is necessary to keep in mind that this force field was obtained using the ab initio optimized geometry. To minimize any errors associated with different geometries, it is therefore necessary to use the ab initio geometry, as listed in Table 1 of our previous paper [8], when this force field is incorporated in a normal coordinate calculation. The force constants for an unsymmetric disulfide bridge can be obtained in a manner similar to that described previously [8].

For most disulfide groups, $\tau(SS)$ is found in the range $75^\circ-105^\circ$. Our calculations indicate that the SS bond length in $C_2H_5SSC_2H_5$ varies with $\tau(SS)$ in a manner similar to that in CH_3SSCH_3 . Therefore, to obtain more accurate

results, the SS stretch force constant, $f(\text{SS})$, can be modified according to $\tau(\text{SS})$, using the same relationship as in CH_3SSCH_3 [9]. If $\tau(\text{SS})$ changes by 15° from the optimal calculated value for the conformer (see Table 2 of our previous paper [8]), then $f(\text{SS})$ should be corrected by -1.0% from the value given in Table 2 of this paper, for a 30° change the correction should be -3.0% . The CS stretch force constant does not change much with $\tau(\text{SS})$, and therefore no correction is necessary.

To calculate the normal modes of peptide molecules containing the disulfide bridge, this scaled ab initio force field would have to be combined (at present) with empirical force fields for the peptide chain [11]. The determination of the force constants in the junction between the disulfide group and the peptide chain may not be so straightforward. This is because of the significantly different bases of the two force fields: the ab initio, derived from the potential energy surface, has many more force constants than the empirical, which aims to reproduce the observed frequencies with as small a force constant set as possible. This can make the determination of the force constants in the junction region somewhat arbitrary. Another complication is that the ab initio force field is canonic [12], whereas the empirical force field is generally not. If the latter is transformed to a canonic force field, the junction force constants can be initially taken as the average of the two values.

NORMAL COORDINATE CALCULATION FOR DIISOBUTYL DISULFIDE

In an initial model to simulate the disulfide bridge in proteins, two H atoms on each side of the bridge in $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ were replaced by masses equal to carbon atoms. This model is very similar to that used by Kuptsov and Trofimov [6]. Our calculations on this model indicate that $\nu(\text{CS})$ for any $\tau(\text{CS})$ is always lower than 670 cm^{-1} , which agrees with the result of Kuptsov and Trofimov [6]. However, this is not consistent with the experimental data [2], which show that $\nu(\text{CS})$ for diisobutyl disulfide is at 710 or 745 cm^{-1} if any methyl C atom is *trans* to an S atom. This difficulty is resolved if we use the complete structure of $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$, i.e. if we add three H atoms to every terminal C atom in the above model. The H atoms are indispensable for obtaining the proper $\nu(\text{CS})$ in the calculation, and the simplification of replacing the CH_3 group by a single C atom is not a satisfactory approximation.

In these calculations the force field for the disulfide bridge portion was taken from our scaled ab initio force field for $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ [8] and that for the terminal methyl groups was taken from Snyder's force field for paraffins [13]. In the junction regions between these two, the average values of the force constants from these two force fields were used. The geometric parameters of $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ [8] were used for the disulfide group and standard geometric parameters [13] were used for the isobutyl group. The calculated $\nu(\text{CS})$ values

are shown in Fig. 2. This figure shows that when an S atom is *trans* to an H atom (i.e. *gauche* to two methyl C atoms), $\nu(\text{CS})$ is predicted between 662 and 719 cm^{-1} , which is similar to the result of Kuptsov and Trofimov [6]. However, when an S atom is *trans* to a methyl C atom, $\nu(\text{CS})$ is predicted between 704 and 755 cm^{-1} , which was not obtained by Kuptsov and Trofimov. Figure 2 also shows the specific dependence of $\nu(\text{CS})$ on $\tau(\text{CS})$. $\nu(\text{CS})$ increases as $\tau(\text{CS})$ increases. Previous studies have shown that $\tau(\text{CS})$ is usually found at about 68° (*gauche*) or 180° (*trans*). From Fig. 2, this leads to $\nu(\text{CS})$ values of

