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Vibrational studies of the disulfide group in proteins Part IV. SS and CS stretch frequencies of known peptide and protein disulfide bridges

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Abstract

We have used our previously derived ab initio disulfide and empirical polypeptide force fields to calculate SS and CS stretch frequencies of disulfide bridges in a cyclic octapeptide and six proteins of known structure. Comparisons with Raman spectra show that the observed bands are very well reproduced by the normal mode calculations, thus validating the use of these force fields in studying detailed correlations between such spectra and disulfide bridge geometry.

INTRODUCTION

The SS stretch, ν(SS), and CS stretch, ν(CS), frequencies in the Raman spectrum have been empirically correlated with the conformations of S–S bridges in proteins for many years [1–6]. While useful at a preliminary level, a more quantitative analysis of this relationship is needed if reliable spectra–structure correlations are to be made, and this requires normal mode calculations of peptide structures containing the disulfide bridge. Previous calculations to model such structures were done primarily on linear [7] or cyclic [8] dialkyl disulfides, although in one calculation [9] the bridge was simulated by attaching C and N atoms to the C^{α} atoms. However, our ab initio disulfide force field studies [10,11] have shown [12] that ν(SS) and ν(CS) depend on the exact C^{α} atom structures as well as the dihedral angles τ(HCCS) (χ^1, taken as $\tau(NC^{\alpha}C^{\beta}S)$ in the peptide case), τ(CCSS) (χ^2), and τ(CSSC) (χ^3), and thus undoubtedly on the ϕ and ψ dihedral angles of the peptide groups around the disulfide bridge. Therefore, it is necessary to examine a more realistic

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structure in which the effect of the geometry of the polypeptide chain is taken into account. In addition, the scaled ab initio disulfide force field [11,12] should be used in such calculations since it provides more reliable results than previous empirical force fields [7–9].

Prior to such a more general study, we thought it would be useful to test our scaled ab initio force field on peptide and protein disulfide bridges whose structures are known from single crystal X-ray studies and for which comparable Raman data are available. We have chosen to examine the S–S bridges of the cyclic octapeptide cyclo(Cys–Gly–Pro–Phe)$_2$ [13] (c(CGPF)$_2$), for which we have obtained Raman spectra, and several proteins for which *X*-ray structures [14] and Raman data are available: actinoxanthin [9], bovine pancreatic trypsin inhibitor [4] (BPTI), lysozyme [15], ribonuclease A [16], insulin [17] and phospholipase A_2 [9]. As will be seen, the predicted frequencies agree very well with the observed bands, thus providing a secure base for investigating the more general dependence of spectra on conformation [18].

DISULFIDE BRIDGE MODEL

In modeling the peptide disulfide bridge in a normal mode calculation, the first question to be answered is how large a structure is needed to reproduce the observed ν(SS) and ν(CS). The earlier approximate model with 16 atoms [9] cannot reproduce the ν(CS) when just a heavy atom (C or N) is placed trans to an S atom across the CC bond [12]. This difficulty is resolved by a model with 28 atoms, in which a CH_3 group is used instead of a single C atom [12]. However, as we used more realistic models to simulate the disulfide bridge in proteins, namely more atoms in the calculation, we found that ν(SS) and especially ν(CS) changed by dozens of wavenumbers. Therefore, we increased the number of atoms until ν(SS) and ν(CS) did not change by more than a couple of wavenumbers with further additions of atoms. In this way, we found that the 32-atom model $(SCH_2CH\langle^{NHCOC}_{CONHC})_2$ was a satisfactory representation of the S–S bridge. A total of 102 internal coordinates are involved in this model.

For the disulfide group, the optimized ab initio geometry of diethyl disulfide was used, since the scaled ab initio force field was determined for this structure [11]. Since these calculations were only done for conformers having τ(CS) near 0° (cis, C), 60° (gauche, G), 120° (skew, S), 180° (trans, T), −120° (S′) and −60° (G′) (the prime indicating a negative angle), and actual values in proteins cover a much broader range, we have defined other conformational states and used average geometries for them. Following earlier practice [5], we designate states for τ(CS) near 30° (A) and 90° (B); we also introduce another state near 150° (D). (Note that we still follow the convention of labeling the preferred conformation about τ(SS) as G, even though this dihedral angle

is near 90°.) The geometries of the A, B and D states were taken as averages of those of their neighboring conformers, the $\boldsymbol{B}$ matrix being calculated from such averaged geometries (averaging the corresponding $\boldsymbol{B}$ matrices did not give reasonable results). In the case of the peptide group, since we used an empirical force field [19], we utilized the standard peptide geometry [19] for which this force field was refined.

