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Transition dipole coupling in Amide I modes of β polypeptides

(vibrational spectra/normal vibration analysis/intermolecular interactions/protein structure)

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ABSTRACT **Our previous introduction of transition dipole coupling helped to explain the splittings in the Amide I modes of antiparallel chain pleated sheet polyglycine I. This mechanism has now been applied to the more likely rippled sheet structure of this polypeptide as well as to the pleated sheet structure of poly(L-alanine). A satisfactorily consistent explanation of the splittings in both polypeptides is obtained. Since a previously incorporated interaction constant has not been used in the present treatment, these results show that transition dipole coupling alone can provide the physical basis for understanding these splittings. It is therefore now possible to predict with confidence the hitherto unidentified $\nu(\pi,\pi)$ frequency of the antiparallel chain pleated sheet as well as the characteristic frequencies of the parallel chain pleated sheet.**

Although the observed frequencies and splittings of Amide I bands have been used extensively to identify chain conformations in polypeptides and proteins, the theoretical basis for these splittings has not been well understood. Miyazawa (1) developed a perturbation treatment for the interaction between amide group modes in polypeptides which has been generally used to interpret the vibrational spectra of polypeptides and proteins (2, 3). It was shown, however, by Abe and Krimm (4), on the basis of a detailed normal vibration analysis of an antiparallel chain pleated sheet structure of polyglycine I, that the perturbation terms required by the Miyazawa theory could not be achieved by a general valence force field. They showed that, if direct coupling between C=O stretching vibrations was introduced, the observed splittings could be reproduced in a normal vibration calculation. Furthermore, Krimm and Abe (5) were able to demonstrate that transition dipole coupling provides a physical basis for these interactions, and more effectively explains the Amide I splittings in the infrared and Raman spectra of β polypeptides and proteins.

This development, while representing a significant advance in the understanding of Amide I splittings, has presented two problems which need resolution before this theory can be considered completely satisfactory. First, in attempting to extend our force field to β polypeptides with beta carbon atoms [such as poly(L-alanine), poly(L-alanylglycine), and poly(L-valine)] we have encountered difficulties in reconciling differences in transition dipole coupling between these polypeptides and polyglycine I, even though the intramolecular force field was readily transferable. Second, evidence has recently been presented from electron diffraction (6) and energy calculations (7) that polyglycine I is more likely to have an antiparallel chain *rippled* sheet structure rather than the *pleated* sheet structure heretofore assumed. We have recently obtained evidence from normal vibration calculations which supports this proposal (8), thus making it necessary to re-examine the transition dipole coupling theory as it applies to polyglycine I.

In this paper we present the results of a normal vibration analysis of Amide I splittings in polyglycine I and in poly(L-alanine) as determined by transition dipole coupling. It will be seen that, when the structural differences between these two polypeptides are taken into account, the differences between interaction terms can be very satisfactorily explained. It is also not necessary to invoke the f(C=O, O···H) interaction force constant which was used previously (9, 5). These conclusions add support to our previous proposals (4, 5) that transition dipole coupling is the likely physical mechanism that accounts for the splittings in the Amide I modes of β polypeptides.

PERTURBATION THEORY

In the perturbation theory of Miyazawa (1), the frequency of an Amide I mode is given by

$$\nu(\delta,\delta') = \nu_0 + \sum_{s,t} D_{st} \cos(s\delta) \cos(t\delta') \qquad [1]$$

In this equation: ν_0 is the unperturbed peptide group frequency, D_{st} is the interaction constant between peptide groups separated by t chains and s groups along the t^{th} chain, and δ and δ' are the phase angles between the vibrations in the appropriate peptide groups (see Fig. 1).

In the early use of this theory (1–3) it had been assumed that only the D_{10} and D_{01} terms were important, but a detailed normal vibration calculation for polyglycine I (4) showed that $D_{10} \cong 0$. Only by introducing the D_{11} term is it possible to explain the observed splittings (5), and it has been shown (5) that transition dipole coupling provides a reasonable explanation for the physical origin of this interaction.

