

Vibrational Analysis of Peptides, Polypeptides and Proteins

XII—Fermi Resonance Analysis of the Unperturbed ND Stretching Fundamental in Polypeptides

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We have obtained the spectrum of β -poly(L-alanine-ND), and find three bands in the ND stretching region. By comparison with Fermi resonance analyses of deuterated derivatives of polyglycine I as well as with α -poly(L-alanine-ND), we conclude that a three-level Fermi resonance is involved in the β polypeptide, and that the unperturbed ND stretch fundamental occurs near 2428 cm^{-1} .

INTRODUCTION

The presence in the infrared spectra of polypeptides of two bands involving the NH stretch mode has long been known.¹ These bands, a strong one near 3300 cm^{-1} (referred to as amide A) and its weak counterpart near 3100 cm^{-1} (referred to as amide B), have been associated with NH stretch because of their disappearance on N-deuteration.² The suggestion³ that these two bands arise from Fermi resonance between the NH stretch fundamental and the first overtone of the (primarily) NH inplane bend mode near 1550 cm^{-1} (amide II) was supported by studies on an N¹⁵-substituted molecule.⁴ Subsequent work⁵ provided a quantitative treatment for this interaction and for the observed intensity ratios of the bands. An analysis of these bands in polyglycine I⁶, however, showed that from symmetry considerations, the first overtone of an amide II mode could not be involved, and that the level interacting with the NH stretch fundamental was most probably a combination band between an observed amide II mode (at 1517 cm^{-1}) and a hitherto unobserved and unpredicted amide II mode (which was computed to be at about 1588 cm^{-1}). This proposal received strong support from a detailed normal mode calculation on polyglycine I⁷, in which it was shown that a band is predicted near 1588 cm^{-1} when transition dipole coupling interactions^{8,9} are incorporated in the force field. Similar results were obtained for other β polypeptides.⁷ In the case of α -helical poly(L-alanine), however, it was shown^{7,10} that an interaction between NH stretch and a first overtone of amide II could indeed be the origin of the amide A and B bands.

The analysis of the NH stretching region of the spectra of polypeptides is thus relatively secure. A much less systematic study has been made, however, of the ND stretching region. Such an analysis is of importance in terms of developing reliable force fields for these molecules. In connection with a calculation of the normal modes of isotopically substituted polyglycine I molecules¹¹ and of β -poly(L-alanine-ND),¹² we have

undertaken a more detailed Fermi resonance analysis of the ND stretching region. We present these results here.

EXPERIMENTAL

Data on the isotopic polyglycine I species were obtained from published band frequencies and qualitative intensities.¹³ Since no spectra were published, nor quantitative intensities given, our analysis is based on assumed intensity ratios (discussed below).

The spectra of β -poly(L-alanine-ND) were obtained in our laboratory.¹² Deuteration (which was never complete, although repeated treatments were used) was performed by dissolving the polypeptide in deuterated trifluoroacetic acid (TFA-D) or by heating in D_2O . The room and liquid N_2 temperature spectral contours in the ND stretching region of KBr pellets of a D_2O -treated polypeptide are shown in Fig. 1.

DISCUSSION

Polyglycine I

An analysis of the NH stretching region of polyglycine I has already been given.⁷ We review this here, in the light of a new normal mode calculation,¹¹ and to provide a background to the subsequent analysis of the ND stretching region.

