Vibrational Analysis of Peptides, Polypeptides, and Proteins. VI. Assignment of β-Turn Modes in Insulin and Other Proteins

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Synopsis

The normal modes have been calculated for structures having the dihedral angles of the four β-turns of insulin. Frequencies are predicted in the amide I region near 1652 and 1680 cm^{-1}. The former overlaps the α-helix band at 1658 cm^{-1} in the Raman spectrum, while the latter accounts for the hitherto unassignable band at 1681 cm^{-1}. Calculated amide III frequencies extend above 1300 cm^{-1}, providing a compelling assignment of the 1303-cm^{-1} band in insulin and similar bands in other globular proteins.

INTRODUCTION

Past efforts to interpret the ir and Raman spectra of proteins have generally been based on the assumption of a three-state model, viz., an ordered component consisting of α-helix and β-sheet structures and an unordered component usually referred to as a "random coil." Such attempts, which are based on the supposedly known characteristic frequencies of the above structures, have often led to controversial assignments[1–4] or to incomplete assignments[5–9] for bands in the amide I and III regions of the Raman spectrum. Part of the reason for this is that another relatively specific structure, the β-turn, has been neglected as an important contributor to the vibrational spectrum, despite the fact that it constitutes a significant portion of the protein: it has been observed[10] that 32% of the residues in 29 globular proteins reside in β-turns.

In order to incorporate their presence in the analysis, it is necessary to know the characteristic frequencies of β-turns. However, since a variety of such structures exist,[10–12] it is not easy to identify these frequencies by a study of model compounds. This is to be contrasted with the cases of the α-helix and the β-sheet, which have well-defined structures and could be studied extensively by analyzing model polypeptides. We have, therefore, chosen to analyze the spectra of β-turns by calculating their normal vibration frequencies. In a preliminary publication[13] we presented results on the amide I–III modes of standard[11] type I and II β-turns, and showed that the calculated frequencies of an appropriate model of the type I turn agreed quite well with the ir bands observed for a related compound.[14] In a more detailed publication[15] we have included calculations of the type III

Biopolymers, Vol. 19, 31–36 (1980)

0006-3525/80/0019-0031$01.00

β-turn, and of the mirror-image turns, and have examined the effects of variation in dihedral angles on the frequencies of the amide modes.

In the present paper we provide a further test for such calculations by an analysis of the β-turns in insulin. This protein is a particularly suitable one for such a study, since its structure has been solved,[16] it is relatively small, with only four β-turns, and Raman spectra of single crystals have been reported.[2] Our calculations permit us to correlate previously unassignable bands in the Raman spectrum with β-turns in the structure. Since some of the computed β-turn frequencies lie in spectral regions associated with α-helix modes, these results reemphasize our previous remarks[13,15] that caution must now be exercised in proposing unique assignments of bands to α-helix and β-sheet structures in proteins. We also consider the relevance of these results to the interpretation of previously unassigned amide III bands in some other globular proteins.

NORMAL VIBRATION CALCULATIONS

The β-turns in insulin are of four different types and have the following dihedral angles[16,17]:

1. A7–10: Cys-Thr-Ser-Ile (type IV)

   $(\phi,\psi)_1 = -98,-61$; $(\phi,\psi)_2 = -89,20$; $(\phi,\psi)_3 = -141,-134$; $(\phi,\psi)_4 = -142,160$

2. A12–15: Ser-Leu-Tyr-Glu (type III)

   $(\phi,\psi)_1 = -109,180$; $(\phi,\psi)_2 = -76,-40$; $(\phi,\psi)_3 = -69,-47$; $(\phi,\psi)_4 = -76,30$

3. B7–10: Cys-Gly-Ser-His (type II′)

   $(\phi,\psi)_1 = -114,109$; $(\phi,\psi)_2 = 84,-107$; $(\phi,\psi)_3 = -92,-24$; $(\phi,\psi)_4 = -53,-17$

4. B20–23: Gly-Glu-Arg-Gly (type I)

   $(\phi,\psi)_1 = 96,-87$; $(\phi,\psi)_2 = -143,11$; $(\phi,\psi)_3 = -96,-40$; $(\phi,\psi)_4 = 114,168$