If we assume that $D_{10} = 0$ in Eq. [1], then we find that for the observed frequencies of polyglycine I, namely, $\nu(0,0)$ = 1674, $\nu(0,\pi)$ = 1685, and $\nu(\pi,0)$ = 1636 cm^{-1}, the constants are: ν_0 = 1679.5, D_{01} = −24.5, and D_{11} = 19.0 cm^{-1}. For poly(L-alanine), whose observed frequencies are $\nu(0,0)$ = 1669, $\nu(0,\pi)$ = 1695, and $\nu(\pi,0)$ = 1630 cm^{-1}, we find similarly that ν_0 = 1682.0, D_{01} = −32.5, and D_{11} = 19.5 cm^{-1}. It is clear that differences in structure or in nature of Amide I modes between these two β polypeptides are reflected in the similarities and differences between these parameters,

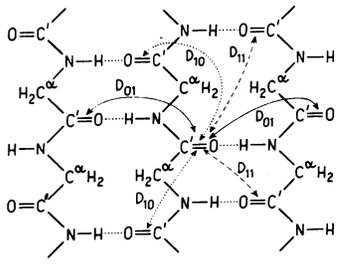

FIG. 1. Interaction constants included in the transition dipole coupling treatment of the Amide I modes of the antiparallel chain sheet structure.

and the question is whether we can explain the latter in terms of the former.

A general intramolecular valence force field has been developed for polyglycine I (4, *) and for poly(L-alanine) and other β polypeptides*. If intermolecular terms are added to this force field, then it is possible to express the above perturbation theory parameters directly in terms of the structure and such force constants (5). Thus, if we let F_{st} represent the force constants associated with the D_{st} terms, and include the f(C=O, O···H) interaction constant introduced previously, then we find from a complete normal vibration analysis of a polyglycine I sheet that

$$\begin{aligned} \nu_0 &\cong \nu_{00} + a_1 f(\text{C=O,O}\cdots\text{H}) \\ D_{10} &\cong c_1 + 65F_{10} \\ D_{01} &\cong b_1 f(\text{C=O,O}\cdots\text{H}) + 65F_{01} + \Delta\epsilon_1 \\ D_{11} &\cong 65F_{11} + \Delta\epsilon_2 \end{aligned} \qquad [2]$$

For the pleated sheet, the various parameters in [2] depend on the calculated frequencies of the intramolecular force field or on the structure. Thus:

$$\nu_{00} = \frac{1}{4}[\nu(0,0) + \nu(0,\pi) + \nu(\pi,0) + \nu(\pi,\pi)]$$

$$c_1 = \frac{1}{4}[\nu(0,0) + \nu(0,\pi) - \nu(\pi,0) - \nu(\pi,\pi)]$$

$$\Delta\epsilon_1 = \frac{1}{4}[\nu(0,0) - \nu(0,\pi) + \nu(\pi,0) - \nu(\pi,\pi)]$$

$$\Delta\epsilon_2 = \frac{1}{4}[\nu(0,0) - \nu(0,\pi) - \nu(\pi,0) + \nu(\pi,\pi)]$$

Both $\Delta\epsilon_1$ and $\Delta\epsilon_2$ are numerically very small ($|\Delta\epsilon| < 1.0$ cm^{-1}) and can be neglected. It was reported previously (5) that a_1 was a function of the relative axial translation of the two chains in the unit cell, while b_1 was insensitive to such shifts. We find that both a_1 and b_1 are sensitive to changes in crystal structure, including not only the relative axial shift but the angle which a chain makes with the hydrogen bonded sheet.

In order to proceed further, we need to obtain the values of the F_{st}s and f(C=O, O···H). It has been shown (5) that the former can be obtained reasonably from a transition dipole coupling mechanism; the latter has had to be assumed (9, 5). We have found, however, that if antiparallel chain pleated sheets are used for both polyglycine I and poly(L-alanine), it is not possible to obtain self-consistent predictions. If we assume that polyglycine I is in the antiparallel rippled sheet structure (6), however, a satisfactory description is possible. This is described below, where we have assumed for simplicity that f(C=O, O···H)=0, an assumption which is justified by the results.

TRANSITION DIPOLE COUPLING

Following our earlier formulation (5), we take the energy between two transition dipole moments, $\partial\mu/\partial S$, as

$$V = \frac{1}{\epsilon} \cdot \left|\frac{\partial\mu_1}{\partial S_1}\right| \cdot \left|\frac{\partial\mu_2}{\partial S_2}\right| \cdot \frac{\cos\alpha - 3\cos\beta\ \cos\gamma}{R^3} \cdot S_1 \cdot S_2 \quad [3]$$

where ϵ is the dielectric constant (taken as 1), R is the distance between transition moment centers, and the angles α, β, and γ are as previously defined (5). From this it follows that the force constant for this interaction is (5), in mdyn/Å

$$F = 0.1 \cdot \left|\frac{\partial\mu_1}{\partial S_1}\right| \cdot \left|\frac{\partial\mu_2}{\partial S_2}\right| \cdot \frac{\cos\alpha - 3\cos\beta\ \cos\gamma}{R^3} \qquad [4]$$

For interactions between identical Amide I (essentially C=O stretching) modes this becomes

$$F = 0.1(\partial\mu/\partial S_{(C=O)})^2 X \qquad [5]$$

where

$$X = (\cos\alpha - 3\cos\beta\ \cos\gamma)/R^3 \qquad [6]$$

is the geometrical factor.

