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Vibrational analysis of peptides, polypeptides, and proteins

XXX. Normal mode analyses of γ-turns

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The normal modes have been calculated for three kinds of low energy γ-turn structures resulting from recent conformational energy calculations by Némethy. Frequencies have been computed for a γ-turn, a mirror-related γ-turn, and an inverse γ-turn of CH_3-CO-(L-Ala)$_n$-NH-CH_3, with n = 3 and n = 5, and for certain ^{14}C and ^{15}N derivatives of the n = 3 molecule. Correlations are evident between amide frequencies and γ-turn structures, and it is found that only amide I modes of peptide groups in the turn are relatively insensitive to the lengths of attached chains.

Key words: C_7 conformation; normal modes; γ-turns

Reverse turns, in which a polypeptide chain changes direction by ~ 180°, give a protein its compact shape. Thus, an understanding of the structures and characteristics of such turns is of importance in understanding how proteins fold. Of the reverse turns, the β-turns have been clearly identified in various globular proteins (1), and we have carried out extensive vibrational spectroscopic studies on the different types of β-turns (2–8). The result of these studies has been to provide useful correlations between vibrational spectrum and structure, and thus to give insight into the interpretation of protein spectra.

Encouraged by the success of these studies, we have now done normal mode analyses on another type of reverse turn, namely the γ-turn (9, 10). The γ-turn is formed by three amino acid residues, i, i + 1, and i + 2, and is characterized by two hydrogen bonds (see Fig. 1). That between the CO of residue i and the NH of residue i + 2 forms a C_7 structure, since the ring of atoms enclosed by the peptide

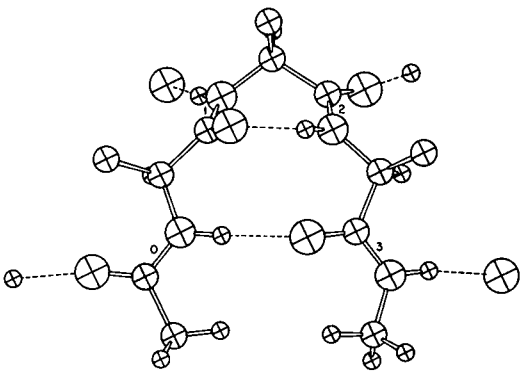

FIGURE 1

CH_3-CO-(L-Ala)$_3$-NH-CH_3 model of a γ-turn. CH_3 groups of L-Ala residues are represented by point masses. External hydrogen bonds are formed by NH (to O atoms) and CO (to H atoms) groups.

backbone and the hydrogen bond contains seven atoms. Although the C_7 structure was discussed in the literature as early as 1943 (11), a specific γ-turn was proposed for the first time in 1972 by Némethy & Printz (9). Since this early study, the γ-turn structure has been further refined using improved energy parameters (G. Némethy, private communication), and energetically stable conformations of three different types have been proposed: a) the γ-turn, γ, b) the mirror-related γ-turn, γ_M, and c) the inverse γ-turn, γ_I. In γ and γ_M there is a second hydrogen bond between the NH of residue i and the CO of residue i + 2; in γ_I this bond is between the CO of residue $i-1$ and the NH of residue i + 3.

The γ-turn does not occur as frequently as the β-turn, but it has been identified in some peptides and proteins. The cyclic tetrapeptide dihydrochlamydocin is known (12) to contain a γ-turn. Host-specific toxin from *Helminthosporium carbium* is claimed from n.m.r. studies to have a γ-turn (13). N.m.r. work from Urry's group (14) has shown that the repeat unit of the elastin polypentapeptide helix contains a γ-turn. And γ-turns have been found in at least 10 proteins (15). Finally, the C_7^{ax} and C_7^{eq} structures that have been discussed in the literature (16–19) are actually the normal and mirror-related γ-turns, respectively. (γ_I is also a C_7^{eq} conformation). The present studies, therefore are expected to be useful in interpreting spectra of such structures.

We report here results of our normal mode analyses on model structures of the γ, γ_M, and γ_I turns. The modes of the three-residue structure, modeled by CH_3-CO-(L-Ala)$_3$-NH-CH_3 (see Fig. 1), are relevant to the spectra of small molecules having γ-turn structures. In addition, we have also calculated the normal modes of CH_3-CO-(L-Ala)$_5$-NH-CH_3. This was done in order to examine the effects of nearest neighbor interactions, such as are likely to be found in proteins.