We have used these dihedral angles in our calculations, which were done on a CH_3-CO-$(Ala)_4$-NH-CH_3 model system, as in our previous studies.[13,15]

The force field used in these calculations was the same as that used previously.[13,15] Internal hydrogen bonds were given f(H···O) force constants related to their length.[15] We have assumed that all external C=O and N-H groups are hydrogen bonded and have included appropriate atoms and force constants in the calculation.[15] Transition dipole coupling[18] must be included in order to account for frequency splittings.[13,15] We have incorporated such coupling for amide I modes (amide II modes are very weak in the Raman spectrum, and such coupling does not have an appreciable

effect in perturbing amide III modes), using an effective dipole moment $\Delta\mu_{eff} = 0.45$ D, a value found to give best agreement for a known β-turn compound.[13]

RESULTS AND DISCUSSION

The amide I and III frequencies of the above four β-turns are given in Table I. Although frequencies are given for all of the peptide groups, we will direct our attention to the modes of groups 2–4, which comprise the β-turn. As in previous calculations,[13,15] it is important to include groups 1 and 5 in the calculation in order to provide the proper environment for the three peptide groups in the turn.

The predicted amide I modes for groups 2–4 center around two frequencies, 1652 ± 3 and 1680 ± 4 cm^{-1}. Bands are observed near these frequencies in the Raman spectra of single crystals,[2] viz., at 1658 and 1681 cm^{-1}. The 1658-cm^{-1} band has been assigned,[1,3] on the basis of previous correlations, to the known[16] 40–50% α-helix component of insulin. This is a reasonable interpretation, except that our calculations would now suggest that the β-turn component of the insulin structure also contributes in this region. While no experimental information is presently available about the relative intensities of these bands, the absence of symmetry in β-turns suggests that all of its modes will be ir and Raman active.

The origin of the observed band at 1681 cm^{-1} has until now been per-

TABLE I
Amide I and III Frequencies of β-Turn CH_3-CO-$(Ala)_4$-NH-CH_3 with Insulin Dihedral Angles

	Amide I		Amide III	
β-Turn	Group	Frequency (cm^{-1})	Group	Frequency (cm^{-1})
A7–10	1 + 2	1697	1 + 3	1311
(type IV)	4 + 5	1677	4	1302
	5 + 4	1660	2	1296
	3	1656	3 + 1	1290
	2 + 1	1650	5	1281
A12–15	1 + 2	1696	1	1310
(type III)	4 + 3	1683	3 + 4	1305
	5	1674	2 + 4	1299
	2 + 1	1655	4 + 3	1289
	3 + 4	1648	5	1283
B7–10	1	1680	1 + 3	1319
(type II′)	5	1677	3 + 1	1311
	2	1675	4	1307
	3	1655	2	1289
	4	1650	5	1281
B20–23	2 + 1	1684	1	1315
(type I)	3 + 4	1683	3 + 1	1296
	5	1671	4 + 5	1290
	4 + 3	1653	2 + 3	1287
	1 + 2	1646	5 + 2	1281

plexing. It has been assigned[1,4] to a "random-coil" component, but this is hardly defensible, since it disappears in denaturned insulin.[1] Other authors[3] have commented that "the shoulder at 1681 cm^{-1} might be due to a state not encountered in model studies." The results of our calculations make a strong case for assigning this band to the β-turns in the native insulin structure. The disappearance of this band on denaturation[1] is certainly consistent with this assignment, as is its continued presence in a deuterated single crystal of insulin.[2]

The predicted amide III modes of groups 2–4 fall roughly into two groups: at 1289 ± 1 cm^{-1} and fairly uniformly distributed in the range of 1296–1311 cm^{-1}. (If external hydrogen bonding is not included, these frequencies are 1280 ± 2 and 1287–1298 cm^{-1}.) The observed amide III modes in the Raman spectrum[2] are found at 1240, 1269, 1284, and 1303 cm^{-1}. (These were determined by subtracting the deuterated from the native spectrum, thus leaving only bands with NH in-plane band contributions.) These bands have been assigned as follows: 1240 to random coil and β-sheet,[1] 1269 and 1284 to α-helix,[1] and 1303 to "the α-helical category."[2] On the other hand, while essentially agreeing with the assignments of the first three bands, other authors[3] note that "the band at 1303 cm^{-1} cannot be assigned on the basis of what is presently known from model studies." The reason for this is that although high-frequency amide III modes have been correlated with α-helix structures, the highest frequency of such band observed to date is at 1295 cm^{-1} in solid poly(L-lysine)·HCl at 50% relative humidity.[19] Thus amide III bands near 1300 cm^{-1} and above cannot be correlated with α-helix, β-sheet, or unordered structures.