Table 1. Eigenvectors and potential energy distributions for Amide I $\nu(0,0)$ in polyglycine I (PGI) and poly(L-alanine) (PLA)

Coordinate	Eigenvector		Potential energy distribution	
	PGI	PLA	PGI	PLA
C=O	0.357	0.354	75.1	74.9
C—N	−0.214	−0.210	17.2	16.8
C^α—C	−0.112	−0.099	3.4	2.7
C^α—C—N	0.392	0.401	12.5	13.1
C—N—C^α	0.263	0.264	2.9	2.9
N—C^α—C	0.206	0.195	2.5	2.0

Our general approach is as follows: From the observed Amide I splittings we obtain the relationships among the F_{ij} which are required in order to reproduce the observed frequencies in a normal vibration calculation. The F_{ij} can be calculated from [5] and [6] once a structure is assumed and the appropriate interactions included. The interactions we have incorporated are shown in Fig. 1, and should be sufficient (10). We also assume that the transition dipole is located at the same point on the C=O bond in both polyglycine I and poly(L-alanine). This is justified by our normal vibration calculations (8, *), which show that the eigenvectors for Amide I are essentially identical in these two polypeptides (see Table 1). We then calculate the F_{ij} as a function of three parameters: the relative axial shift, Δz, between adjacent antiparallel chains; the orientation angle, θ, of the transition dipole moment with respect to the C=O bond direction; and the location, ΔC, of the center of this moment along the C=O bond with respect to the C atom (positive ΔC is in the direction C → O). The best fit is taken as that which gives the most constant $(\partial\mu/\partial S)$ for all F_{ij}.

For polyglycine I we find that the following relationships are required by the observed Amide I frequencies:

$$\begin{aligned} F_{11} + F_{01} &= 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{11} + X_{01}) = -0.0809 \\ F_{10} - F_{01} &= 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{10} - X_{01}) = 0.3249 \\ F_{10} + F_{11} &= 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{10} + X_{11}) = 0.2436 \end{aligned} \qquad [7]$$

These relationships do not, of course, permit determining the F_{ij} since there are only three observed frequencies and

* W. H. Moore and S. Krimm, to be published.

therefore only two independent splittings. For the generalized rippled sheet structure (6), with a repeat per residue of 3.522 Å and a chain rotation angle with respect to the sheet (6) of 76°, we have made calculations for -0.7 Å $\leq \Delta z \leq 0$, $0° \leq \theta \leq 75°$ (from the C=O bond in the direction N → C), and $0 \leq \Delta C \leq 1.24$ Å. These parametrizations resulted in the testing of over 1100 sets of geometric factors.

The results on polyglycine I can be summarized as follows: (*1*) If $\Delta C < 0.62$ Å (the center of the C=O bond), Eqs. [7] cannot be satisfied for any Δz or θ. (*2*) For $\Delta z = -0.7$ Å, as suggested by conformational energy calculations (7), a satisfactory solution exists for $\theta = 55°$. This implies, however, that $(\partial\mu/\partial S)$ is essentially parallel to the N—C bond, which is very unlikely. (*3*) As Δz increases from -0.7 Å toward 0 Å, θ decreases from 55°. (*4*) An optimum fit to Eqs. [7] is obtained for $\Delta z = -0.1$ Å, $\theta = 18.5°$, and $\Delta C = 0.87$ Å. For this case $(\partial\mu/\partial S) = 11.5$ Debye/Å. These results are very satisfying on two counts: the electron diffraction data (6) require $\Delta z \cong 0$, and the value we obtain for θ is within the range of 17°–25° deduced from experimental studies on a number of molecules containing the peptide group (11). From the above optimum parameters we obtain: $F_{10} = -0.00524$, $F_{01} = -0.333$, and $F_{11} = 0.250$, from which it follows that $\nu_0 = 1676.0$, $D_{10} = 1.6$, $D_{01} = -21.6$, and $D_{11} = 16.2$ cm^{-1}.

