

Energetics of the Disulfide Bridge: An Ab Initio Study

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SYNOPSIS

The energetics of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge have been obtained from an ab initio study of diethyl disulfide. Calculations at the 3-21G* level were done on relaxed structures at every $\sim 30^\circ$ in χ_1^2 and χ_2^2 , and the additional energies for small $\Delta\chi^3$ were obtained. Complete $E(\chi_1^2, \chi_2^2)$ and $\chi_0^3(\chi_1^2, \chi_2^2)$ maps were computed from Fourier series expansions. These results have been used to calculate the energetics of 92 disulfide bridges in known protein structures, and to compare ab initio and molecular mechanics energies for some observed and predicted bridges. The differences found in relative energies and in χ_0^3 values suggest that present energy functions give a limited description of the structural and energetic properties of the disulfide bridge. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

The disulfide bridge, $(\text{NH})(\text{CO})\text{C}^\alpha\text{H}-\text{C}^\beta\text{H}_2-\text{S}-\text{S}-\text{C}^\beta\text{H}_2-\text{C}^\alpha\text{H}(\text{NH})(\text{CO})$, is one of the basic elements of the three-dimensional structures of proteins.¹ Knowledge of its conformational energies is therefore of importance in assessing the stabilities of protein structures, whether with native² or engineered³⁻⁶ disulfides. While there have been several studies on the stereochemical modeling of disulfide bridges,⁷⁻¹⁰ very few^{4,8} have dealt with the energetics of such structures, and these have used rough molecular mechanics energy functions to evaluate the conformations. Recent ab initio calculations¹¹ have been restricted to studies of the barriers and dihedral angle of dimethyl disulfide, which provides only restricted information on the full disulfide bridge.

To reliably characterize the energetics of the main part of the disulfide bridge requires at a minimum an ab initio analysis of diethyl disulfide, which can provide the dependence of the parameters on the $\text{C}^\beta\text{SSC}^\beta$, χ^3 , and the two $\text{C}^\alpha\text{C}^\beta\text{SS}$, χ^2 , dihedral angles. As a preliminary step¹² in deriving an ab initio vibrational force field to relate SS and CS stretch frequencies of the disulfide bridge to its conformation,¹³ we had calculated the energies of 20 stationary state conformers of this molecule. We have now

extended these calculations to produce a $\chi_1^2-\chi_2^2$ energy map for χ_0^3 , i.e., at its minimum, and determined the dependence of this energy on small values of $\Delta\chi^3$. A map of χ_0^3 as a function of χ_1^2 and χ_2^2 shows that this angle departs significantly from $\sim 90^\circ$ in many regions of the conformational space. These results represent an initial step in providing an accurate basis for determining relative energies and structures of disulfide bridge conformations.

CALCULATIONS AND RESULTS

Mapping out the entire conformation space of the disulfide bridge by ab initio calculations would be an extravagant task. This is particularly true if we were to include the dependence of energy on the $\text{NC}^\alpha\text{C}^\beta\text{S}$, χ^1 , dihedral angles. For the present, we assume that the latter energy is associated with the polypeptide chain, and that the dependence on χ_1^2 , χ^3 , and χ_2^2 is not significantly affected by χ^1 . Nor do we scan the entire three-dimensional $\chi_1^2\chi^3\chi_2^2$ space. Rather, we fully optimize geometries at given $\chi_1^2\chi_2^2$, obtaining energies E and χ_0^3 values, and then explore ΔE as a function of small $\Delta\chi^3$ in selected regions. As we will see, there seems to be a regularity to this variation.

As in our previous studies,¹² the ab initio calculations were done with the 3-21G* basis set. In addition to the previous 20 conformers at energy minima, maxima, and saddle points, we have computed

Table I Energies, Dihedral Angles, and Some Geometric Parameters^a of Conformers of Diethyl Disulfide

Conformer ^b	E^c	χ_1^2	χ_0^3	χ_2^2	$r(\text{C}_1\text{S})$	$r(\text{SS})$	$r(\text{SC}_2)$	$\theta(\text{CC}_1\text{S})$	$\theta(\text{C}_1\text{SS})$	$\theta(\text{SSC}_2)$	$\theta(\text{SC}_2\text{C})$
TT	0.40	177.6	86.6	177.6	1.828	2.044	1.828	109.0	102.9	102.9	109.0
TD'	1.41	177.6	86.8	-150.0	1.827	2.044	1.834	109.0	102.6	103.6	109.9
TS'	2.09	176.5	86.9	-123.8	1.828	2.043	1.838	109.0	103.0	104.2	111.5
TB'	1.28	177.6	93.0	-90.0	1.829	2.044	1.830	108.7	104.0	103.9	113.9
TG'	0.82	174.8	97.5	-68.6	1.830	2.046	1.827	108.7	103.6	103.7	114.6
TA'	3.01	177.6	93.6	-30.0	1.828	2.042	1.842	108.8	103.4	107.1	116.4
TC	4.03	174.0	90.8	-4.7	1.828	2.039	1.849	108.9	103.4	107.8	116.7
TA	2.50	177.6	85.3	30.0	1.828	2.039	1.840	109.0	103.4	106.7	115.9
TG	0.20	177.6	86.6	69.4	1.828	2.043	1.828	109.0	102.7	103.3	113.6
TB	0.92	177.6	86.9	90.0	1.827	2.044	1.831	108.9	102.7	103.2	112.7
TS	1.98	177.1	87.4	120.0	1.828	2.043	1.838	109.0	103.1	104.1	111.4
TD	1.12	177.6	86.4	150.0	1.828	2.042	1.833	109.0	103.4	103.7	110.0
D'D'	2.51	-150.0	87.0	-150.0	1.834	2.044	1.834	109.8	103.4	103.4	109.8
D'S'	3.28	-150.0	87.8	-123.8	1.835	2.044	1.838	109.8	103.9	104.1	111.6
D'B'	2.32	-150.0	94.3	-90.0	1.835	2.045	1.830	109.7	104.6	103.4	113.8
D'G'	1.72	-150.0	96.7	-69.5	1.835	2.045	1.827	109.8	104.2	103.2	114.5
D'A'	4.06	-150.0	92.9	-30.0	1.835	2.042	1.843	109.9	104.0	106.7	116.4
D'C	5.14	-150.0	89.8	-4.7	1.835	2.039	1.850	109.9	104.2	107.5	116.7
D'A	3.66	-150.0	86.0	30.0	1.835	2.039	1.840	109.9	104.1	106.5	116.0
D'G	1.23	-150.0	86.7	68.2	1.834	2.045	1.827	110.0	103.4	103.0	113.6
D'B	2.02	-150.0	87.3	90.0	1.834	2.045	1.831	109.8	103.4	102.9	112.7
D'S	3.13	-150.0	88.3	117.4	1.835	2.044	1.838	109.8	103.9	103.9	111.5
D'D	2.20	-150.0	87.5	150.0	1.835	2.043	1.833	109.8	104.1	103.4	109.6
S'S'	3.98	-125.5	88.6	-125.5	1.839	2.043	1.839	111.4	104.4	104.4	111.4
S'B'	2.79	-125.5	92.3	-90.0	1.839	2.044	1.829	111.5	105.0	103.7	113.7
S'G'	2.42	-125.5	95.6	-74.4	1.839	2.045	1.826	111.8	105.1	104.0	114.4
S'A'	4.82	-125.5	95.0	-30.0	1.839	2.043	1.842	112.1	104.7	107.4	116.4
S'C	6.09	-115.0	97.3	-6.7	1.838	2.041	1.849	113.2	104.7	108.6	117.0
S'A	4.27	-125.5	88.2	30.0	1.839	2.039	1.840	111.7	104.7	106.9	115.9
S'G	1.93	-123.9	86.5	68.2	1.838	2.044	1.827	111.5	104.1	103.4	113.6
S'B	2.78	-125.5	87.8	90.0	1.838	2.044	1.831	111.5	104.1	103.4	112.7
S'S	3.83	-125.5	89.2	118.6	1.839	2.043	1.838	111.5	104.5	104.3	111.4
S'D	2.84	-125.5	88.0	150.0	1.839	2.042	1.833	111.4	104.6	103.6	109.6
B'B'	3.00	-90.0	106.5	-90.0	1.831	2.051	1.831	114.9	104.9	104.9	114.9
B'G'	2.73	-90.0	111.5	-69.5	1.831	2.053	1.828	115.0	103.9	104.3	115.4
B'A'	4.88	-90.0	107.6	-30.0	1.831	2.048	1.843	115.0	103.7	107.8	117.2
B'C	5.41	-90.0	102.5	-4.7	1.831	2.043	1.849	114.6	103.7	108.5	117.1
B'A	3.16	-90.0	91.6	30.0	1.829	2.041	1.840	113.8	103.9	107.4	115.8
B'G	1.12	-90.0	93.5	68.2	1.830	2.045	1.828	113.9	103.7	104.3	113.4
B'B	1.86	-90.0	94.6	90.0	1.830	2.045	1.832	113.9	103.5	104.1	112.6
B'S	2.64	-90.0	92.4	117.4	1.829	2.043	1.839	113.6	103.7	104.8	111.5
B'D	1.75	-90.0	90.6	150.0	1.829	2.042	1.833	113.6	104.1	104.4	109.5
G'G'	2.36	-71.0	114.5	-71.0	1.829	2.056	1.829	115.5	103.7	103.7	115.5
G'A'	4.46	-69.5	109.8	-30.0	1.829	2.047	1.844	115.5	103.3	107.3	117.3
G'C	4.97	-70.5	105.7	-12.6	1.829	2.045	1.849	115.2	103.4	108.2	117.3
G'A	2.70	-69.5	94.4	30.0	1.827	2.042	1.841	114.3	103.8	107.3	115.8
G'G	0.66	-69.1	98.0	68.6	1.827	2.047	1.829	114.6	103.5	103.8	113.4
G'B	1.30	-69.5	97.6	90.0	1.827	2.046	1.833	114.6	103.3	103.7	112.5
G'S	2.06	-69.5	94.6	117.4	1.827	2.044	1.839	114.3	103.6	104.6	111.4
G'D	1.30	-69.5	95.0	150.0	1.827	2.044	1.834	114.5	104.0	104.2	109.4
A'A'	6.64	-30.0	105.0	-30.0	1.845	2.042	1.845	117.3	107.1	107.1	117.3
A'C	6.92	-30.0	98.6	-4.7	1.844	2.038	1.850	116.7	107.0	107.9	117.1
A'A	4.83	-30.0	90.2	30.0	1.842	2.038	1.839	115.9	107.1	107.1	115.9
A'G	2.90	-30.0	93.8	68.2	1.843	2.043	1.828	116.5	106.9	103.7	113.4