As before,^{5,7} we assume a two-level Fermi resonance, that is, the interaction of the NH stretch fundamental (which for the antiparallel chain rippled sheet structure has B_u symmetry⁷) with a combination band involving two amide II modes. Under these circumstances, the observed (perturbed) frequencies, $\nu_{A,B}$, are given in terms of the unperturbed frequencies, $\nu_{A,B}^0$, by⁵

$$\begin{aligned}\nu_A &= \frac{1}{2}[(\nu_A^0 + \nu_B^0) + s] \\ \nu_B &= \frac{1}{2}[(\nu_A^0 + \nu_B^0) - s]\end{aligned}\quad (1)$$

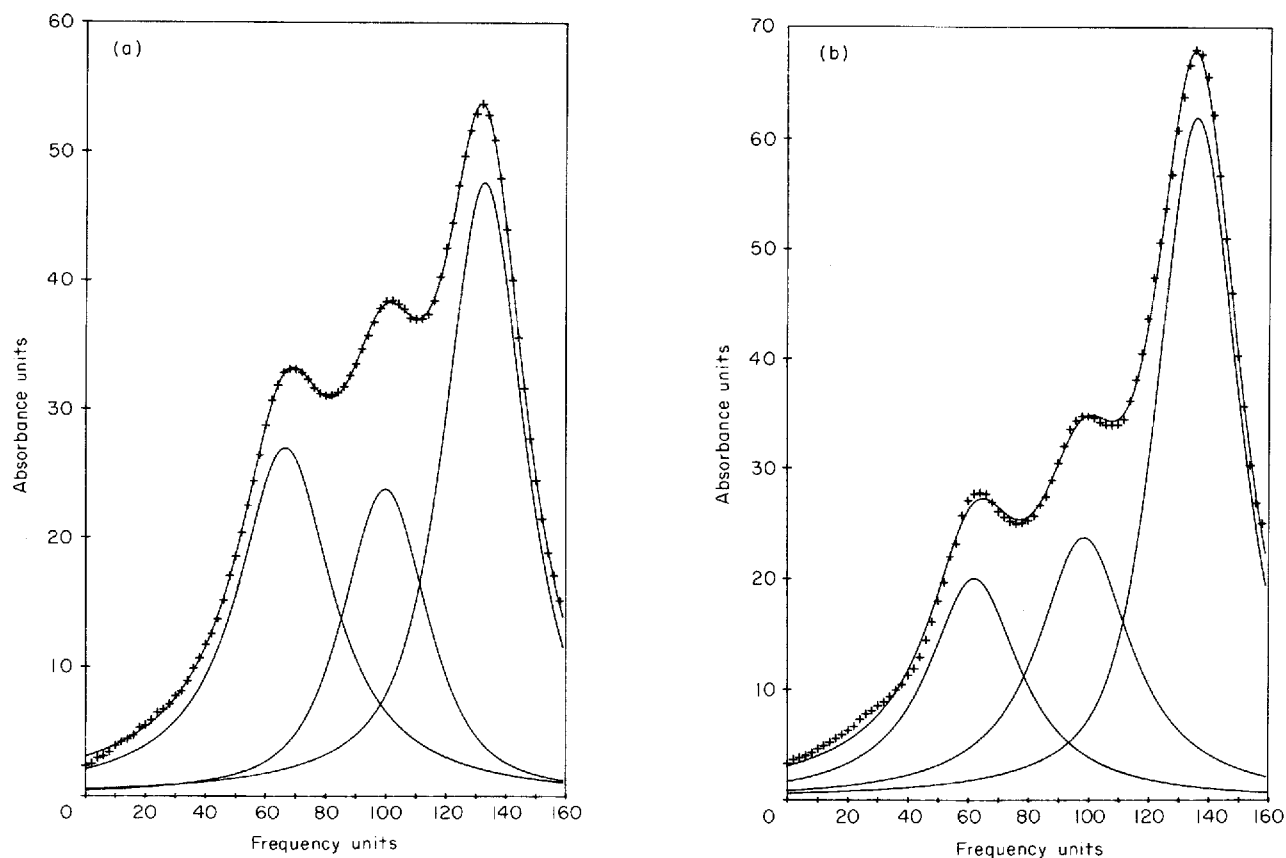


Figure 1. Spectral contours in ND stretching region of β -poly(L-alanine-ND) prepared by exchange in D_2O . Crosses are observed absorbances (on an arbitrary scale). The curve through them is the sum of indicated component bands. Frequency scale: $0.00 = 2548 \text{ cm}^{-1}$, $160.00 = 2388 \text{ cm}^{-1}$. (a) Spectrum at room temperature, (b) spectrum at liquid N_2 temperature.