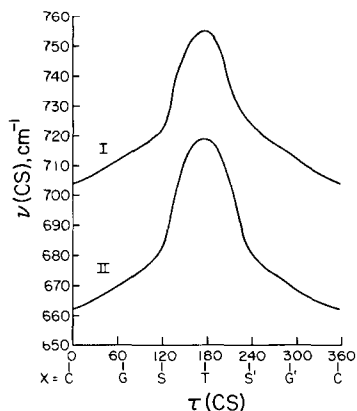


Fig. 2 Dependence of $\nu(\text{CS})$, the CS stretch frequency, on $\tau(\text{CS})$, the CS dihedral angle, for GXG (I) and --GXT (II) $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$. The central G refers to $\tau(\text{SS})$ and the terminal G and T to $\tau(\text{CC})$, the HCCS dihedral angle

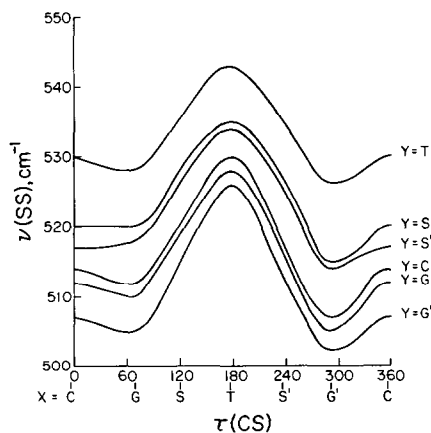


Fig. 3 Dependence of $\nu(\text{SS})$, the SS stretch frequency, on $\tau(\text{CS})$, the CS dihedral angle, for GXGYG $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$. The G, X, G, Y and G in GXGYG refer to $\tau(\text{HCCS})$, $\tau(\text{CCSS})$, $\tau(\text{CSSC})$, $\tau(\text{SSCC})$ and $\tau(\text{SCCH})$, respectively

about $671, 713 \text{ cm}^{-1}$ (*gauche*) or about $719, 755 \text{ cm}^{-1}$ (*trans*), which are consistent with the observed values of Sugeta et al [2], ($666, 710$ and 745 cm^{-1}). However, our calculations also show that the observed frequency, 710 cm^{-1} , arises not only from the conformation with a methyl C atom *trans* to an S atom, as predicted by Sugeta et al. [2], but also from the conformation with two methyl C atoms *gauche* to an S atom, provided that $\tau(\text{CS})$ is about 180°

The correlation of $\nu(\text{SS})$ of $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$ with $\tau(\text{CS})$ is much more complicated and cannot be shown in a single figure. In Figs 3, 4

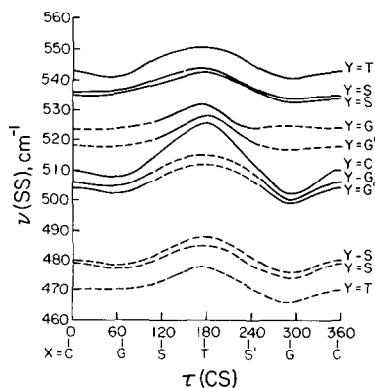


Fig 4 Dependence of $\nu(\text{SS})$, the SS stretch frequency, on $\tau(\text{CS})$, the CS dihedral angle, for GXGYT $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$. The G, X, G, Y and T in GXGYT refer to $\tau(\text{HCCS})$, $\tau(\text{CCSS})$, $\tau(\text{CSSC})$, $\tau(\text{SSCC})$ and $\tau(\text{SCCH})$, respectively. When $\nu(\text{SS})$ makes significant contributions to two normal modes, we have indicated the frequencies containing the larger (—) and smaller (----) contributions to the potential energy distribution.