Table I (Continued)

Conformer ^b	E^c	χ_1^2	χ_0^3	χ_2^2	$r(\text{C}_1\text{S})$	$r(\text{SS})$	$r(\text{SC}_2)$	$\theta(\text{CC}_1\text{S})$	$\theta(\text{C}_1\text{SS})$	$\theta(\text{SSC}_2)$	$\theta(\text{SC}_2\text{C})$
A'B	3.62	-30.0	93.8	90.0	1.843	2.042	1.832	116.5	106.8	103.5	112.5
A'S	4.32	-30.0	91.8	117.4	1.842	2.041	1.837	116.1	106.9	104.3	111.3
A'D	3.42	-30.0	92.0	150.0	1.842	2.041	1.832	116.1	107.2	103.9	109.4
CC	7.54	-11.6	95.7	-11.6	1.849	2.035	1.849	116.9	107.7	107.7	116.9
CA	5.94	-4.7	88.5	30.0	1.848	2.036	1.838	116.4	108.0	107.1	115.9
CG	3.93	-5.2	90.8	68.3	1.850	2.039	1.828	116.8	107.7	103.8	113.5
CB	4.70	-4.7	90.5	90.0	1.850	2.039	1.832	116.7	107.6	103.7	112.6
CS	5.52	-1.8	89.3	116.5	1.849	2.038	1.837	116.5	107.7	104.5	111.5
CD	4.56	-4.7	90.2	150.0	1.849	2.038	1.832	116.5	108.0	104.0	109.5
AA	4.42	30.0	84.6	30.0	1.839	2.036	1.839	115.8	107.0	107.0	115.8
AG	2.36	30.0	85.1	68.2	1.828	2.040	1.840	113.6	103.7	106.6	115.9
AB	3.19	30.0	86.1	90.0	1.840	2.040	1.832	116.0	106.6	103.7	112.7
AS	4.10	30.0	87.0	117.4	1.840	2.039	1.838	115.9	106.8	104.5	111.5
AD	3.10	30.0	85.9	150.0	1.840	2.039	1.832	115.8	106.9	104.0	109.6
GG	0.00	69.4	86.3	69.4	1.827	2.045	1.827	113.5	103.1	103.1	113.5
GB	0.74	68.2	86.9	90.0	1.827	2.045	1.831	113.6	103.0	103.0	112.7
GS	1.80	68.2	87.2	119.9	1.827	2.043	1.838	113.6	103.5	104.0	111.4
GD	0.93	68.2	86.5	150.0	1.828	2.043	1.832	113.6	103.7	103.5	109.6
BB	1.53	90.0	87.4	90.0	1.831	2.045	1.831	112.7	103.0	103.0	112.7
BS	2.64	90.0	88.2	117.4	1.831	2.044	1.838	112.8	103.4	104.0	111.5
BD	1.86	90.0	87.4	150.0	1.831	2.044	1.833	112.7	103.6	103.4	109.7
SS	3.69	118.7	89.8	118.7	1.838	2.042	1.838	111.4	104.4	104.4	111.4
SD	2.71	117.4	88.4	150.0	1.837	2.042	1.832	111.6	104.5	103.8	109.6
DD	1.79	150.0	87.1	150.0	1.833	2.021	1.833	109.7	104.0	104.0	109.7

^a Bond lengths, r , in Å. Bond angles, θ , in degrees. Dihedral angles, $\chi_1^2 = \text{CC}_1\text{SS}$, $\chi_0^3 = \text{C}_1\text{SSC}_2$, $\chi_2^2 = \text{SSC}_2\text{C}$, in degrees.

^b Conformer, designated by $\chi_1^2\chi_2^2$. $T = 180^\circ$, $D = 150^\circ$, $S = 120^\circ$, $B = 90^\circ$, $G = 60^\circ$, $A = 30^\circ$, $C = 0^\circ$ (for $\chi^3 > 0$). Prime indicates negative angle.

^c Energy, in kcal/mole.

fully relaxed structures at the remaining 30° intervals in χ_1^2 and χ_2^2 , for a total of 78 structures. (Calculations were for $\chi^3 > 0$; these results apply to $\chi^3 < 0$ if the signs of χ_1^2 , χ^3 , and χ_2^2 are reversed.) The resulting values of E (with respect to the global minimum), χ_0^3 , and selected geometric parameters are given in Table I. The conformations are designated by the closest values of the χ^2 dihedral angle: 0° (C), 30° (A), 60° (G), 90° (B), 120° (S), 150° (D), 180° (T), and their negative values A', G', B', S', and D'.

In order to present a relatively accurate energy contour map as a function of χ_1^2 and χ_2^2 , we make use of the fact that E is a periodic function of these variables and can therefore be represented as a two-dimensional Fourier series whose coefficients can be determined from the calculated energies (see Appendix). The energy contours are then found by a program that uses the Fourier series as an analytic function to search by 0.5° – 1.0° steps along the tangents and with 0.001 kcal accuracy along the normals of the contours. The resulting map is given in Figure

1, with energies relative to the GG minimum, E_{GG} ; the exact energy at any $\chi_1^2\chi_2^2$ for χ_0^3 can be obtained from Eq. (A4) using the coefficients of Table AI. The same procedure can be used for χ_0^3 , the contours being determined by a search with 0.01° accuracy along the normals. The resulting map is given in Figure 2, with exact values obtainable from Eq. (A4) using the coefficients of Table AII.