where $s = \nu_A - \nu_B$. It can also be shown⁵ that

$$I_R \equiv I_B/I_A = \frac{s - \delta}{s + \delta} \quad (2)$$

where $\delta = \nu_A^0 - \nu_B^0$. Thus, a measurement of ν_A , ν_B , and I_R permits the determination of ν_A^0 and ν_B^0 .

In Table 1, we present the results of such calculations for the various isotopic species of polyglycine I. For $(NHCH_2CO)_n$, the only other possible combination besides $A_u + B_g$ is $1515(A_g) + 1572(B_u) = 3087(B_u)$ (the underlined frequency is an observed band), but this is a poorer match to the observed ν_B^0 of 3108 cm^{-1} than the combination given. For $(NHCD_2CO)_n$, no quantitative intensity data were given,¹³ only the designations s (strong) and m (medium) for ν_A and ν_B , respectively. Since these were the same designations as those given

for $(NHCH_2CO)_n$,¹³ we have used the same nominal value for I_R , recognizing that this may not be correct. In fact, there is no reason to think that ν_A^0 should be different for these two molecules, and a value of $I_R = 0.12$ would give $\nu_A^0 = 3271$ (and $\nu_B^0 = 3081$). (A common problem with values of I_R given in the literature heretofore is that an accurate quantitative value for this ratio is not available, because resolution of band contours has not been done. We estimate, however, that this introduces an error of no more than about $\pm 3 \text{ cm}^{-1}$ in most computed values of ν_A^0 and ν_B^0 .) The most important point, however, is that ν_B^0 is significantly lower here than for $(NHCH_2CO)_n$, and this is well accounted for by the somewhat lower frequency amide II bands of the C-deuterated molecule. For $(N^{15}HCH_2CO)_n$, we have again used the same value of I_R , and we see that

Table 1. Fundamental NH and ND stretching frequencies, and combinations, for polyglycine I.

Molecule	ν_A (cm^{-1})	ν_B (cm^{-1})	I_R	ν_A^0 (cm^{-1})	$\nu_A^0(C)^a$ (cm^{-1})	ν_B^0 (cm^{-1})	Combination ^b
$(NHCH_2CO)_n$	3300 ^c	3080 ^c	0.146 ^c	3272	3272 ^d	3108	<u>1517</u> (A_u) + 1602(B_g) = 3119(B_u)
$(NHCD_2CO)_n$	3297 ^e	3055 ^e	0.146 ^f	3266	3272 ^d	3086	<u>1498</u> (A_u) + 1588(B_g) = 3086(B_u)
$(N^{15}HCH_2CO)_n$	3293 ^e	3064 ^e	0.146 ^f	3264	3263 ^d	3093	<u>1503</u> (A_u) + 1592(B_g) = 3095(B_u)
$(NDCH_2CO)_n$	2462 ^e	2419 ^e	0.146 ^f	2457	2405 ^d	2425	<u>1475</u> (A_u) + 939(B_g) = 2414(B_u)
$(ND_2CD_2CO)_n$	2464 ^e	2416 ^e	0.146 ^f	2458	2405 ^d	2422	<u>1486</u> (B_g) + <u>950</u> (A_u) = 2436(B_u)

^a Calculated frequency, from Ref. 11.

^b Underlined frequencies are observed infrared bands; others are calculated values (Ref. 11).

^c From Ref. 7.

^d Force constant adjusted to give $\nu_A^0(NH) = 3272 \text{ cm}^{-1}$ (Ref. 11).

^e From Ref. 13.