NORMAL MODE CALCULATIONS

The dihedral angles used for the various γ-turn conformations of CH_3-CO-(L-Ala)$_3$-NH-CH_3 are given in Table 1. These values were kindly supplied to us by Dr. George Némethy, and are the result of energy calculations using the latest form of ECEPP on terminally blocked (L-Ala)$_3$ (G. Némethy, private communication). The relative energies of the above structures are (in kcal/mol) a) γ: 8.32, b) γ_M: 2.80, c) γ_I: 3.09. The γ-turn, although of relatively high energy, forms a good chain reversal: the $H_{i+2} \ldots O_i$ and $H_i \ldots O_{i+2}$ distances are 1.86 Å and 1.91 Å, respectively. The γ_M-turn is of relatively low energy but forms a very poor chain reversal: the $H_{i+2} \ldots O_i$ and $H_i \ldots O_{i+2}$ distances are 2.13 Å and 6.06 Å, respectively. The γ_I-turn is characterized by both relatively low energy and good chain reversal: the $H_{i+2} \ldots O_i$ and $H_{i+3} \ldots O_{i-1}$ distances are 2.03 Å and 1.92 Å, respectively.

TABLE 1

Dihedral angles for γ-turn structures of CH_3-CO-(L-Ala)$_3$-NH-CH_3

	γ-Turn	γ_M-Turn	γ_I-Turn
ω_0 [a]	180 [b]	180	180
ϕ_1	−152	−155	59
ψ_1	90	−53	−177
ω_1	−161	177	172
ϕ_2	58	−81	−77
ψ_2	−74	74	68
ω_2	178	−179	−176
ϕ_3	−76	−154	−163
ψ_3	149	158	−55
ω_3	−179	−180	179

[a] See Fig. 1 for designation of angles. [b] In degrees.

The dihedral angles for the CH_3-CO-(L-Ala)$_5$-NH-CH_3 structure (see Fig. 2) were the same in the turn region as for the smaller structure, the additional residues being taken to correspond to those of the antiparallel-chain pleated sheet, viz. $(\phi, \psi, \omega)_0 = (\phi, \psi, \omega)_4 = -139°, 133°, 180°$ (again $\omega_{\bar{1}} = 180°$). Although such a conformation is not necessarily present in all γ-turns, and the frequencies will vary somewhat with these angles (see below), the ϕ, ψ values in many γ-turns in proteins are close to the β-sheet values (15). For both structures, in order to use our force fields, we used the bond lengths and bond angles of β-poly(L-alanine) (20), while retaining the above dihedral angles. (This led to essentially identical $H_{i+2} \ldots O_i$ distances, viz. 1.86, 2.14, and 2.04 Å,

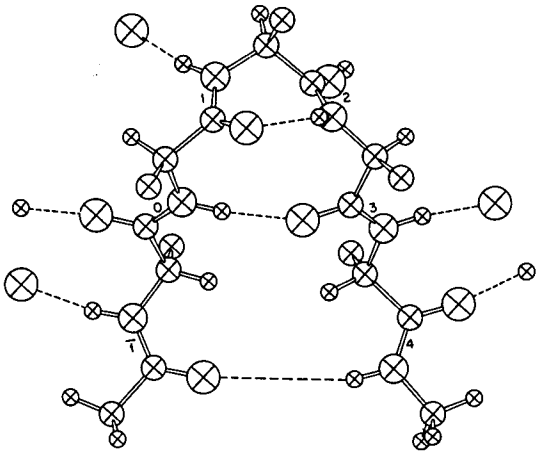

FIGURE 2
CH_3-CO-(L-Ala)$_5$-NH-CH_3 model of γ-turn. CH_3 groups of L-Ala residues are represented by point masses. External hydrogen bonds are formed by NH (to O atoms) and CO (to H atoms) groups.

and slightly different $H_i \ldots O_{i+2}$ distances, viz. 1.99, 6.24, and 2.03 Å, for γ, γ_M, and γ_I, respectively, which is not expected to have a significant effect on the frequencies.)