The ab initio force constants were obtained on diethyl disulfide with right-handed (G) chirality [11], but disulfide bridges in proteins occur with about equal probability in left-handed (G′) chiral arrangements [20]. It is therefore necessary to derive a force field for the left-handed disulfide bridge from that obtained for the right-handed bridge [12]. This is straightforward, however, since the potential function in internal coordinates, $V=\sum_{ij}F_{ij}R_iR_j$, is invariant to such a mirror reflection, which only inverts the signs of the torsion and out-of-plane bend coordinates. Since the latter do not occur in the S–S bridge, we only need to change the sign of the F_{ij} in the original force field [12] when only one of the R values is a torsion internal coordinate in order to obtain the corresponding F_{ij} for the left-handed bridge. The peptide moiety is always in the L configuration, and thus our previously refined peptide force field [19] is applicable as it is.

The values of the S–S bridge force constants used in the calculations were obtained by the method previously used [11]. For example, the force constants for SG′B′c(CGPF)$_2$ (representing the $\chi_1^2\chi^3\chi_2^2$ angles) were taken as average values of those of the S′GG and S′GS conformers, those of S′GG, for instance, being appropriate averages of the ab initio values calculated for the S′GS′ and GGG conformers [11].

The peptide group force constants were taken from our empirical force field [19] (the values for β-poly(L-alanine) were used). The diagonal force constants for the region where the S–S bridge and the polypeptide chain join, for example $C^\alpha C^\beta$ and $C^\alpha H$ stretch, and for the interactions corresponding to the two parts, for example $C^\alpha C^\beta$ stretch/$NC^\alpha H$ bend and $C^\alpha C^\beta$ stretch/$CC^\alpha H$ bend, were taken as the average of the scaled ab initio force constant for diethyl disulfide and the empirical force constant for the polypeptide chain.

RESULTS AND DISCUSSION

We compare below the ν(SS) and ν(CS) calculated for the 27 S–S bridges in the seven molecules studied. Only modes with contributions from SS or CS stretch $\geqslant 15$ are included. In the case of CS stretch, the other components are mainly from peptide-group amide V contributions (NH out-of-plane bend and CN torsion), which usually give rise to very weak bands in the Raman but probably gain intensity from the CS stretch contribution. At present, independent assignments are not available for the ν(CS) modes, so our correlations based on frequency proximity should be regarded as suggestive in the light of

the agreement for alkyl disulfides [11] and the discussions concerning other expected modes.

Cyclo(Cys-Gly-Pro-Phe)$_2$

A Raman spectrum of crystalline c(CGPF)$_2$ (Spex 1403 spectrometer, Ar^+ laser 514.5 nm excitation) in the region from 450–850 cm^{-1} is shown in Fig. 1. A comparison of observed and calculated bands is given in Table 1.

This cyclic peptide has one S–S bridge whose conformation is SG'B' [13]. The main calculated ν(SS) of 506 cm^{-1} is obviously in excellent agreement with the very strong, sharp and symmetrical band at the same frequency. Another mode with a smaller (but predominant) contribution of ν(SS) is predicted at 534 cm^{-1}, with the only other calculated mode in this region being 527 $C^{\alpha}CN$ d(25) $C^{\alpha}CO$ d(11) (d=deformation). Since Phe exhibits a band at 525–530 cm^{-1} [1], it is possible that the three weak bands observed at 515, 527 and 541 cm^{-1} should be assigned to Phe and the two predicted modes of the S–S bridge. On this basis, we associate the 541 cm^{-1} band with the second ν(SS) mode.