^f Assumed value (see text).

both the expected drop in the NH stretch fundamental and the intermediate value of the combination band are well explained. These results confirm the original analysis⁷ that the origin of the combination band is in $A_u + B_g$ amide II modes rather than $A_g + B_u$ modes; in fact, calculations¹¹ show that the latter combination has a smaller value for $(N^{15}HCH_2CO)_n$ than for $(NHCD_2CO)_n$, in disagreement with observation. In all of the above cases, the amide modes consist mainly of NH in-plane bend with some CN stretch and $C^\alpha C$ stretch.

For the analysis of the ND stretching region, we have again had to use a nominal value of I_R because of the absence of quantitative information.¹³ However, reasonable changes in I_R result in only $\pm 2 \text{ cm}^{-1}$ changes in ν_A^0 and ν_B^0 . For $(NDCH_2CO)_n$, we see first that the calculated value for ν_A^0 is about 50 cm^{-1} below the observed value. This is probably a result of the real difference in anharmonicities between the NH and ND stretch modes, which is not accounted for in our harmonic calculation (the difference between observed and calculated ND stretch frequencies is in the right direction for this effect). Second, the most reasonable combination band is again $A_u + B_g$, with the former involving mainly $C^\alpha C$ stretch and CN stretch, and the latter having a significant ND in-plane bend component.¹¹ In this respect, there is a certain analogy with the combination bands for the NH cases. (The only other possible combination of this kind is¹¹ $1485(A_g) + 932(B_u) = 2417(B_u)$, which cannot be completely excluded.) For $(NDCD_2CO)_n$, no combination of the above form (i.e., an A_u or A_g mode near 1480 cm^{-1}) is possible if we wish the band near 900 cm^{-1} to have an ND in-plane bend component: for this molecule, there are no B_g or B_u modes in the $900\text{--}1000 \text{ cm}^{-1}$ region that have ND in-plane bend contributions.¹¹ The $B_g + A_u$ combination, however, seems reasonable (as would a comparable $B_u + A_g$ combination¹¹).

Thus, the two bands in the ND stretching region of N-deuterated polyglycine I molecules can be assigned,

as in the NH species, to a Fermi resonance doublet in which the strong, high frequency component is predominantly ND stretch and the weaker low frequency component is a combination band involving a mode with $C^\alpha C$ and CN stretch near 1480 cm^{-1} and a mode with significant ND in-plane bend near 950 cm^{-1} . The necessary presence of the latter component seems reasonable in terms of the localized nature of the ND stretch mode.

Poly(L-alanine)

An examination of the ND stretching region of β -poly(L-alanine-ND) (Fig. 1) shows two significant differences when compared with the spectra of polyglycine I-ND: there are three bands instead of two and the more intense band seems to be the one at lowest frequency. If Fermi resonance accounts for this triplet, then this resonance is presumably not exactly the same as that in polyglycine I; for example, a three-level resonance may be involved. Before examining this situation in detail, we will review the analyses for the hydrogenated molecules, both α -helical as well as β .

For α -poly(L-alanine), it has been established^{7,10} that a Fermi resonance between NH stretch (A species) and an overtone of amide II (E_1 species) can explain the observed amide A and B bands. This is reproduced in Table 2, for both room temperature and low temperature spectra.¹⁰ (We note that an overtone of the A species amide II mode, found^{10,14} at 1516 cm^{-1} , cannot account for the observed ν_B^0 .) It will be seen, incidentally, that ν_A^0 decreases significantly, by 29 cm^{-1} , when the temperature is lowered, undoubtedly reflecting the significant increase in hydrogen bond strength as the helix contracts axially. For β -poly(L-alanine), ν_A and ν_B are accounted for by a Fermi resonance entirely analogous to that in polyglycine I, involving combinations between amide II modes of similar symmetry (the NH stretch fundamental for the antiparallel chain

Table 2. Fundamental NH and ND stretching frequencies, and combinations, for α - and β -poly(L-alanine)