It should be noted that the dihedral angles for the γ-turns given here are very different from those originally proposed (9). This is partly because of the improved potential functions used in the recent calculations (G. Némethy, personal communication), but is more a result of the early assumption (9) that the i + 1 residue is in the $C_7^{ax}(\gamma)$ conformation. It was thought that this conformational region was only of moderately high energy for L-Ala, but subsequent studies on CH_3-CO-L-Ala-NH-CH_3 (21, 22) have shown that it is in fact of very high energy compared to the $C_7^{eq}(\gamma_M)$ conformation. As indicated above, γ_M is more stable than γ by 5.52 kcal/mol in the (L-Ala)$_3$ structure, thus indicating that, in the absence of other factors, it is a more likely conformation in small peptides and possibly proteins. We also note that the dihedral angles for γ_I differ considerably from those originally reported for thermolysin and in the later refined structure deposited in the Protein Data Bank (23). This is undoubtedly a result of the influence of environmental interactions in the protein (G. Némethy, personal communication).

In the calculations, the side-chain Ala CH_3 groups were replaced by point masses at the C^β atom, the force field being a β force field appropriate to this approximation (24) (the terminal CH_3 groups were treated completely, using the detailed force field (20)). The force constants for the internal hydrogen bonds were assumed, as previously (2–4), to be 0.15 mdyn/Å at r(H . . . O) = 1.80 Å, decreasing linearly to zero at 5 Å. The external hydrogen bonds were all taken as equivalent, and the same as those for the β-turns (2–4). Transition dipole coupling was incorporated for amide I and amide II modes (25, 26), using $\Delta\mu_{eff} = 0.450$D for amide I and 0.279D for amide II.

RESULTS AND DISCUSSIONS

CH_3-CO-(L-Ala)$_3$-NH-CH_3

We present in Table 2 the calculated frequencies of the main amide modes for three γ-turn conformations of the (L-Ala)$_3$ molecule. The main contribution to the potential energy distribution (PED) is given for each frequency, CO stretch for amide I, NH in-plane bend for amide II and amide III, and NH out-of-plane bend for amide V. The peptide groups have been numbered such that group i comprises CO_i and NH_{i+1}.

Some of the amide I modes are significantly different for the three γ-turn structures, although it would be difficult to distinguish these conformations based on frequency ranges alone. It might be expected that the amide I mode associated with group 1, amide I(1), would be most sensitive to conformation, since the CO of peptide group 1 is directly involved in the hydrogen bond at the turn. Indeed, this mode shifts from 1670 cm^{-1} in γ to 1656 cm^{-1} in γ_M to 1660 cm^{-1} in γ_I. The frequency of amide I(2) is also sensitive to conformation, being predicted at 1656 cm^{-1} in γ, 1668 cm^{-1} in γ_M, and 1667 cm^{-1} in γ_I. The latter result and, in part, the predictions for amide I(1) strongly suggest that the main conformational sensitivity is to whether the turn conformation is $C_7^{ax}(\gamma)$ or C_7^{eq} (γ_M and γ_I). The frequency of amide I(0) seems (perhaps unexpectedly) to support this observation: it is predicted at 1684 cm^{-1} for γ and at 1675 cm^{-1} for both γ_M and γ_I.

TABLE 2

Calculated amide frequencies (in cm^{-1}) of CH_3-CO-(L-Ala)$_3$-NH-CH_3 in γ-turn conformations