The 623 cm^{-1} band is clearly the ring mode of Phe side chain [1]. In the region between this band and 700 cm^{-1}, five bands are predicted for our S–S bridge model: 654 $C^{\alpha}C$ s(17) CS s(16); 658 $C^{\alpha}C$ s(21) CO ob(13); 672 CS s(54) CO ob(27); 679 CO ob(63) CS s(13); and 690 CO ob(65) (s=stretch, ob=out-of-plane bend). The assignments of the 656 and 666 cm^{-1} bands to the indicated ν(CS) modes are clearly reasonable. Between 700 and 800 cm^{-1}, bands are predicted at 751 CS s(55), 762 CO ob(37) and 772 CO ob(21). It is difficult to be certain of the assignment of the observed bands at 748, 759, 766 and 786 cm^{-1} to these modes, but if the 748 cm^{-1} band is associated with the Phe side chain [1] then the 759 cm^{-1} band is reasonably assigned as a ν(CS) and the others as CO ob modes.

Actinoxanthin

This protein has two S–S bridges, B'GS' and G'G'T, and its calculated modes are given in Table 2. Only two bands in the ν(SS) region were reported [9], and it can be seen that the observed bands at 510 and 524 cm^{-1} are in excellent agreement with the calculated frequencies of the main SS s modes. The early empirical correlation [2] of a GGT conformation with a ν(SS) near 525 cm^{-1} is well reproduced here, as it was for the dialkyl disulfides [11]. Another (weaker) ν(SS) mode is predicted near 530 cm^{-1}, but current data [9] are not available to determine if it is observed.

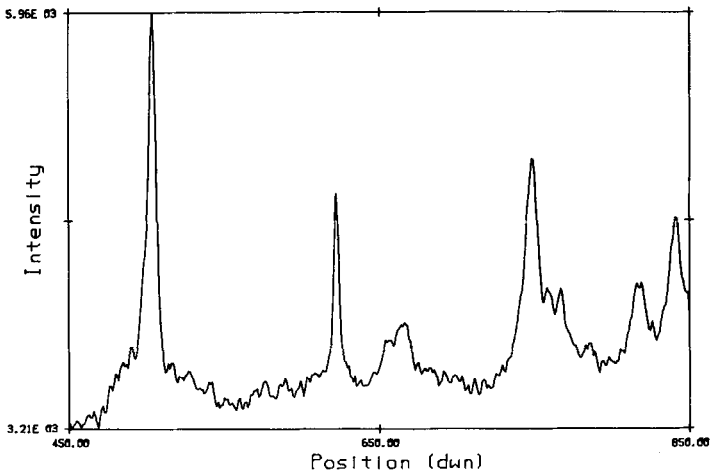

Fig. 1. Raman spectrum of crystalline cyclo(Cys-Gly-Pro-Phe)$_2$ from 450–850 cm^{-1}.

TABLE 1

Disulfide bridge frequencies (in cm^{-1}) of cyclo(Cys-Gly-Pro-Phe)$_2$

τ[a]	ν_{obs}[b]	Cys 1–Cys 5 SG′B′[c]	
		H_t[d]	N_t
ϕ		−133.7	−125.9
ψ		11.1	−59.7
χ^1		59.2	177.4
χ^2		117.2	−98.5
χ^3		−89.3	
CS	759 W		751 (55)[e]
	666 MW	672 (54)	
	656 W	654 (16)	
SS	541 VW	534 (26)	
	506 VS	506 (60)	

[a]Dihedral angles: $\phi=\tau(NC^\alpha)$; $\psi=\tau(C^\alpha C)$; $\chi^1=\tau(C^\alpha C^\beta)$; $\chi^2=\tau(C^\beta S)$; $\chi^3=\tau(SS)$. CS=CS stretch frequencies, SS=SS stretch frequencies.
[b]Our spectra; S=strong, M=medium, W=weak, V=very.
[c]XYZ=$\chi^2_1\chi^3\chi^2_2$.
[d]Atom trans to S across $C^\alpha C^\beta$ bond; H_t, $\chi^1\approx 60°$; C_t, $\chi^1\approx -60°$; N_t, $\chi^1\approx 180°$.
[e]Potential energy contribution from appropriate bond in parentheses: ≥15 for SS and CS stretch.