Molecule	ν_A (cm^{-1})	ν_B (cm^{-1})	I_R	ν_A^0 (cm^{-1})	$\nu_A^0(C)^a$ (cm^{-1})	ν_B^0 (cm^{-1})	Combination ^b
α -NH: RT ^c	3307 ^d	3058 ^d	0.127 ^d	3279	3279 ^a	3086	$2 \times 1545(E_1) = 3090(A)$
LT ^c	3286 ^d	3070 ^d	0.200 ^d	3250		3106	$2 \times 1553(E_1) = 3106(A)$
β -NH: RT	3280 ^f	3072 ^f	0.223 ^f	3242	3242 ^d	3109	$1524(B_1) + 1593(B_3) = 3117(B_2)$
RT	3276 ^h	3070 ^h	0.146 ^h	3250		3096	$1538(A) + 1555(B_2) = 3093(B_2)$
LT	3263 ^h	3086 ^h	0.128 ^h	3243		3101	
α -ND: RT	2464 ^d	2407 ^d	0.35 ^h	2449	2410 ^a	2422	$1439(E_1) + 974(E_1) = 2413(A)$
LT	2462 ^d	2403 ^d	0.99 ^d	2433		2432	$1430(A) + 1000(A) = 2430(A)$
	ν_A (cm^{-1})	ν_B (cm^{-1})	ν_C (cm^{-1})	ν_A^0 (cm^{-1})	$\nu_A^0(C)$ (cm^{-1})		Combinations
β -ND: RT	2415	2448	2482	2428	2381 ^d		$1488(B_3) + 999(B_1) = 2487(B_2)$
							$1488(B_3) + 942(B_1) = 2430(B_2)$
LT	2411	2449	2486	2419			

^a Calculated frequency.

^b Underlined frequencies are observed bands; others are calculated values (Ref. 12 for β , Ref. 14 for α).

^c RT = room temperature, LT = liquid N_2 temperature.

^d From Ref. 10.

^e Force constant adjusted to give $\nu_A^0(NH) = 3279 \text{ cm}^{-1}$ (Refs. 10, 14).

^f From Ref. 7.

^g Force constant adjusted to give $\nu_A^0(NH) = 3242 \text{ cm}^{-1}$ (Ref. 12).

^h This work.

pleated sheet structure of β -poly(L-alanine) has B_2 symmetry⁷). Our present measurements of I_R would also permit an $A+B_2$ combination. The much lower value of ν_A^0 reflects the much stronger hydrogen bond in the β ($I(N\cdots O) = 2.73 \text{ \AA}$) as compared with the α ($I(N\cdots O) = 2.83 \text{ \AA}$) polypeptide. The smaller shift on lowering the temperature is undoubtedly a result of the small lateral contraction within the sheet, thus only slightly altering the hydrogen bond strength. Therefore, the analysis of the NH stretch fundamental in poly(L-alanine) follows directly from that of polyglycine I.

In the case of α -poly(L-alanine-ND), we have re-examined the data¹⁰ for I_R and feel that the previous room temperature ratio does not adequately account for band overlaps. We believe that a value of I_R closer to half of that originally quoted,¹⁰ is more realistic. In addition, our new normal mode calculation¹⁴ indicates that the observed frequency at $\sim 1000 \text{ cm}^{-1}$ should be assigned to an A species rather than an E_1 species mode, the latter being associated with a band near 974 cm^{-1} . Both of these observations provide a more consistent explanation in terms of an E_1+E_1 combination, although an $A+A$ combination can perhaps not be excluded. The situation is complicated by the fact that a weak third band, at $\sim 2450 \text{ cm}^{-1}$, is seen as a shoulder in the low temperature spectrum,¹⁰ thus raising the possibility that a three-level resonance may be involved. We have not pursued this point further.