Our calculations clearly indicate that the band at 1303 cm^{-1}, and probably part of that at 1284 cm^{-1}, can be assigned to β-turns. Our previous calculations,[13,15] as well as the present one, have shown that β-turns have characteristic amide III modes above 1300 cm^{-1}, in a region where such modes are not found for α-helix and β-sheet structures. The above assignment is thus strongly supported by these results. While the 1284-cm^{-1} band could be associated with the α-helix, the fact that a band is predicted near this position for the β-turns of insulin indicates that, at the very least, this band should be considered to be partly due to the presence of the latter structures. Thus our predictions of the characteristic frequencies for β-turns are strongly supported by the observations on the Raman spectrum of insulin.

The assignment of amide III modes with frequencies near 1300 cm^{-1} to β-turns helps to interpret many such bands in other proteins that have heretofore been unassignable on the basis of a three-state model. A band at 1300 cm^{-1} in lysozyme[7] may be due to β-turns. Similar assignments may be appropriate for the 1305-cm^{-1} band in human carbonic anhydrase,[9] the 1305- and 1317-cm^{-1} bands in ribonuclease,[20] the 1314-cm^{-1} band in ovalbumin,[20] and the 1279-cm^{-1} band in concanavalin A (unpublished results). In a recent study of the Raman spectra of Bence-Jones proteins,[21] amide III bands were observed at 1242, 1262, and 1318 cm^{-1} in the solid

state and at 1245, 1265, and 1322 cm^{-1} in aqueous solution for the type λ protein. Since crystal structure analysis of this protein[22] shows that it contains about 50% β-sheet structure and no α-helix, it is appropriate to assign the strong 1242–1245-cm^{-1} band to β-sheet structure,[21] but there are no reasonable assignments for the weak bands at 1262–1265 and 1318–1322 cm^{-1} on the basis of the three-state model. Since this protein has nine β-turns, and since bands are predicted[15] above 1300 cm^{-1} for β-turn types I–III, we propose that the bands at 1262–1265 and 1318–1322 cm^{-1} of the Bence-Jones are assignable to its β-turn component. These assignments are supported by the disappearance of the three bands on thermal denaturation of the protein and on deuteration.[21] (Note that β-turn frequencies can occur in the 1262–1265-cm^{-1} region if the conformation results in weak internal and/or external hydrogen bonds.[13,14])

CONCLUSIONS

Normal vibration calculations on CH_3-CO-$(Ala)_4$-NH-CH_3 having dihedral angles corresponding to the four β-turns of insulin lead to the prediction of amide I and III frequencies that correspond to hitherto unassignable bands. In particular, an observed amide I band at 1681 cm^{-1} agrees well with calculated bands in this region, and the observed amide III band at 1303 cm^{-1} is in similar agreement with predicted frequencies. In the former case, calculated frequencies near 1680 cm^{-1} arise as a result of transition dipole coupling, which emphasizes (as we have noted in the earlier studies on β-turns[13,15]) the importance of this component of the force field. A similar detailed agreement is found for the amide I modes of a tetrapeptide known to have a type I β-turn structure,[23] which shows that this interaction provides a sensitive probe of local conformation in peptide and polypeptide structures.

The prediction that amide III modes of β-turns have characteristic frequencies above 1300 cm^{-1} is seen to account reasonably for the assignment of such bands in a number of other proteins. It seems likely that this region will be a particularly good diagnostic for the presence of β-turns.

This research was supported by National Science Foundation Grants PCM76-83047 and CHE78-00753. One of us (J.B.) wishes to acknowledge support from a postdoctoral fellowship from the Macromolecular Research Center.

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Received April 6, 1979
Accepted August 6, 1979