Mode	γ			γ_M			γ_I		
	ν	PED[a]		ν	PED[a]		ν	PED[a]	
Amide I[b]	1684	0(42)	3(35)	1675	0(45)	3(31)	1675	0(69)	3(9)
	1670	1(74)		1668	2(73)		1667	2(68)	1(9)
	1655	2(81)		1656	1(79)		1660	1(69)	2(8)
	1653	3(39)	0(38)	1654	3(42)	0(31)	1649	3(72)	0(9)
Amide II[c]	1552	0(47)		1551	2(27)	1(15) 0(9)	1546	1(26)	2(24)
	1529	1(36)	3(8)	1540	0(32)	2(13)	1540	1(25)	2(24)
	1526	3(21)	1(11)	1527	3(30)		1512	3(30)	
	1509	2(47)		1518	1(40)		1503	0(31)	
Amide III[d]	1390	0(12)	1(6)	1387	0(13)		1375	1(13)	
	1367	1(7)		1352	2(10)		1371	2(7)	
	1336	1(11)		1331*	1(7)		1351	3(5)	
	1327	3(9)		1310*	2(12)	3(7) 1(6)	1346	0(26)	
	1297*	3(14)	2(13)	1261*	0(7)		1323	1(7)	
	1282	1(9)		1254*	3(21)		1308*	2(8)	1(6)
	1242*	0(19)		1248*	0(6)	1(6)	1268	3(12)	1(5)
	1225*	3(13)	2(9)				1243*	3(23)	2(6)
							1232*	0(21)	
Amide V[e]	709	3(7)		729	2(17)		718	1(16)	
	706	3(27)		719	1(14)		712	2(18)	3(7)
	676	1(20)		707	3(34)		706	3(25)	
	655	1(12)	2(6)	677	2(12)		698	0(27)	1(5)
	608	2(18)		643	1(12)		[644]	[2(6)]	
	602	2(5)		570	0(40)				
	548	0(32)		562	0(7)				
	517	0(14)							
	493	0(12)							

[a] Potential energy distribution, with number of peptide group (see Fig. 1) and (in parentheses) the percentage of the contributing coordinate.
[b] Percentage of CO stretch.
[c] Percentage of NH in-plane bend. All modes also have a CN stretch contribution appropriate to the indicated peptide group.
[d] Percentage of NH in-plane bend. Only frequencies with * have a CN stretch contribution, in all cases appropriate to the peptide group except the 1268 cm^{-1} band of γ_I, which has a CN(2) stretch contribution.
[e] Percentage of NH out-of-plane bend. All modes have a relevant CN torsion contribution, except 644 cm^{-1} of γ_I which has a CN(1) torsion contribution.

The relative sensitivity of the amide I(1) mode to conformation suggests that a more definitive structural assignment could be achieved by studying a suitable isotopically substituted molecule. We have therefore calculated the normal modes of CH_3-CO-(L-Ala)$_3$-NH-CH_3 with ^{14}C substituted at the CO(1) position. The results are shown in Table 3. As might be expected, the other amide I frequencies stay the same or decrease by several cm^{-1} (although amide I(3, 0) of γ_M is predicted to increase by 5 cm^{-1}). The frequency of amide I(1) of course decreases, to 1588 cm^{-1} for γ, 1570 cm^{-1} for γ_M, and 1580 cm^{-1} for γ_I. The location of the shifted band in the original sequence of amide I frequencies, plus the magnitude of the shift (82, 98, and 80 cm^{-1} for γ, γ_M, and γ_I, respectively), could thus serve to identify the conformation with increased certainty. This serves to emphasize a

TABLE 3

Calculated amide I and amide II frequencies (in cm^{-1}) of ^{14}C- and ^{15}N-substituted CH_3-CO-(L-Ala)$_3$-NH-CH_3 [a]

Mode	γ		γM		γI	
	^{14}C	^{15}N	^{14}C	^{15}N	^{14}C	^{15}N
I	1683 [0, 3] [b]	1683 [0, 3]	1675 [0, 3]	1675 [0, 3]	1675 [0, 3]	1676 [0, 3]
	1654 [2]	1670 [1]	1666 [2]	1664 [2]	1664 [2(76)] [c]	1667 [2(45), 1(33)]
	1653 [3, 0]	1653 [3, 0]	1659 [3, 0]	1656 [1]	1648 [3, 0]	1658 [1(45), 2(32)]
	1588 [1]	1653 [2]	1570 [1]	1655 [3, 0]	1580 [1(78)]	1649 [3, 0]
II	1551 [0]	1552 [0]	1540 [2(46)]	1542 [1(33), 2(17)]	1546 [2(45)]	1542 [1(42), 2(9)]
	1527 [3(29)]	1528 [1(45)]	1530 [3(25), 0(6)]	1534 [3(22), 0(6)]	1515 [3]	1540 [2(32), 1(9), 3(6)]
	1509 [2(43), 1(7)]	1525 [3(27)]	1526 [0(32), 3(5)]	1526 [2(24), 1(10), 3(7), 0(5)]	1511 [1(54)]	1506 [3(26), 2(9)]
	1499 [1(55), 2(6)]	1497 [2]	1501 [1(50)]	1508 [0(26), 1(12)]	1503 [0]	1503 [0]

[a] ^{14}C-substitution on CO(1), ^{15}N-substitution on NH(2).