For poly(L-alanine) we find that the following relationships are required by the observed Amide I frequencies:

$$F_{11} + F_{01} = 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{11} + X_{01}) = -0.2193$$

$$F_{10} - F_{01} = 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{10} - X_{01}) = 0.4797 \qquad [8]$$

$$F_{10} + F_{11} = 0.1\left(\frac{\partial\mu}{\partial S}\right)^2 (X_{10} + X_{11}) = 0.2604$$

Scanning the same range of parameters as before, we find an optimum fit for $\Delta z = -0.3$ Å, $\theta = 23°$, and $\Delta C = 0.87$ Å. This corresponds to: $F_{10} = 0$, $F_{01} = -0.480$, and $F_{11} = 0.260$, with $(\partial\mu/\partial S) = 12.2$, and $\nu_0 = 1682.4$, $D_{10} = 2.0$, $D_{01} = -31.2$, and $D_{11} = 16.9$ cm^{-1}. These results are also satisfying, in that θ is again quite satisfactory, and Δz is in the range required by x-ray structure refinement, namely, > -0.45 Å, in order that there be good van der Waals contacts (12).

DISCUSSION

Our results show that transition dipole coupling, without the introduction of the f(C=O, O···H) interaction constant (5), can provide a physical basis for explaining the observed Amide I mode splittings in the rippled sheet of polyglycine I and the pleated sheet of poly(L-alanine). In fact, the differences in splittings are directly related to the differences in these two structures, and are well explained by the transition dipole coupling mechanism. We find that this theory also predicts an orientation of the transition dipole which is in accord with experimental determinations, as well as a relative axial shift between adjacent chains which is consistent with values derived from x-ray diffraction studies. In fact, it may now be possible to derive the latter more accurately from spectroscopic studies, since there are uncertainties in the x-ray method (6, 7, 12). It should be noted that the four independent splittings for these two different structures are in effect accounted for by three adjustable parameters: the two magnitudes and the common location of the transition dipole moment. The other geometric parameters are reasonably known from experimental studies, and could have been incorporated as known constants; however, it is significant that, when allowed to vary, they are reproduced by our optimization procedure.

The results of the present theory permit the prediction, as previously noted (5), of the very weak $\nu(\pi,\pi)$ mode. From the parameters given above, we find that $\nu(\pi,\pi) = 1712.2$ cm^{-1} for polyglycine I and $\nu(\pi,\pi) = 1728.5$ cm^{-1} for poly(L-alanine). It is possible that a band observed near the latter position in the Raman spectrum of β-poly(L-valine) (13, 14) is assignable to this mode.

As has been shown earlier (5), it is also possible to predict the frequencies of the parallel chain pleated sheet. If we take all interactions between a chain and its two nearest neighbors, similar to those shown in Fig. 1, and use the structure proposed by Pauling and Corey (15) with $\Delta z = 0$, $\theta = 20°$, and $\Delta C = 0.87$ Å, then we find that (*a*) for $(\partial\mu/\partial S) = 11.5$: $F_{10} = 0.0397$, $F_{01} = -0.3517$, $F_{11} = 0$, and (*b*) for $(\partial\mu/\partial S) = 12.2$: $F_{10} = 0.0446$, $F_{01} = -0.395$, $F_{11} = 0$. From relationships similar to those in Eqs. [2] it follows that (*a*) $D_{10} = 3.2$ and $D_{01} = -22.9$ cm^{-1}, and (*b*) $D_{10} = 3.5$ and $D_{01} = -25.7$ cm^{-1}. This gives for (*a*) polyglycine I, $\nu_0 = 1676.0$ cm^{-1}: $\nu(0,0) = 1656.3$, $\nu(\pi,0) = 1649.9$ cm^{-1}, and (*b*) poly(L-alanine), $\nu_0 = 1682.4$ cm^{-1}: $\nu(0,0) = 1660.2$, $\nu(\pi,0) = 1653.2$ cm^{-1}. These results differ slightly from those previously presented (5), primarily in that we have not assumed that $D_{10} = 0$ in the present case. The strong $\nu(\pi,0)$ mode of a β polypeptide with beta carbon atoms is, however, again predicted to occur near 1655 cm^{-1} (5).

Transition dipole coupling is thus seen to provide a completely consistent explanation for the Amide I splittings in polyglycine I and in β poly(L-alanine), and to account for differences in these splittings which arise from structural differences between these polypeptides. It therefore should provide a sound basis for predicting hitherto unidentified frequencies, such as the $\nu(\pi,\pi)$ mode of the antiparallel chain pleated sheet and the characteristic frequencies of the parallel chain pleated sheet.

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