In order to determine the additional energy associated with a departure from χ_0^3 , we have calculated ab initio energies at selected $\chi_1^2\chi_2^2$ values for $\Delta\chi^3 = \pm 10^\circ$ and $\pm 20^\circ$. The underlying assumption is that the $\chi_1^2\chi_2^2$ dependence for such small $\Delta\chi^3$ (which is in the range observed for 92 protein disulfide bridges¹⁴) is not significant. Calculations were done for 15 conformers that span a range of E and χ_0^3 . We found that $E(\Delta\chi^3)$ depended essentially only on χ_0^3 : for 7 conformers with $\chi_0^3 < 90^\circ$ (GA, GG, TD, TT, TS', BD, and SD), $E(\Delta\chi^3)$ had a value at any $\Delta\chi^3$ that was independent of E_{GG} (which varied from 0.00 to 2.71 kcal/mole); the same was true for 8 conformers with $\chi_0^3 > 90^\circ$ (S'B', B'G, G'S,

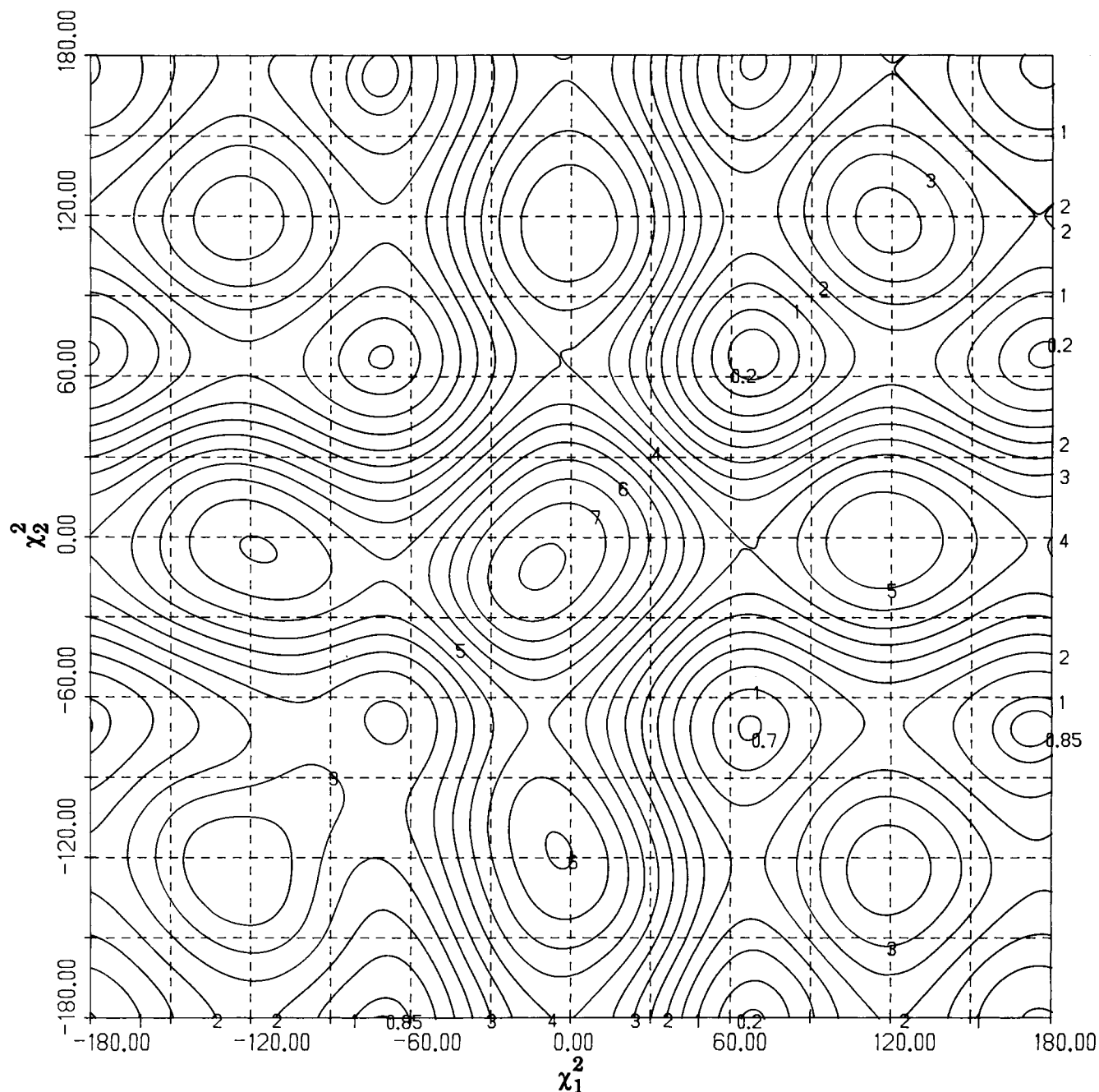


Figure 1. Energies (in kcal/mole) of relaxed diethyl disulfide structures as a function of χ_1^2 and χ_2^2 . Contours are at intervals of 0.5 kcal/mole, except where otherwise designated.

S'A', S'G', GB, G'G, and G'G'), although the specific $E(\Delta\chi^3)$ is different (E_{GG} varied from 0.66 to 4.82 kcal/mole for these conformers). By averaging the energies of the conformers in each group of $\Delta\chi^3 = \pm 10^\circ$ and $\pm 20^\circ$, and fitting the energy variation with a three-term Fourier series, we can accurately obtain $E(\Delta\chi^3)$ for intermediate $\Delta\chi^3$. These values

are given in Table II, together with average deviations at 5° intervals. As can be seen, the latter are ≤ 0.2 kcal/mole for all $\Delta\chi^3$ except $\Delta\chi^3 = -20^\circ$, -25° , and -30° for $\chi_0^3 > 90^\circ$, suggesting that the assumption that $E(\Delta\chi^3)$ has a small dependence on $\chi_1^2\chi_2^2$ is substantially valid for most χ_0^3 , particularly when $|\Delta\chi^3| < 20^\circ$.