TABLE 2

Disulfide bridge frequencies (in cm^{-1}) of actinoxanthin

τ[a]	ν_{obs}[b]	Cys 34–Cys 43 B′GS′[c]		Cys 83–Cys 88 G′G′T	
		C_t[d]	C_t	C_t	C_t
ϕ		−124.6	−116.0	−58.2	−82.0
ψ		174.1	161.7	−2.9	154.8
χ^1		−50.6	−36.4	−66.4	−79.0
χ^2		−101.4	−113.3	−50.2	177.9
χ^3		108.0		−76.8	
CS				811 (24)[e]	
		797 (20)			
			787 (20)		
					723 (38)
		708 (21,19)[f]			
		707 (22,18)			
		671 (16)			
			668 (16)		668 (17)
				663 (24)	
				656 (36)	
SS				532 (18)	
	524			523 (70)	
	510	510 (96)			

[a,c,d,e]See Table 1.
[b]Ref. 9.
[f]First number refers to CS(χ_1^2), second to CS (χ_2^2).

Bovine pancreatic trypsin inhibitor

BPTI has three S–S bridges, whose conformations can be designated B′G′G′, SGS′ and B′G′B′. The calculated frequencies are given in Table 3, and compared with the observed bands in the ν(SS) and ν(CS) regions [4]. As before, the predicted ν(SS) modes are in excellent agreement with the observed band at 508 cm^{-1}.

A complication arises in the assignments of the ν(CS) modes, since BPTI has one methionine residue that gives rise to such modes in similar regions to those of the S–S bridge. Using model compounds, it has been possible to relate the ν(CS) frequencies to the $C^{\alpha}C^{\beta}$–$C^{\gamma}S$ and $C^{\beta}C^{\gamma}$–SCH_3 dihedral angles in this side chain [21]: TG, 698 S and 748 W cm^{-1}; TT, 718 S and 762 W cm^{-1}; GG, 648 S and 724 S cm^{-1}, and GT, 667 W cm^{-1} (relative intensities are for liquid methyl propyl sulfide [21]). The Met residue in BPTI can adopt two confor-

TABLE 3

Disulfide bridge frequencies (in cm^{-1}) of bovine pancreatic trypsin inhibitor

τ[a]	ν^{b}_{obs}	Cys 5–Cys 55 B′G′G′[c]		Cys 14–Cys 38 SGS′		Cys 30–Cys 51 B′G′B′	
		C_t[d]	C_t	C_t	H_t	C_t	N_t
ϕ		−65.3	−113.9	−84.4	−146.3	−86.7	−57.9
ψ		−16.2	−15.9	154.8	158.2	133.2	−52.2
χ^1		−62.2	−64.5	−71.9	61.0	−72.3	178.1
χ^2		−75.5	−66.0	105.9	−114.3	−102.8	−95.9
χ^3		−82.8		95.1		−89.9	
CS		821 (15)[e]					
			817 (20)				
	726 W						727 (69)
				717 (39)		715 (34)	
		670 (15,13)[f]					
	669 W	667 (16)	661 (49)	666 (20)	663 (53)	664 (28)	
		654 (29)					
SS	508 MS					513 (92)	
		506 (94)		507 (66)			

[a,c,d,e,f]See Table 2.

[b]Ref. 4; S = strong, M = medium, W = weak.

mations [14], with $\tau(C^{\beta}C^{\gamma})$ and $\tau(C^{\gamma}S)$ of −64.9° and −69.8°, or of −170.0° and −49.6°, corresponding to conformations of GG and TG. We thus expect their ν(CS) contributions to be near 648 and 724 cm^{-1} and near 698 and 748 cm^{-1}, respectively. While it is possible that the observed 726W cm^{-1} band of BPTI could be contributed to by ν(CS) of both Met and the S–S bridge (the presence of a $\approx$650 cm^{-1} band would give support to the former assignment), it is clear that the 669 cm^{-1} band should be associated only with the S–S bridge. Its frequency is quite consistent with predicted ν(CS) modes. We note that this value is considerably lower than the 700–745 cm^{-1} range previously suggested [2] for such C_t conformations (C trans to S across the $C^{\alpha}C^{\beta}$ bond).

Lysozyme

Lysozyme has four S–S bridges, designated G′G′A′, B′G′G′, BGG′ and TGG; their calculated frequencies are given in Table 4 and compared with observed bands [15]. There are two Met residues in lysozyme [14], whose conformations are −76.3° and −98.8° (GG) and −68.8° and −64.4° (GG), which should give rise to ν(CS) bands near 648 and 724 cm^{-1} [21].