On the basis of the above results, we should have certain general expectations about the ND stretch frequency in β -poly(L-alanine-ND). First, since the hydrogen bond in β is stronger than in α , resulting in $\nu_A^0(\text{NH}) = 3242\text{--}3250 \text{ cm}^{-1}$ of β being lower than the $\nu_A^0(\text{NH}) = 3279 \text{ cm}^{-1}$ of α , we should expect that $\nu_A^0(\text{ND})$ of β should be lower than 2449 cm^{-1} (2441 cm^{-1} if the earlier value¹⁰ of I_R were used). Second, following the relatively small downward shift ($\sim 7 \text{ cm}^{-1}$) on cooling that occurs for $\nu_A^0(\text{NH})$, we would anticipate a similarly small shift for $\nu_A^0(\text{ND})$, certainly less than the $\sim 16 \text{ cm}^{-1}$ shift found for $\alpha\text{-}\nu_A^0(\text{ND})$. Third, by analogy with polyglycine I-ND and perhaps α -poly(L-alanine-ND), we should expect that the observed $\nu_A^0(\text{ND})$ should be no more than $40\text{--}50 \text{ cm}^{-1}$ higher than the calculated value based on using the force constant adjusted for $\nu_A^0(\text{NH})$. It is useful to keep these guidelines in mind as we analyze the ND stretching region of β -poly(L-alanine-ND).

As can be seen from Fig. 1, there are three clearly defined bands in the ND stretching region of the spectrum of the β polypeptide. Furthermore, the more intense band is now at the lowest frequency, in distinction to all of the previous cases. We have resolved the observed band contour, using a previously described computer program,¹⁵ and find three component bands (labelled ν_A , ν_B , and ν_C) at the frequencies given in Table 2 (see Fig. 1 for results of the resolution). It is clear that if all three bands are associated with β -poly(L-alanine-ND), then we are probably dealing with a three-level Fermi resonance situation, which has already been proposed for the amide I–amide II region of solid *N*-methylacetamide.¹⁶ In distinction to that case, we presumably have a resonance between a fundamental and two combination bands, and we therefore expect the strongest observed band to be most closely related to the fundamental.¹⁶

We believe that the evidence supports the proposal that the three bands are associated with the β structure, although there can be a contribution in this region from another (perhaps unordered) component. First, the residual NH stretch band for the D_2O -treated sample is at the same frequency (3276 cm^{-1}) as the original β band, thus suggesting that the treatment has not produced drastic structural changes. Second, in the sample deuterated with TFA-D the residual NH stretch band is at about 3302 cm^{-1} , suggesting contributions from another structure, but the main change is a downward shift in the high frequency component (from 2482 cm^{-1} to 2478 cm^{-1}) and an increase in its intensity. In fact, as will be seen from Table 3, this strong broad component shifts downward by about 30 cm^{-1} at low temperature, far more than the observed 2 cm^{-1} shift for the α -helix (cf. Table 2), but consistent with what might be expected for a disordered structure. Third, the intensity ratios $I(\nu_B)/I(\nu_A)$ and $I(\nu_C)/I(\nu_A)$ remain about the same between samples, although the other ratios vary in a manner consistent with a contribution from an unordered component. Thus, for both samples at room temperature $I(\nu_B)/I(\nu_A) = 0.41 \pm 0.10$, whereas $I(\nu_C)/I(\nu_A)$ is 0.73 for the D_2O sample and 1.21 for the TFA-D sample (this reversal of relative intensity shows that an independent component must be contributing near 2478 cm^{-1} in the latter sample). Similarly, at low temperature $I(\nu_C)/I(\nu_A) = 0.55 \pm 0.13$ for both samples, but $I(\nu_B)/I(\nu_A)$ is 0.50 for the D_2O sample and 1.12 for the TFA-D sample (consistent with an independent contribution near 2449 cm^{-1} in the latter sample). As we will see below, in addition to the general expectations mentioned above as well as the demonstrated persistence of three bands in this region, we also find two appropriate combination bands that can interact with the ND stretch fundamental.