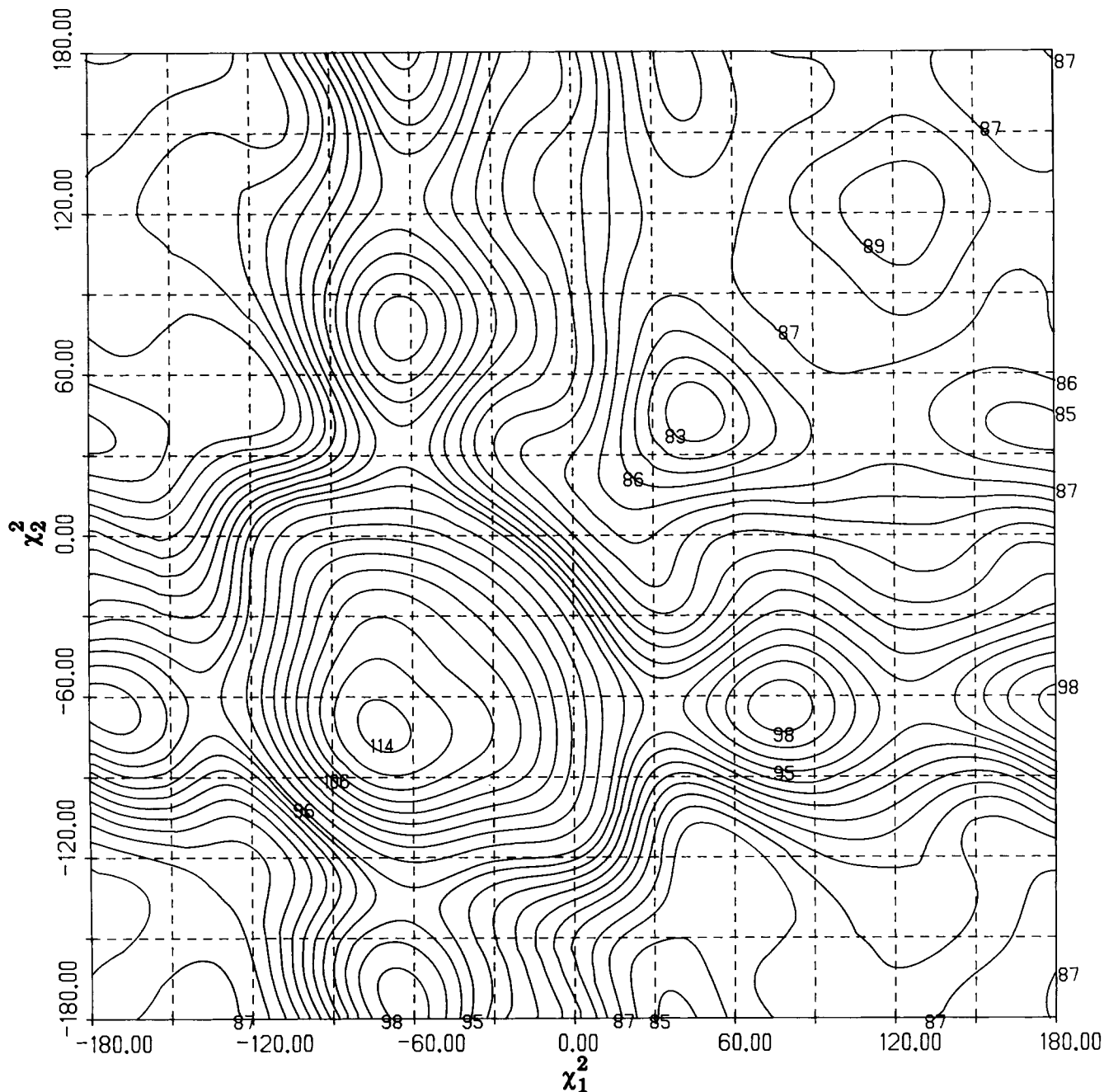


Figure 2. χ_0^3 (in degrees) of relaxed diethyl disulfide structures as a function of χ_1^2 and χ_2^2 . Contours are at intervals of 1° , except above 96° , where the interval is 2° .

DISCUSSION

The results represented by Figures 1 and 2 provide a view of the energetic and conformational landscape of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge. The maps are of course symmetrical about the χ_1^2 - χ_2^2 diagonal. It can be seen from Figure 1 that the 20 stationary structures are divided into three groups:

6 conformers are at energy minima (GG, GT, TT, GG', TG', and G'G'), another 6 conformers, SS, SS', S'S', CS, CS', and CC, are at maxima, and the remainder are all at transition states, surrounded by two minima and two maxima. This clearly shows that the conformer G'S', which could not be fully optimized in the region around -74° and -125° ,¹² is not at a saddle point, and a transition structure

Table II Energy Variation^a with $\Delta\chi^3$ ^b from χ_0^3 for Diethyl Disulfide

$\Delta\chi^3$	$\chi_0^3 < 90^\circ$			$\chi_0^3 > 90^\circ$			
	E^c	$\Delta\chi^3$	E^d	$\Delta\chi^3$	E^e	$\Delta\chi^3$	E^f
1	0.00	-1	0.00	1	0.01	-1	0.01
2	0.01	-2	0.01	2	0.02	-2	0.02
3	0.02	-3	0.02	3	0.04	-3	0.04
4	0.04	-4	0.04	4	0.06	-4	0.07
5	0.07	-5	0.07	5	0.10	-5	0.11
6	0.10	-6	0.11	6	0.14	-6	0.15
7	0.13	-7	0.15	7	0.18	-7	0.21
8	0.18	-8	0.20	8	0.24	-8	0.28
9	0.22	-9	0.25	9	0.30	-9	0.35
10	0.27	-10	0.31	10	0.36	-10	0.44
11	0.33	-11	0.38	11	0.43	-11	0.53
12	0.38	-12	0.45	12	0.51	-12	0.63
13	0.45	-13	0.53	13	0.59	-13	0.75
14	0.51	-14	0.62	14	0.67	-14	0.87
15	0.58	-15	0.72	15	0.76	-15	1.01
16	0.66	-16	0.82	16	0.85	-16	1.15
17	0.74	-17	0.93	17	0.95	-17	1.30
18	0.82	-18	1.04	18	1.05	-18	1.47
19	0.90	-19	1.17	19	1.15	-19	1.64
20	0.99	-20	1.29	20	1.26	-20	1.82
21	1.07	-21	1.43	21	1.37	-21	2.02
22	1.16	-22	1.57	22	1.48	-22	2.22
23	1.26	-23	1.72	23	1.59	-23	2.43
24	1.35	-24	1.87	24	1.70	-24	2.65
25	1.45	-25	2.04	25	1.82	-25	2.89
26	1.55	-26	2.20	26	1.93	-26	3.13
27	1.65	-27	2.38	27	2.05	-27	3.38
28	1.75	-28	2.55	28	2.17	-28	3.63
29	1.85	-29	2.74	29	2.29	-29	3.90
30	1.95	-30	2.93	30	2.41	-30	4.17

^a In kcal/mole.^b In degrees.^c With average deviations: 5°: ±0.00; 10°: ±0.01; 15°: ±0.02; 20°: ±0.02; 25°: ±0.03; 30°: ±0.06.^d With average deviations: -5°: ±0.01; -10°: ±0.01; -15°: ±0.04; -20°: ±0.08; -25°: ±0.13; -30°: ±0.20.^e With average deviations: 5°: ±0.02; 10°: ±0.04; 15°: ±0.06; 20°: ±0.10; 25°: ±0.13; 30°: ±0.15.^f With average deviations: -5°: ±0.01, -10°: ±0.05; -15°: ±0.12; -20°: ±0.23; -25°: ±0.38; -30°: ±0.58.

predicted by the Fourier series may be located in the region around -72° , -99° . Calculations on a finer grid are not likely to be very helpful, both because no uncertain region was found in the map and the higher term Fourier coefficients are already very small. The maps also show an interesting relationship between $E(\chi_1^2, \chi_2^2)$ and χ_0^3 . We see from Figure 2 that χ_0^3 is quite variable, taking values from $< 83^\circ$ to $> 114^\circ$. It might be thought that the departure from the expected equilibrium value near 90° (86.3° for the GG conformer, Table I; 87.4° for dimethyl disulfide¹¹) could be attributed to nonbonded interactions in the bridge, which would imply that highly

“distorted” χ^3 should be associated with high energies. This is clearly not the case. The largest χ_0^3 , $> 114^\circ$ at $\chi_1^2, \chi_2^2 \sim -70^\circ, -70^\circ$, is associated with an energy of ~ 2.5 kcal/mole, whereas energies of > 7.5 kcal/mole (at $\sim -10^\circ, -10^\circ$) have $\chi_0^3 \sim 96^\circ$. Similarly, energies of ~ 0.7 kcal/mole can be associated with $\chi_0^3 \sim 97.5^\circ$ (at $\sim 69^\circ, -70^\circ$) as well as χ_0^3 in the range of $\sim 83.5^\circ$ – 87.3° (near $\sim 70^\circ, 70^\circ$). It would seem that these results reflect the possibility that interactions occur between the χ_1^2 , χ^3 , and χ_2^2 torsions, a feature not taken into account by calculations based on simple torsion energy functions.^{4,8}