TABLE 4

Disulfide bridge frequencies (in cm^{-1}) of lysozyme

τ[a]	ν^{b}_{obs}	Cys 6–Cys 128 G′G′A′[c]		Cys 30–Cys 116 B′G′G′		Cys 65–Cys 81 BGG′		Cys 77–Cys 95 TGG	
		C_t[d]	C_t	N_t	C_t	H_t	C_t	C_t	N_t
ϕ		−78.4	−87.6	−63.1	−131.8	−129.0	−67.4	−85.0	−76.4
ψ		−24.6	−5.5	−38.6	−40.9	156.6	−23.8	−7.7	−42.6
χ^1		−69.0	−60.0	−175.4	−58.1	62.6	−71.0	−71.0	−176.3
χ^2		−51.8	−44.3	−95.7	−72.3	81.2	−58.1	178.0	48.3
χ^3			−65.3		−95.7		95.0		83.0
CS								831 (27)[e]	
		820 (20)					819 (22)		
			808 (20)		813 (21)				
	721 W			727 (69)					728 (72)
					678 (23)			681 (39)	
		670 (36)			666 (40)		669 (36)		
	663 W,bd	658 (21)				658 (59)			
			653 (54)				656 (12,25)[f]		
SS	525 W								524 (83)
	507 S		511 (92)		508 (88)		506 (82)		

[a,c,d,e,f] See Table 2.

[b] Ref. 15; S = strong, W = weak, bd = broad.

The Raman spectrum of lysozyme exhibits one strong band in the ν(SS) region, at 507 cm^{-1}, that has been assigned to this mode [15]. Our calculations reproduce this band quite well, but also suggest that there should be a ν(SS) band near 524 cm^{-1}. Such a band is observed but was not so assigned [15]; we suggest this assignment, consistent with our similar assignment to such a conformation in actinoxanthin (see Table 2).

In the ν(CS) region, the observed band at 663 cm^{-1} is unusually broad, consistent with the relatively large range of expected modes for the S–S bridges. The only other predicted modes in this region are 663 CO ob(56) for Cys 6–Cys 128; 695 CO ob(62) for Cys 30–Cys 116; 692 CO ob(72) for Cys 65–Cys 81; and 695 CO ob(62) for Cys 77–Cys 95, the last three of which may be assignable to the observed band at 696 cm^{-1}. Again a band is observed at 721 cm^{-1} that could be associated with ν(CS) modes of two of the S–S bridges. However, it may also derive from the Met residues; the presence of the 644 cm^{-1} Tyr band makes it difficult to determine if the 648 cm^{-1} counterpart is seen.

Ribonuclease A

Ribonuclease A has four S–S bridges, B′G′G′, G′G′G′, G′G′S′ and G′GB, whose calculated frequencies are compared with observed bands [16] in Table 5. There are four Met residues in this protein [14], whose conformations are −49.8° and −60.2° (GG); −72.1° and −59.1° (GG); −59.5° and −68.0° (GG); and −171.4° and 176.3° (TT). These should give rise to bands at 648, 718–724, and 762 cm^{-1} [21].

The single strong band at 514 cm^{-1} is consistent with our calculated ν(SS) modes. The calculated ν(CS) modes in the 650–700 cm^{-1} region comprise all the predicted vibrations for our bridge model, and it therefore seems reasonable to assign observed bands at 654 and 670 cm^{-1} to these modes, as is the case for the 724 cm^{-1} band. The only problem is that Met ν(CS) modes may also be contributing to the 654 and 724 cm^{-1} bands. Thus, only the assignment of the 670 cm^{-1} ν(CS) mode is reasonably secure at present.

Insulin

The three S–S bridge pairs of insulin have the following comparable conformations: G′GT and G′GD′, GGB′ and GGB′, and G′G′G′ and A′G′G′. Their calculated frequencies are given in Table 6 and compared with observed bands [17]. Insulin has no Met residues, so the ν(CS) region is not complicated by its modes.