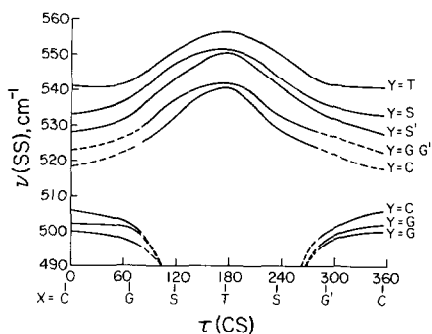


Fig 5 Dependence of $\nu(\text{SS})$, the SS stretch frequency, on $\tau(\text{CS})$, the CS dihedral angle, for TXGYT $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$. The T, X, G, Y and T in TXGYT refer to $\tau(\text{HCCS})$, $\tau(\text{CCSS})$, $\tau(\text{CSSC})$, $\tau(\text{SSCC})$ and $\tau(\text{SCCH})$, respectively. When $\nu(\text{SS})$ makes significant contributions to two normal modes, we have indicated the frequencies containing the larger (—) and smaller (----) contributions to the potential energy distribution.

and 5 we show $\nu(\text{SS})$ for GXGYG, GXGYT and TXGYT, respectively, where the terminal G and T refer to $\tau(\text{HCCS})$ and $\tau(\text{SCCH})$ and the X and Y refer to $\tau(\text{CCSS})$ and $\tau(\text{SSCC})$, respectively. By comparing with the observed $\tau(\text{SS})$ of $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$, 512 and 525 cm^{-1} [2], it is found that the $-\text{GGG}-$ conformation of this molecule may exist. However, it is clear from Figs 3, 4 and 5 that other structures could also account for the 512 cm^{-1} band. Several structures could also account for the 525 cm^{-1} band.

A SIMPLE WAY TO IDENTIFY LOCAL C_2 SYMMETRY OF THE DISULFIDE BRIDGE

By analyzing the calculated results for CH_3SSCH_3 , $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ and $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$ we have found that the symmetric $\nu(\text{CS})$ is

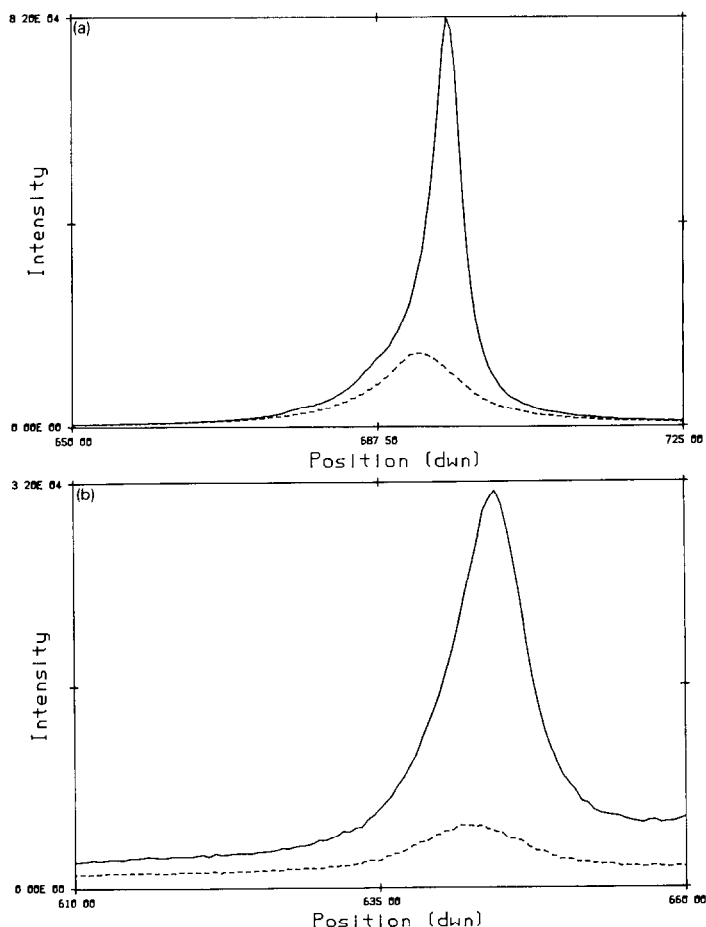


Fig 6 Raman spectra of the CS stretch bands of (a) CH_3SSCH_3 and (b) $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ —, parallel spectrum, ----, perpendicular spectrum