As noted above, the energy associated with small deviations from χ_0^3 , $E(\Delta\chi^3)$, depend essentially only on χ_0^3 , indicating that the $E(\chi_1^2, \chi_2^2)$ surface is not changed significantly for small $\Delta\chi^3$. To assess this as well as other aspects of the energetics of disulfide bridges in proteins, we have computed the various energies for the 92 S-S bridges that we studied previously.¹⁴ The results are given in Table III, and the positions of these structures on a $E(\chi_1^2, \chi_2^2)$ map are shown in Figure 3. The following will be seen: (1) Many of the structures fall in the low energy regions of the $E(\chi_1^2, \chi_2^2)$ map: 37% have energies of < 1 kcal/mole and 77% have energies of < 2 kcal/

mole. All of them fall in the 2 kcal contours centered at 5 minimum energy structures, and the numbers in each region seem fairly consistent with their relative energies: GG (29), GT (16), TT (7), GG' (13), and TG' (7), although such energy relationships may be slightly different at higher levels of theory. (2) $E(\Delta\chi^3)$ is generally fairly small: for 65% of the structures, this energy is < 0.2 kcal/mole and for 86% of the structures it is < 0.5 kcal/mole. (3) There is no apparent correlation between $E(\Delta\chi^3)$ and χ_0^3 , either for small χ_0^3 [cf. $\chi_0^3 = 82.9^\circ$ and $E(\Delta\chi^3) = 0.00$ for no. 43 with $\chi_0^3 = -82.7^\circ$ and $E(\Delta\chi^3) = 0.97$ for no. 44] or large χ_0^3 [cf. $\chi_0^3 = 97.1^\circ$

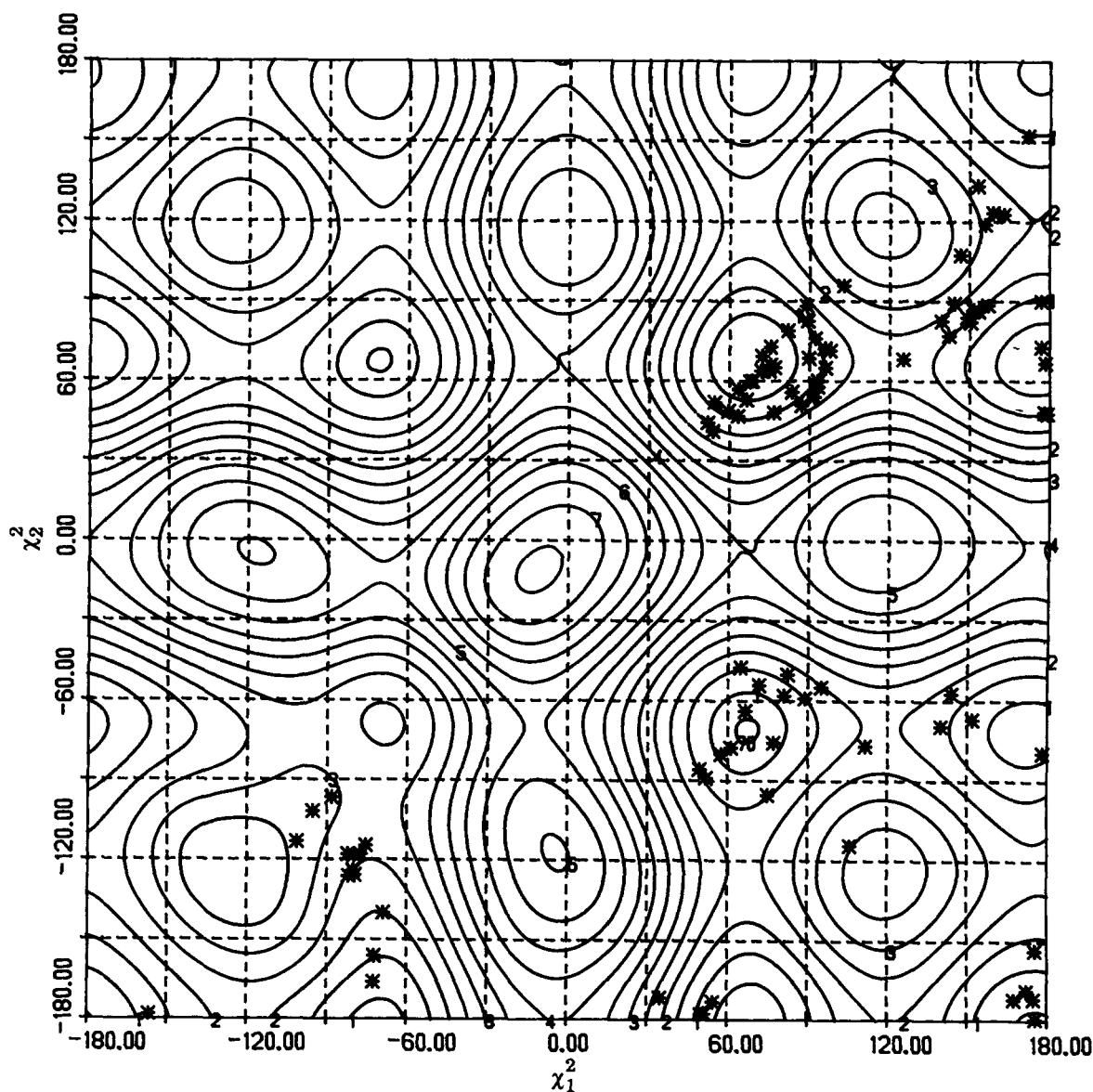


Figure 3. Locations of 92 disulfide bridges of known protein structures on $E(\chi_1^2, \chi_2^2)$ map.