The strong 514 cm^{-1} band is consistent with calculated ν(SS) modes of four of the S–S bridges. The other two are expected to have significantly higher frequencies, and we suggest that the very weak band at 530 cm^{-1} should be

TABLE 5

Disulfide bridge frequencies (in cm^{-1}) of ribonuclease A

τ[a]	ν_{obs}[b]	Cys 26–Cys 84 B′G′G′[c]		Cys 40–Cys 95 G′G′G′		Cys 58–Cys 110 G′G′S′		Cys 65–Cys 72 G′GB	
		C_t[d]	C_t	C_t	C_t	C_t	C_t	C_t	C_t
ϕ		−59.1	−120.8	−83.7	−76.1	−66.9	−113.9	−71.9	−96.0
ψ		−46.7	119.0	108.4	137.7	−3.6	149.9	160.0	153.8
χ^1		−68.6	−66.1	−55.0	−76.1	−64.6	−46.5	−59.3	−81.3
χ^2		−87.1	−50.8	−52.9	−66.4	−68.1	−125.2	−59.1	88.9
χ^3			−81.4		−77.6		−86.4		107.8
CS		824 (15)[e]							
									799 (18)
			790 (20)		792 (19)		795 (20)	793 (17)	
				782 (21)					
	724 W			715 (27)	718 (31)			719 (38)	713 (38)
			709 (34)				708 (34)		
		675 (26)							
	670 VW		667 (25)				667 (19)	667 (8,15)[f]	
				661 (29)	664 (25)	661 (22)		665 (14,10)	
	654 W	654 (27)				656 (36)			
SS	514 S						514 (91)		
			506 (84)		507 (87)			504 (85)	

[a,c,d,e,f]See Table 2.

[b]Ref. 16; S = strong, W = weak, V = very.

TABLE 6

Disulfide bridge frequencies (in cm^{-1}) of insulin

τ[a]	ν^{b}_{obs}	Cys A6-Cys A11 G′GT[c]		Cys C6-Cys C11 G′GD′		Cys A7-Cys B7 GGB′		Cys C7-Cys D7 GGB′		Cys A20-Cys B19 G′G′G′		Cys C20-Cys D19 A′G′G′	
		C_t[d]	H_t	C_t	H_t	N_t	C_t	N_t	C_t	C_t	C_t	C_t	C_t
ϕ		−59.4	−153.6	−109.4	−147.6	−84.8	−122.3	−105.6	−129.3	−72.4	−90.8	−76.9	−94.4
ψ		−36.8	160.0	−19.8	158.7	−43.2	153.8	−55.0	153.4	161.0	−32.7	146.1	−44.2
χ^1		−70.7	74.4	−68.8	71.5	−169.4	−57.6	−178.8	−59.3	−52.2	−56.9	−55.4	−63.5
χ_2		−72.5	−166.0	−72.3	−156.1	51.9	−88.9	57.4	−80.0	−48.7	−59.6	−41.1	−53.8
χ^3		104.3		103.1		98.2		92.1		−78.8		−83.0	
CS	814 sh	819 (20)		818 (23)[e]									816 (17)
	802 sh						799 (21)		799 (21)				
										783 (19)		787 (15)	
	731 W					727 (71)		732 (66)					
										718 (36)		717 (35)	
	697 sh						708 (37)		706 (37)				
	678 sh	675 (7,47)[f]		670 (15,30)					670 (21)		672 (35)		675 (34)
	667 W	672 (24,12)			668 (30)		669 (21)			665 (21)			
		656 (28)		661 (44)							659 (26)	663 (25)	660 (24)
SS	562 W			549 (18)									
	530 VW	524 (55)		519 (54)									
	514 S					507 (93)		505 (90)		508 (93)		510 (86)	
	492 W	468 (22)		469 (21)									

[a,c,d,e,f] See Table 2.

[b] Ref. 17; S = strong, W = weak, V = very, sh = shoulder.