[b] If PED is the same as in original molecule (Table 1), bracketed numbers give contributing peptide groups only.

[c] If PED differs from original molecule, percentage contributions are given in parentheses.

point not usually realized: spectra of backbone isotopically substituted molecules in conjunction with normal mode analysis provide a very powerful method for the determination of polypeptide chain conformation.

The calculated amide II modes have frequency distributions that are significantly different for the three conformations; this might be expected to permit a distinction between the structures (assuming the bands can be observed in the infrared, since Raman amide II modes are usually weak). The highest frequency, near 1550 cm^{-1}, is about the same for all three structures, but a band near 1540 cm^{-1} is predicted only for γ_M and γ_I. Similarly, only γ and γ_M have bands near 1527 cm^{-1}, only γ_M has a band near 1518 cm^{-1}, and, whereas both γ and γ_I have bands near 1510 cm^{-1}, only γ_I has a band as low as 1503 cm^{-1}. The situation is more complex than for the amide I modes because, whereas the conformation-sensitive amide I(1) and amide I(2) are relatively pure modes, the expected conformation-sensitive amide II(1) and amide II(2) modes are generally mixed, and differently for the three conformations.

As in the case of amide I, we have considered the sensitivity of amide II to isotopic substitution, both the ^{14}C substitution discussed above and ^{15}N substitution at NH(2). The results of such normal mode calculations are shown in Table 3. We note first that, as expected (since amide II involves CN stretch as well as NH in-plane bend), amide II(1) is significantly affected by the ^{14}C substitution, the predominant mode decreasing by 30, 17, and ~32 cm^{-1} in γ, γ_M, and γ_I, respectively. The ^{15}N substitution does not, of course, influence the amide I modes significantly, but it does have the expected effect on amide II(2). However, while it leads to an unambiguous drop of 12 cm^{-1} for γ, where the mode is relatively pure in NH in-plane bend, the situation is more complex for γ_M and γ_I, where the already-mixed modes alter their state of mixing. Despite these complexities, such isotopic substitutions provide a new dimension of analysis of spectra in terms of conformation, and should help significantly in assigning structures.

A relatively large number of bands in the 1400–1200 cm^{-1} region contain NH in-plane

bend contributions at the $\geqslant 5\%$ level, which we have found to be significant in determining N-deuteration sensitivity (8). Although only three or four of these also have related CN stretch contributions, which are usually associated with "standard" amide III modes, we list all of these bands in Table 2. The patterns of both of these kinds of modes differ from one conformation to another, and whether this region can be used to distinguish between the structures depends on whether all or most of the modes appear in the Raman and infrared spectra. (Isotopic substitution does not appear to help much in this case, since the shifts are relatively small.) With this proviso, some points of difference should still be noted with respect to such N-deuteration-sensitive modes: the lowest frequency is significantly different for the three conformations, viz. 1225, 1248, and 1232 cm^{-1} for γ, γ_M, and γ_I, respectively; while all structures have bands near 1305 cm^{-1} (1297, 1310, 1308 cm^{-1}), the next lowest frequency is significantly different in the three conformations, viz. 1282, 1261, and 1268 cm^{-1} for γ, γ_M, and γ_I, respectively; while all structures have four bands in the region $\lesssim 1305\,cm^{-1}$, the number above differ, viz. 4, 3, and 5 for γ, γ_M, and γ_I, respectively. Considering the extent of these differences, it seems probable that the amide III region could be used effectively to distinguish between these γ-turn conformations.