Table III Energies of 92 Disulfide Bridges in Proteins

No.	Code ^a	Cys-Cys	χ_1^{2b}	χ_2^{2b}	$E(\chi_1^2, \chi_2^2)^c$	χ^{3b}	χ_0^{3b}	$E(\chi^3)^c$	$E(\chi^2, \chi^3)$
1	1ACX	34-43	-101.4	-113.3	3.35	108.0	92.1	0.85	4.20
2	1ACX	83-88	-50.2	177.9	0.87	-76.8	-85.5	0.23	1.10
3	2AZA	A3-A26	-60.0	-68.6	0.17	-96.9	-85.6	0.35	0.52
4	2AZA	B3-B26	-69.3	-72.3	0.07	-82.4	-86.6	0.05	0.12
5	1REI	A23-A88	-153.1	-133.3	2.26	-91.0	-88.0	0.02	2.28
6	1REI	B23-B88	123.3	158.7	2.28	81.1	87.9	0.14	2.42
7	2RHE	22-89	122.6	162.8	2.16	87.3	87.7	0.00	2.16
8	5CPA	138-161	144.0	-57.2	1.81	92.9	95.2	0.02	1.83
9	4CHA	A42-A58	-150.4	-82.1	1.41	-87.6	-87.1	0.00	1.41
10	4CHA	A136-A201	-114.7	-75.5	2.64	108.9	98.8	0.37	3.01
11	4CHA	A168-A182	153.3	-175.5	1.30	-75.4	-86.7	0.40	1.70
12	4CHA	A191-A220	54.7	-173.1	0.70	76.3	86.0	0.29	0.99
13	4CHA	B42-B58	-149.1	-84.0	1.58	-88.9	-87.2	0.00	1.58
14	4CHA	B136-B201	-118.0	-77.5	2.58	101.3	97.1	0.07	2.65
15	4CHA	B168-B182	156.7	178.3	1.20	-78.1	-86.9	0.24	1.44
16	4CHA	B191-B220	51.3	-177.5	0.80	72.8	85.6	0.51	1.31
17	2CGA	A42-A58	-144.3	-89.2	2.09	-92.2	-87.6	0.06	2.15
18	2CGA	A136-A201	-124.2	-80.2	2.47	99.1	94.9	0.07	2.54
19	2CGA	A168-A182	168.5	-172.4	0.70	-84.7	-86.9	0.01	0.71
20	2CGA	A191-B220	178.2	-79.4	0.92	100.1	96.1	0.07	0.99
21	2CGA	B42-B58	-142.2	-77.0	1.54	-98.6	-87.2	0.35	1.89
22	2CGA	B136-B201	-126.2	-81.9	2.48	105.4	94.2	0.44	2.92
23	2CGA	B168-B182	171.7	-175.0	0.60	-74.2	-87.0	0.52	1.12
24	2CGA	B191-B220	-176.9	-72.7	0.19	-89.3	-86.9	0.01	0.20
25	1CRN	3-40	-72.7	-75.4	0.17	-80.4	-86.9	0.13	0.30
26	1CRN	4-32	-82.8	-118.0	2.67	105.6	95.9	0.34	3.01
27	1CRN	16-26	-93.1	-58.0	1.11	-86.4	-86.9	0.00	1.11
28	3EBX	3-24	-67.8	-59.8	0.17	-85.4	-85.5	0.00	0.17
29	3EBX	17-41	-54.1	-91.8	1.25	-82.7	-86.7	0.04	1.29
30	3EBX	43-54	66.7	178.5	0.16	83.6	86.7	0.02	0.18
31	3EBX	55-60	88.9	88.1	1.36	85.6	87.1	0.00	1.36
32	3GRS	58-63	79.9	118.4	2.60	-133.4	-96.5	3.22	5.82
33	3FAB	L22-L87	118.9	155.8	2.43	110.5	88.0	1.21	3.64
34	3FAB	L136-L195	179.4	-175.6	0.45	-94.4	-87.0	0.15	0.60
35	3FAB	H22-H95	171.9	-167.6	0.66	-66.1	-86.7	1.37	2.03
36	3FAB	H144-H200	-171.9	-152.0	1.05	-79.7	-86.3	0.13	1.18
37	3FAB	L213-H220	111.6	-76.9	2.10	166.8	94.7	5.83	7.93
38	3INS	A6-A11	-72.5	-166.0	1.21	104.3	98.2	0.14	1.35
39	3INS	C6-C11	-72.3	-156.1	1.53	108.4	97.4	0.43	1.96
40	3INS	A7-B7	51.9	-88.9	1.44	98.2	91.8	0.16	1.60
41	3INS	A20-B19	-48.7	-59.6	0.87	-78.8	-83.5	0.06	0.93
42	3INS	C7-D7	57.4	-80.0	0.94	98.7	95.0	0.05	0.99
43	3INS	C20-D19	-41.1	-53.8	1.66	-83.0	-82.9	0.00	1.66
44	1LZ1	6-128	-51.9	-44.3	1.50	-65.3	-82.7	0.97	2.47
45	1LZ1	30-116	-95.7	-72.3	1.08	-95.7	-87.2	0.20	1.28
46	1LZ1	65-81	81.1	-58.1	1.23	95.0	98.3	0.05	1.28
47	1LZ1	77-95	178.0	48.3	0.95	83.0	85.2	0.01	0.96
48	2ALP	42-58	-156.9	-88.5	1.44	-88.4	-87.0	0.00	1.44
49	2ALP	137-159	-96.6	-88.2	3.05	102.5	103.9	0.01	3.06
50	1SN3	12-65	-63.8	66.5	0.75	88.5	98.1	0.40	1.15
51	1SN3	16-41	97.7	71.5	1.16	91.4	87.2	0.05	1.21
52	1SN3	25-46	-54.5	-51.9	0.86	-76.3	-83.2	0.14	1.00
53	1SN3	29-48	63.5	56.9	0.32	74.6	84.7	0.31	0.63
54	1NXB	3-24	-96.1	-65.0	1.07	-82.4	-87.1	0.06	1.13

Table III (Continued)

No.	Code ^a	Cys-Cys	χ_1^{2b}	χ_2^{2b}	$E(\chi_1^2, \chi_2^2)^c$	χ^{3b}	χ_0^{3b}	$E(\chi^3)^c$	$E(\chi^2, \chi^3)$
55	1NXB	17-41	-63.8	-74.9	0.16	-84.3	-86.4	0.01	0.17
56	1NXB	43-54	90.1	176.9	0.92	84.5	87.0	0.01	0.93
57	1NXB	55-60	82.5	89.3	1.10	86.6	87.1	0.00	1.10
58	2OVO	8-38	-46.8	-63.2	0.93	-75.6	-83.9	0.21	1.14
59	2OVO	16-35	-55.8	-83.5	0.74	-79.8	-86.5	0.13	0.87
60	2OVO	24-56	64.7	-47.5	1.57	98.9	96.2	0.03	1.60
61	9PAP	22-63	85.8	-49.4	1.47	-88.5	-92.4	0.06	1.53
62	9PAP	56-95	-95.3	75.0	1.46	97.4	92.7	0.09	1.55
63	9PAP	153-200	171.6	-34.9	2.31	-85.2	-85.0	0.00	2.31
64	1BP2	11-77	78.9	81.9	0.57	90.3	87.1	0.03	0.60
65	1BP2	27-123	-65.0	-77.0	0.19	-90.3	-86.6	0.04	0.23
66	1BP2	29-45	-66.9	151.9	1.23	97.6	95.5	0.02	1.25
67	1BP2	44-105	-68.5	-89.9	0.73	-78.6	-87.1	0.22	0.95
68	1BP2	51-98	-93.2	-60.0	1.04	-79.0	-87.0	0.19	1.23
69	1BP2	61-91	-54.1	71.7	1.17	108.4	97.8	0.40	1.57
70	1BP2	84-96	-76.0	-92.0	0.98	-96.8	-87.1	0.25	1.23
71	2SGA	42-58	-146.7	-107.5	2.76	-87.3	-88.1	0.00	2.76
72	2SGA	191-220	95.0	-54.8	1.96	101.4	96.9	0.08	2.04
73	3RP2	A42-A58	-153.3	-86.2	1.49	-88.2	-87.0	0.00	1.49
74	3RP2	A136-A201	-125.8	-79.6	2.43	113.3	94.7	1.11	3.54
75	3RP2	A168-A182	82.4	-50.1	1.72	158.9	97.4	5.37	7.09
76	3RP2	B42-B58	-151.5	-86.8	1.62	-80.5	-87.1	0.13	1.75
77	3RP2	B136-B201	-140.0	-68.8	2.00	98.9	95.6	0.05	2.05
78	3RP2	B168-B182	76.7	48.1	0.98	116.0	85.5	2.01	2.99
79	2APP	249-283	-75.7	77.1	0.88	103.8	97.7	0.14	1.02
80	5RSA	26-84	-87.1	-50.8	1.22	-81.4	-86.4	0.07	1.29
81	5RSA	40-95	-52.9	-66.4	0.50	-79.6	-84.7	0.07	0.57
82	5RSA	58-110	-68.1	-125.2	1.76	-86.4	-87.2	0.00	1.76
83	5RSA	65-72	-59.1	88.9	1.50	107.8	97.9	0.36	1.86
84	1TPP	22-157	-77.6	61.1	0.79	109.4	96.1	0.61	1.40
85	1TPP	42-58	-139.5	-82.5	1.91	-77.3	-87.6	0.33	2.24
86	1TPP	128-232	-69.4	140.0	1.61	99.5	94.4	0.10	1.71
87	1TPP	136-201	-101.8	-95.3	3.17	100.5	98.3	0.02	3.19
88	1TPP	168-182	63.8	72.0	0.10	68.7	86.2	0.98	1.08
89	1TPP	191-220	47.7	179.1	1.00	83.3	85.1	0.01	1.01
90	5PTI	5-55	-75.5	-66.0	0.14	-82.8	-86.6	0.04	0.18
91	5PTI	14-38	105.9	-114.3	3.47	95.1	89.8	0.08	3.55
92	5PTI	30-51	-102.8	-95.9	2.55	-89.9	-87.9	0.01	2.56

^a 1ACX: actinoxanthin; 2AZA: azurin; 1REI, 2RHE: Bence-Jones protein; 5CPA: carboxypeptidase; 4CHA: α -chymotrypsin; 2CGA: chymotrypsinogen; 1CRN: crambin; 3EBX: erabutoxin; 3GRS: glutathione reductase; 3FAB: immunoglobulin; 3INS: insulin; 1LZ1: lysozyme; 2ALP: lytic protease; 1SN3, 1NXB: neurotoxin; 2OVO: ovomucoid; 9PAP: papain; 1BP2: phospholipase A2; 2SGA, 3RP2, 2APP: proteinase; 5RSA: ribonuclease; 1TPP: trypsin complex; 5PTI: trypsin inhibitor.