always higher than the antisymmetric $\nu(\text{CS})$ by 5–20 cm^{-1} for any symmetric conformation of these molecules. For these conformations, the symmetric $\nu(\text{CS})$ interacts with $\nu(\text{SS})$, resulting in an increase in frequency of the former and a decrease in the latter. The antisymmetric $\nu(\text{CS})$ has a different symmetry from $\nu(\text{SS})$ and therefore there is no interaction between them.

As a result of this, a local C_2 symmetry of the disulfide bridge can be recognized easily from a Raman polarization experiment. If $\nu(\text{CS})$ in the parallel orientation is a few wavenumbers higher than the frequency in the perpendicular orientation, the disulfide bridge can be identified as having a local C_2 symmetry environment. We carried out such Raman polarization experiments for CH_3SSCH_3 and $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$. The frequency shifts between the different polarization orientations for these two molecules were 3.5 and 2.0 cm^{-1} , respectively (see Fig. 6). These values are smaller than those obtained from our calculations, partly because of the overlap of the symmetric and antisymmetric $\nu(\text{CS})$ bands (in $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$, also because of the overlap with one of the $\nu(\text{CS})$ bands of the GGT conformer).

CONCLUSIONS

By eliminating about 75% of the force constants in our full scaled ab initio force field for $\text{C}_2\text{H}_5\text{SSC}_2\text{H}_5$ [8], we have produced a force field that still reproduces the observed frequencies very well. This force field will make the application of normal mode analysis to the study of the disulfide bridge in proteins more manageable.

As a model for this disulfide bridge, we have performed complete normal mode analyses of the possible conformers of $(\text{CH}_3)_2\text{CHCH}_2\text{SSCH}_2\text{CH}(\text{CH}_3)_2$. Additional correlations have been demonstrated beyond those obtained previously [6, 10].

Our theoretical and experimental studies show that a local C_2 symmetry of the disulfide bridge can be revealed from Raman polarization measurements on the $\nu(\text{CS})$ modes: since the symmetric $\nu(\text{CS})$ is expected to be higher than the antisymmetric $\nu(\text{CS})$, polarized spectra of such structures show a higher frequency intense band in parallel polarization and a lower frequency weak band in perpendicular polarization.

ACKNOWLEDGEMENT

This research was supported by the Monsanto Company, St. Louis, MO, and by NSF grants DMB-8816756 and DMR-8806975.

REFERENCES

- 1 R C Lord and N -T Yu, *J Mol Biol* , 50 (1970) 509
- 2 H Sugeta, A Go and T Miyazawa, *Chem Lett* , (1972) 83, *Bull Chem Soc Jpn* , 46 (1973) 3407
- 3 H Brunner and M Holz, *Biochim Biophys Acta*, 379 (1975) 408
- 4 H E Van Wart and H A Scheraga, *J Phys Chem* , 80 (1976) 1812, 1823
- 5 T Takamatsu, I Harada and K Hayashi, *Biochim Biophys Acta*, 622 (1980) 189
- 6 A H Kuptsov and V I Trofimov, *J Biomol Struct Dyn* , 3 (1985) 185
- 7 H E Van Wart and H A Scheraga, *Proc Natl Acad Sci USA*, 83 (1986) 3064
- 8 W Zhao and S Krimm, *J Mol Struct* , 224 (1990) 7
- 9 W Zhao, J Bandekar and S Krimm, *J Am Chem Soc* , 110 (1988) 6891
- 10 H Sugeta, *Spectrochim Acta*, part A, 31 (1975) 1729
- 11 S Krimm and J Bandekar, *Adv Protein Chem* , 38 (1986) 181
- 12 K Kuczera and R Czerminski, *J Mol Struct* , 105 (1983) 269
- 13 R G Snyder, *J Chem Phys* , 47 (1967) 1316