TABLE 7

Disulfide bridge frequencies (in cm^{-1}) of phospholipase A_2

τ^a	ν^b_{obs}	Cys 11-Cys 77 BGB[c]		Cys 27-Cys 123 G′G′B′		Cys 29-Cys 45 G′GD		Cys 44-Cys 105 G′G′B′		Cys 51-Cys 98 B′G′G′		Cys 61-Cys 91 G′GG		Cys 84-Cys 96 B′G′B′	
		N_t[d]	N_t	C_t	C_t	C_t	C_t	C_t	N_t	N_t	C_t	C_t	N_t	C_t	N_t
ϕ		−65.4	−124.5	−90.3	−117.2	−75.4	−69.4	−59.0	−54.6	−61.9	−58.8	−70.0	−70.4	−90.6	−60.1
ψ		−43.9	125.0	−22.3	−100.5	100.4	−35.5	−44.6	−49.7	−43.7	−47.0	−37.7	−44.5	134.3	−41.9
χ^1		−179.1	175.0	−66.7	−54.6	−46.4	−68.4	−64.6	−162.7	−151.7	−71.0	−64.6	176.1	−77.3	−166.8
χ^2		78.9	81.9	−65.0	−77.0	−66.9	151.9	−68.5	−89.9	−93.2	−60.0	−54.1	71.7	−76.0	−92.1
χ^3		90.3		−90.3		97.6		−78.6		−79.0		108.4		−96.8	
CS							833 (25)[e]								
				819 (17)								821 (22)			
														794 (15)	
						773 (26)									
					761 (47)										
			744 (58)												
		729 (68)							730 (61)	731 (58)			725 (70)		729 (65)
						718 (25)								715 (35)	
							680 (29)	675 (27)			675 (27)	674 (32)			
				668 (36)										664 (28)	
				659 (26)	661 (20)	659 (27)		656 (29)			657 (27)	656 (27)			
							653 (22)								
SS	525					516 (77)									
				511 (82)											
	509		505 (87)						508 (92)		508 (90)		503 (91)		508 (86)

[a,c,d,e] See Table 2.

[b] Ref. 9.

assigned to them. Two other (weak though predominantly ν(SS)) modes are predicted for these bridges, near 470 and 550 cm^{-1}; it is tempting to suggest (though with caution) that the observed bands at 492 and 562 cm^{-1} may be assignable to these modes.

The region of calculated ν(CS) modes is rich with observed bands that are readily assigned to these vibrations. This is particularly true of the 667 and 678 cm^{-1} bands since no other modes are predicted in this region. Bands in the $\approx$700–800 cm^{-1} region can also be assigned to predicted ν(CS) modes, although perhaps with less certainty. It is interesting that the 667 and 731 cm^{-1} bands have comparable, relatively high intensities; this may be due to the large number of modes contributing to the former and the relatively pure ν(CS) nature of the latter. This strong ν(CS) character of bands near 730 cm^{-1} is characteristic of the N_t conformation (N trans to S across the $C^{\alpha}C^{\beta}$ bond).

Phospholipase A_2

Phospholipase A_2 has seven S–S bridges, whose conformations are BGB, G′G′B′, G′GD, G′G′B′, B′G′G′, G′GG and B′G′B′. Their calculated frequencies are given in Table 7, and compared with the only reported ν(SS) modes [9]. The 509 cm^{-1} band is in excellent agreement with the mean value of ν(SS) modes predicted for six of the bridges; the 525 cm^{-1} band is consistent with an assignment to the G′GD bridge, whose ν(SS) mode is calculated at 516 cm^{-1}. As in the case of actinoxanthin [9], more detailed studies of the spectra seem desirable.

CONCLUSIONS

The above results show that calculated ν(SS) and ν(CS) frequencies of S–S bridges in seven known peptide and protein structures are in good agreement with observed Raman bands due to these modes. While the ν(SS) modes are easier to assign, knowledge of the contributions due to specific Met conformations in these molecules permits reasonable inferences regarding the assignments of ν(CS) modes of the S–S bridges.

The agreement between observed and calculated ν(CS) frequencies in such a wide range of disulfide bridges enables a more secure correlation to be made of these modes with χ^1. For N_t conformations, bands are predicted and observed in the range of 725–755 cm^{-1}. For C_t conformations, calculated bands have definitely observed counterparts in the regions 650–680 and 700–725 cm^{-1}, with the probability that predicted bands in the 785–830 cm^{-1} region can also be observed (cf. insulin, Table 6). For H_t conformations, the range for observed and calculated frequencies is 650–680 cm^{-1}, which is seen to overlap in part the C_t range.

We believe that this study gives support to the validity of our ab initio disulfide [12] and empirical polypeptide [19] force fields in calculating reliable normal modes of disulfide bridges in proteins, and provides a sound basis for developing more detailed correlations between conformation and spectra for such structures [18].

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