^b Dihedral angle, in degrees. $\chi_1^2 = \text{CC}_1\text{SS}$, $\chi_2^2 = \text{SSC}_2\text{C}$, $\chi^3 = \text{C}_1\text{SSC}_2$, $\chi_0^3 = \text{ab initio minimum}$.

^c Energy, in kcal/mole.

and $E(\Delta\chi^3) = 0.07$ for no. 14 with $\chi_0^3 = 97.4^\circ$ and $E(\Delta\chi^3) = 5.37$ for no. 75]. (4) Although there are as many cases of $\chi_0^3 > 0$ (with an average value of 92.8°) as $\chi_0^3 < 0$ (with an average value of -86.7°), in both cases there is a preponderance of $|\Delta\chi^3| > 0$. These characteristics undoubtedly reflect the special nature of the surroundings of the disulfide bridge in a protein as compared to the simpler diethyl disulfide system.

It is useful to compare the results given by our ab initio energies with those predicted by the torsional potentials of present molecular mechanics functions. In Table IV we show such a comparison for some classes of observed left-handed and right-handed SS bridges,⁴ for two engineered SS bridges in subtilisin,⁴ and for the SS bridges of rat mast cell protease.⁸ In the case of the left-handed bridges, the energies are similarly ordered but differ in relative

Table IV Comparison Between Molecular Mechanics and Ab Initio Energies of Observed Disulfide Bridges

Protein	χ_1^{2a}	χ^{3a}	χ_2^{2a}	χ_0^{3a}	$E_{MM}(\chi^2, \chi^3)^b$	$E_{AI}(\chi^2, \chi^3)^b$
L.H.SS^c						
1	-57	-85	-64	-84.7	0.53	0.31
2	173	-84	-171	-86.8	0.67	0.61
3	171	-82	-171	-86.8	0.71	0.72
4	-65	-80	-93	-87.0	1.70	1.07
5	-87	-89	-148	-87.3	2.52	1.79
R.H.SS^d						
1	-61	98	63	97.6	0.99	0.87
2	-56	103	74	98.1	1.61	1.21
3	-78	103	-166	97.5	1.96	1.45
4	170	92	43	84.9	1.20	1.53
5	-71	103	84	98.0	2.18	1.20
6	-83	101	-121	95.0	3.82	2.76
Subtilisin^e						
Cys 22-Cys 87	121	-98	143	-88.5	4.33	3.90
Cys 24-Cys 87	-50	96	-171	97.1	1.11	1.75
Protease^f						
Cys 42-Cys 58	-153.3	-88.2	-86.2	-87.6	1.94	2.73
Cys 136-Cys 201	-125.8	113.2	-79.6	-86.7	0.89	1.24
Cys 168-Cys 182	82.4	158.9	-50.1	86.1	1.46	2.32
Cys 42-Cys 58	-151.5	-80.5	-86.8	97.0	1.38	2.38
Cys 136-Cys 201	-140.0	98.9	-68.8	86.4	1.70	2.18
Cys 168-Cys 182	76.9	116.0	48.1	95.4	2.35	4.08

^a Dihedral angle, in degrees. $\chi_1^2 = CC_1SS$, $\chi^3 = C_1SSC_2$, $\chi_2^2 = SSC_2C$, $\chi_0^3 =$ ab initio minimum.

^b Energy, in kcal/mole. MM: molecular mechanics (AMBER for L.H.SS, R.H.SS, and subtilisin, GROMOS for protease), AI: = ab initio.

^c From Ref. 4. Numbers refer to classes of proteins with left-handed disulfides.

^d From Ref. 4. Numbers refer to classes of proteins with right-handed disulfides.

^e From Ref. 4. Refined x-ray structures of engineered disulfides.

^f From Ref. 8.

and absolute magnitudes. In the case of the right-handed bridges, the order is significantly inverted in some cases and the magnitudes are substantially different. The subtilisin energies are in the same order but the magnitudes are significantly different. For the protease, the order of energies is again very different and the magnitudes are often widely apart. We think this reflects the oversimplified torsion potentials being assumed in the molecular mechanics functions for what is probably a coupled bridge system. Molecular mechanics energy functions usually assume that the potential function for χ^2 in the SS bridge only has cos terms and is independent for χ_1^2 and χ_2^2 . From Table AI we can see that the sin term coefficients $F_{10}^{sc} = -0.35$, $F_{20}^{sc} = -0.14$, and $F_{30}^{sc} = -0.13$ are all of the same order as the second cos term coefficient $F_{02}^{cc} = 0.54$, and therefore the assumption of an even potential function may not be valid in this case. As to the independence assumption, the cross-term coefficients $F_{11}^{sc} = -0.16$,

$F_{11}^{ss} = 0.22$, and $F_{12}^{ss} = 0.13$ are also of the same order as F^{cc} . It is not likely that such terms can be deleted by separating the nonbonded interactions from the potential function and optimizing them.

CONCLUSIONS

The ab initio energies of diethyl disulfide should provide a more accurate description of the energetics of the $\chi_1^2\chi^3\chi_2^2$ portion of the disulfide bridge in proteins than is obtainable from present molecular mechanics functions. Differences are certainly seen between relative energies calculated by these two methods for observed or predicted bridges, and these may be due to the limited nature of the torsion potentials used in the energy functions. For small departures from χ_0^3 ($|\Delta\chi^3| < 20^\circ$), the energy depends primarily on χ_1^2 and χ_2^2 , with a relatively constant increment for $\Delta\chi^3$ whose value depends only on

whether χ_0^3 is smaller or larger than 90° . This information plus the $E(\chi_1^2, \chi_2^2)$ map makes it possible to readily determine the relevant energy of a disulfide bridge once χ_1^2 , χ_2^2 , and χ^3 are given.

The ab initio studies also show that χ_0^3 is quite variable, having values between $< 83^\circ$ and $> 114^\circ$. Nor are departures from the widely assumed equilibrium value of $\sim 90^\circ$ attributable to higher energies of the bridge, presumably from nonbonded interactions. These studies therefore suggest that the total electronic structure must be taken into account in order to properly describe the energies and structures of the disulfide bridge.