Table 3. Component bands in ND stretching region of β -poly(L-alanine-ND)

		D_2O^a	TFA-D ^a
RT ^b	ν_A (cm^{-1})	2415	2416
	HWHM ^c (cm^{-1})	16	17
	Area ^d	0.448	0.398
	ν_B (cm^{-1})	2448	2449
	HWHM (cm^{-1})	17	14
	Area	0.227	0.120
LT ^b	ν_C (cm^{-1})	2482	2478
	HWHM (cm^{-1})	19	25
	Area	0.325	0.482
	ν_A (cm^{-1})	2411	2411
	HWHM (cm^{-1})	16	14
	Area	0.520	0.356
	ν_B (cm^{-1})	2449	2449
	HWHM (cm^{-1})	19	26
	Area	0.262	0.400
	ν_C (cm^{-1})	2486	2492
HWHM (cm^{-1})	19	20	
Area	0.218	0.244	

^a Sample deuterated with D_2O or TFA-D.

^b RT = room temperature, LT = liquid N_2 temperature.

^c HWHM = half-width at half-maximum.

^d Area relative to that of the total band.

If we accept the existence of three bands in the ND stretching region, then the frequency of the unperturbed ND stretch mode can be obtained in a straightforward way if we can determine the contributing combination bands. This is because we always have¹⁶ $\nu_A + \nu_B + \nu_C = \nu_A^0 + \nu_B^0 + \nu_C^0$. An examination of possible combinations similar to those for β -poly(L-alanine) indicates¹² that, for the room temperature spectrum, the only reasonable ones are (a) $\nu_B^0 = 1464(B_1) + 1020(B_3) = 2484(B_2)$ and $\nu_C^0 = 1488(B_3) + 942(B_1) = 2430(B_2)$, or (b) $\nu_B^0 = 1488(B_3) + 999(B_1) = 2487(B_2)$ and $\nu_C^0 = 2430(B_2)$. Since $\nu_A + \nu_B + \nu_C = 7345 \text{ cm}^{-1}$, this gives (a) $\nu_A^0 = 2431 \text{ cm}^{-1}$ or (b) $\nu_A^0 = 2428 \text{ cm}^{-1}$. Choice (a) is less reasonable than choice (b), since it would put the fundamental at a higher frequency than a combination band, and the relative intensities suggest that ν_A^0 is the lowest frequency. We therefore choose the latter assignments for the contributing combination bands. In the low temperature spectrum, we have observed that the medium intensity band at 1464 cm^{-1} moves to higher frequency by about 5 cm^{-1} , whereas the 999 and 942 cm^{-1} bands remain about the same. If we can assume a similar shift in the very weak 1488 cm^{-1} band (which is hard to detect in the low temperature spectrum), then we find that $\nu_A^0(\text{ND})$ is now 2419 cm^{-1} .

The conclusion that $\nu_A^0(\text{ND})$ for room temperature β -poly(L-alanine-ND) is at about 2428 cm^{-1} is con-

sistent with our expectations. First, it is lower than the 2449 cm^{-1} of the α -ND molecule, which is required by the stronger hydrogen bond. Second, it shifts down by only 9 cm^{-1} on cooling, analogous to the 7 cm^{-1} shift found for β - $\nu_A^0(\text{NH})$. Third, the value of $\nu_A^0(\text{ND})$ is 47 cm^{-1} higher than the calculated ND stretch frequency, as expected by analogy with the other polypeptides. Finally, we can see a justification for the three-level Fermi resonance in β -poly(L-alanine-ND) as compared with the analogous polyglycine I species: whereas in the latter only one combination band is possible in the appropriate region,¹¹ for the former we can have two combination bands in the necessary region,¹² separated by some 55 cm^{-1} . (The expectation of a three-level resonance is, of course, based on the assumption¹⁶ that otherwise a combination band would exhibit essentially no intensity.)

We therefore propose that in deuterated β polypeptides the unperturbed ND stretch frequency is to be found near 2428 cm^{-1} .

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