The NH out-of-plane bend coordinate contributes to about 60% of the complex modes below $\sim 730\,cm^{-1}$. For some of these, mostly in the ~ 730–$500\,cm^{-1}$ region, this is combined with CN torsion in what is called amide V, and which generally gives rise to moderately strong bands in the infrared spectrum. The calculated frequencies of such modes are given in Table 2. The three γ-turn conformations show different amide V features, and again a differentiation based on this mode will depend on the appearance of these features in the spectra and their detection by N-deuteration. Thus, the number of such modes differs between structures, viz. 9, 7, and 4 for γ, γ_M, and γ_I, respectively; the lowest frequency differs significantly, viz. 493, 562, and 698 cm^{-1} for γ, γ_M, and γ_I, respectively; and there are important differences in distribution of modes, viz. 3, 2, and 0 in the $\lesssim 570$ cm^{-1} region, 4, 2, and 0 in the 570–680 cm^{-1} region, and 2, 3, and 4 in the $\gtrsim 700\,cm^{-1}$ region, for γ, γ_M, and γ_I, respectively. It seems possible that the combination of the above characteristics in the amide V region could effectively differentiate between these γ-turn conformations.

CH_3-CO-(L-Ala)$_5$-NH-CH_3

As noted above, we have also calculated the normal modes of γ-turn conformations of CH_3-CO-(L-Ala)$_5$-NH-CH_3 in order to determine the effect of interactions of the added peptide groups on the frequencies of those groups characteristic of the turn. Such additional interactions would be present if the γ-turn occurred in a protein. The results of these calculations are given in Table 4. The peptide groups are labeled (cf. Figs. 1 and 2) so that comparable groups in both molecules have the same number. The appropriate contributions to the PEDs are also given.

A comparison of Tables 2 and 4 shows that some modes are unchanged by the additional interactions whereas others are significantly affected. In the case of the amide I modes, the turn-sensitive frequencies of peptide groups 1 and 2 are, as might be expected, essentially unaffected in all three conformations. However, the frequencies of peptide groups 0 and 3, perhaps as a result of their closer proximity to the added groups, are affected, decreasing for group 0 and increasing for group 3. The situation for amide II is more complex. The conformation-sensitive frequencies of groups 1 and 2 are unaffected for γ but change significantly for γ_M and γ_I. For group 0 there is essentially no change for γ and γ_I but a large change for γ_M, while for group 3 the additional peptide groups cause large changes for all conformations. For amide III and amide V the changes display even greater complexity, and do not seem to furnish useful guidelines.

In order to see if the frequencies are influenced by structural changes beyond the first two hydrogen bonds, we calculated the modes of γ for a structure in which $(\phi, \psi, \omega)_0 = (\phi, \psi, \omega)_4 = -180°, 100°, 180°$. This has the effect of keeping the first two hydrogen bond lengths the same, but increasing the $O(\bar{1})\ldots H(4)$ distance from 3.69 Å to 5.71 Å. The

TABLE 4

Calculated amide frequencies (in cm^{-1}) of CH_3-CO-(L-Ala)$_5$-NH-CH_3 in γ-turn conformations