APPENDIX

Since the energy E is a periodic function of χ_1^2 and χ_2^2 , it can be represented by a truncated two-dimensional Fourier series on the assumption that the highest frequency component is not larger than $N/2$, viz.,

$$E(\chi_1^2, \chi_2^2) = \sum_{m=0}^{N-1} \sum_{n=0}^{N-1} F_{mn} \times \exp[-i\tau(m\chi_1^2 + n\chi_2^2)] \quad (\text{A1})$$

Table AI Coefficients of Fourier Series for $E(\chi_1^2, \chi_2^2)$ [Eq. (A4)]

		F_{mn}^{cc}						
		n						
		0	1	2	3	4	5	6
m	0	3.0147	0.847800	0.536770	1.0409	0.013197	-0.054787	-0.041513
	1		0.057098	-0.079923	-0.076815	0.017264	0.016289	-0.006821
	2			-0.034413	-0.003889	-0.013304	-0.016798	0.003849
	3				0.043571	-0.020427	-0.001367	0.002678
	4					0.000529	0.010502	-0.008309
	5						-0.011344	-0.004365
	6							0.006409

		F_{mn}^{sc}						
		n						
		0	1	2	3	4	5	6
m	1	-0.346550	-0.161950	0.045841	0.077266	-0.037407	0.006122	0.010101
	2	-0.144100	-0.052890	0.059914	0.061352	-0.023006	0.012132	0.011711
	3	-0.131100	-0.029800	-0.003000	-0.021453	0.020863	0.019568	0.006887
	4	0.041358	-0.024509	-0.004729	0.010313	0.008968	-0.009079	-0.001200
	5	-0.016032	-0.021542	0.015050	0.023329	-0.013131	0.005318	0.003513

		F_{mn}^{ss}				
		n				
		1	2	3	4	5
m	1	0.22204	0.127310	0.033926	0.020998	0.037757
	2		0.053418	0.007215	0.024386	0.013913
	3			-0.031389	0.022615	0.008852
	4				0.007189	-0.005376
	5					0.021852

where the sampling interval τ is $360^\circ/N$ and the coefficients F_{mn} are given by the discrete Fourier transformation

$$F_{mn} = \frac{1}{N^2} \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} E(\chi_1^2, \chi_2^2) \exp[ir(m\chi_1^2 + n\chi_2^2)] \quad \times m, n = 0, 1, \dots, N-1 \quad (A2)$$

By substituting (A2) into (A1), expanding the exponentials, and making use of the symmetry and orthogonality properties of the various summed products, we find that

$$E(\chi_1^2, \chi_2^2) = \frac{1}{N^2} \sum_{m=0}^{N-1} \sum_{n=0}^{N-1} [F_{mn}^{cc} \cos m\chi_1^2 \cos n\chi_2^2 + F_{mn}^{ss} \sin m\chi_1^2 \sin n\chi_2^2 + F_{mn}^{cs} \cos m\chi_1^2 \sin n\chi_2^2 + F_{mn}^{sc} \sin m\chi_1^2 \cos n\chi_2^2] \quad (A3)$$

with

$$F_{mn}^{cc} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \cos m\chi_1^2 \cos n\chi_2^2$$

$$F_{mn}^{ss} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \sin m\chi_1^2 \sin n\chi_2^2$$

Table AII Coefficients of Fourier Series for $\chi_0^3(\chi_1^2, \chi_2^2)$ [Eq. (A4)]

		F_{mn}^{cc}						
		n						
		0	1	2	3	4	5	6
m		91.683	2.2608	-1.43410	-0.896220	0.473180	0.376160	0.109440
	0		0.2071	-0.69768	-0.414550	0.305280	-0.179860	-0.083668
	1			-0.32891	0.079237	-0.274650	-0.182360	0.072627
	2				0.541920	-0.294700	-0.167560	0.000847
	3					0.054598	-0.083145	0.074219
	4						-0.111820	-0.058893
	5							0.006335
	6							

		F_{mn}^{sc}						
		n						
		0	1	2	3	4	5	6
m		-4.07290	-1.22270	0.21973	0.484040	-0.541730	-0.006197	0.149150
	1		-0.12894	0.40904	0.473650	-0.167980	0.090300	0.070256
	2		0.14594	0.30129	0.162440	-0.011696	0.147460	0.072773
	3		-0.40549	-0.26651	-0.077994	-0.051936	-0.149070	-0.057131
	4		-0.10019	0.15957	0.152660	-0.138570	-0.042011	0.047482
	5							

		F_{mn}^{ss}				
		n				
		1	2	3	4	5
m		1.4532	0.68126	0.210030	0.15042	0.283370
	1		0.12587	0.037071	0.28062	0.182260
	2			-0.267780	0.23380	0.143020
	3				-0.13210	-0.097876
	4					0.123440
	5					

$$F_{mn}^{cs} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \cos m\chi_1^2 \sin n\chi_2^2$$

$$F_{mn}^{sc} = \sum_{\chi_1^2=0}^{N-1} \sum_{\chi_2^2=0}^{N-1} \sin m\chi_1^2 \cos n\chi_2^2$$

where the units of χ_1^2 and χ_2^2 are taken to be τ .

For a χ_1^2, χ_2^2 symmetric function, Eq. (A3) has the more explicit form

$$\begin{aligned} E(\chi_1^2, \chi_2^2) = & F_{00}^{cc} + \sum_{m=1}^6 [F_{0m}^{cc} (\cos m\chi_1^2 + \cos m\chi_2^2) \\ & + F_{mm}^{cc} \cos m\chi_1^2 \cos m\chi_2^2 \\ & + \sum_{n=m+1}^6 F_{mn}^{cc} (\cos m\chi_1^2 \cos n\chi_2^2 \\ & + \cos n\chi_1^2 \cos m\chi_2^2)] \\ & + \sum_{m=1}^5 [F_{mm}^{ss} \sin m\chi_1^2 \sin m\chi_2^2 \\ & + \sum_{n=m+1}^5 F_{mn}^{ss} (\sin m\chi_1^2 \sin n\chi_2^2 \\ & + \sin n\chi_1^2 \sin m\chi_2^2)] \\ & + \sum_{m=1}^5 [F_{m0}^{sc} (\sin m\chi_1^2 + \sin m\chi_2^2) \\ & + \sum_{n=1}^6 F_{mn}^{sc} (\sin m\chi_1^2 \cos n\chi_2^2 \\ & + \cos n\chi_1^2 \sin m\chi_2^2)] \quad (\text{A4}) \end{aligned}$$

where the coefficients are given by

$$\begin{aligned} F_{mn}^{cc} = & \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2) \cos m\chi_1^2 \cos n\chi_2^2 \\ & \times m, n \geq m = 0, 1, \dots, 6 \quad (\text{A5}) \end{aligned}$$

$$\begin{aligned} F_{mn}^{ss} = & \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2) \sin m\chi_1^2 \sin n\chi_2^2 \\ & \times m, n \geq m = 1, 2, \dots, 5 \quad (\text{A6}) \end{aligned}$$

$$\begin{aligned} F_{mn}^{sc} = & \sum_{\chi_1^2=0}^{11} \sum_{\chi_2^2=0}^{11} E(\chi_1^2, \chi_2^2) \sin m\chi_1^2 \cos n\chi_2^2 \\ & \times m = 1, 2, \dots, 5; n = 0, 1, \dots, 6 \quad (\text{A7}) \end{aligned}$$

In Eq. (A4), the normalization of the F_{mn} has been taken into account: the F_{00}^{cc} , F_{06}^{cc} , and F_{66}^{cc} were divided by 144; the F_{mn}^{cc} , F_{mn}^{ss} , and F_{mn}^{sc} ($m, n = 1, 2,$

$\dots, 5$) were divided by 36; and the F_{0n}^{cc} , F_{m6}^{cc} , F_{m6}^{sc} , and F_{m0}^{sc} ($m, n = 1, 2, \dots, 5$) were divided by 72.

The transformations (A5)–(A7) require that the data be sampled evenly, but the $E(\chi_1^2, \chi_2^2)$ for the stationary state conformers do not occur at the exact 30° intervals (see Table I). It was therefore necessary to refine the F_{mn} , which was done using a least-squares fitting program. The final coefficients reproduced the 78 $E(\chi_1^2, \chi_2^2)$ to the order of 10^{-4} kcal/mole. These coefficients are given in Table AI.

A Fourier series identical to that in Eq. (A4) is appropriate for representing $\chi_0^3(\chi_1^2, \chi_2^2)$, and the coefficients for this series are given in Table AII.

This research was supported by National Science Foundation grants MCB-9115906 and DMR-9110353.

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Received January 13, 1993

Accepted April 8, 1993