Mode	γ			γM			γI		
	ν	PED[a]		ν	PED[a]		ν	PED[a]	
Amide I[b]	1675	0(47)	3(23)	1668	2(32)	3(32) 4(11)	1671	0(74)	
	1670	1(74)		1667	0(74)		1668	2(58)	4(13)
	1666	4(56)	3(19)	1665	4(50)	2(25)	1667	4(62)	2(12)
	1663	3(33)	0(28) 4(14)	1664	3(44)	2(19) 4(14)	1660	1(71)	2(16)
	1655	2(81)		1655	1(80)		1655	3(79)	
	1650	Ī(82)		1649	Ī(82)		1649	Ī(82)	
Amide II[c]	1554	0(45)	3(6)	1552	3(48)		1558	2(29)	1(14) 3(9)
	1548	3(45)	0(6)	1547	Ī(50)		1547	Ī(50)	
	1547	Ī(50)		1547	1(27)	0(15) 2(10)	1541	3(32)	1(21)
	1527	1(47)		1544	2(36)	1(11)	1525	2(21)	1(17) 3(13)
	1521	4(28)		1515	4(26)		1513	4(24)	
	1509	2(48)		1510	0(30)	1(16)	1506	0(38)	
Amide III[d]	1390	Ī(7)	0(5)	1387	Ī(10)		1384	Ī(12)	
	1372	1(13)		1384	3(7)		1376	1(9)	
	1358	3(9)		1362	0(6)		1367	2(6)	
	1329	1(10)	0(5)	1341	2(11)		1328	0(21)	
	1298	2(8)	4(8)	1322*	1(7)		1323	2(5)	
	1288	1(7)		1317	0(5)		1308*	2(8)	1(7)
	1266	0(12)		1305	2(8)	4(5)	1273	3(11)	4(7)
	1250	4(18)	2(8)	1271	3(9)	4(8) 0(5)	1267	0(13)	3(7)
	1238*	Ī(19)		1268	0(14)		1255	1(6)	
	1232*	3(11)	4(7) 2(5)	1254*	1(9)	2(7)	1238	Ī(16)	
				1239*	Ī(17)		1228	4(20)	
				1237	4(19)				
Amide V[e]	704	Ī(7)	3(6)	792	4(26)		710	1(12)	
	698	Ī(10)	3(7)	709	1(18)		707	Ī(14)	
	685	3(12)		701	Ī(18)		702	1(11)	Ī(7)
	674	1(17)		696	3(25)		692	3(20)	
	658	1(12)	2(5)	689	2(10)		679	0(17)	2(11)
	637†	Ī(6)		674	0(5)		674	2(17)	
	627†	Ī(5)		659	2(19)		646	2(7)	
	610	2(9)		646	1(10)		638	3(10)	
	604	2(16)		640	0(10)		619†	Ī(10)	
	581	4(41)		625†	Ī(6)		607†	Ī(8)	
	561	0(21)		615	3(7)	0(5)	580†	1(5)	
	481	0(21)		595	0(18)		557	4(38)	
	478	0(10)							

[a] Potential energy distribution, with number of peptide group (see Fig. 2) and (in parentheses) the percentage of the contributing coordinate.

[b] Percentage of CO stretch.

[c] Percentage of NH in-plane bend. All modes also have a CN stretch contribution appropriate to the indicated peptide group.

[d] Percentage of NH in-plane bend. Only frequencies with * have a CN stretch contribution.

[e] Percentage of NH out-of-plane bend. All modes, except those with †, have a relevant CN torsion contribution.

effects of this change are as follows: the amide I frequencies remain the same for peptide groups 0 and 2, and change by no more than 2 cm^{-1} for the others; all of the amide II frequencies change (one by as much as 13 cm^{-1}), that for group 0 increasing from 1554 to 1563 cm^{-1} and that for group 2 decreasing from 1509 to 1501 cm^{-1}; all of the amide III modes shift slightly (all except one by 4 cm^{-1} or less), that for group 0 remaining the same and that for group 2 decreasing by 1 cm^{-1}; and the pattern of amide V frequencies changes significantly, including those of groups 0 and 2. It appears that most of the amide modes of γ-turns are sensitive to the local environment.

As a result of these comparisons it can be said that the amide I modes of peptide groups 1 and 2 are relatively insensitive to the lengths of the attached chains, and therefore that deductions based on these amide I predictions have some measure of generality. (This may be useful in analyzing spectra of small molecules having C_7 structures (17)). This is not true of other groups and other amide modes, and therefore it is preferable to use calculations that are most relevant to the structure being studied. This is evident from the results presented in the accompanying paper (27).

CONCLUSIONS

The results of our normal mode calculations (Tables 2 and 4) should provide guidelines for identifying γ-turns. Of course, it must be realized that the calculations are restricted in several aspects: we have assumed specific dihedral angles appropriate to energy-minimized $(L\text{-Ala})_3$ structures while dihedral angles in a particular molecule may deviate from these because of interaction effects; although we have allowed for a variation in the f(H. . .O) stretching force constant with hydrogen bond length, we have not taken into account changes in other force constants of the hydrogen bond (of which relatively little is known), which may have an effect particularly on the lower frequencies such as amide V; we have assumed that force constants are the same for all peptide groups, which may not be true in a specific molecule, especially if residues such as proline are present (27, 28). A more general problem in identifying γ-turn conformations involves the overlapping of such frequencies with those for other kinds of structures, such as β-turns (3). This situation, of course, does not have a general solution, but the kind of isotopic substitution discussed above can provide answers in specific cases.

In spite of the above problems, these normal mode analyses should provide useful guidelines in analyzing the vibrational spectra of γ-turns.

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