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## Infrared Spectra and Resonance Interactions of Amide-I and II Vibrations of $\alpha$ -Helix

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### Synopsis

Amide-I and II vibrations of  $\alpha$ -helix have been treated on the basis of the perturbation theory in a dipole-dipole approximation. The infinite helix and its finite fragments have been considered as models. The calculated infrared spectra for the infinite helix are in good agreement with the spectra of synthetic polypeptides. In the case of finite fragments of the  $\alpha$ -helix, the characteristic contours of the amide-I and II bands appear when the number of peptide groups is about 10 and 6, respectively.

### INTRODUCTION

The  $\alpha$ -helix is one of the most widespread regular conformations in synthetic polypeptides and proteins. In the infrared spectra, the  $\alpha$ -helix has a few strong amide bands, which have characteristic frequencies<sup>1,2</sup> and intensities.<sup>3</sup> It has recently been shown that the observed resonance frequency splitting of these bands can be explained by the transition dipole coupling.<sup>4</sup> In our previous papers,<sup>5,6</sup> resonance interactions of the amide-I vibration have been considered for the pleated sheet with antiparallel- and parallel-chain packings. Resonance interactions and infrared spectra of amide-I and II vibrations of the  $\alpha$ -helix are analyzed in this paper.

### THEORY

The model of the right-handed  $\alpha$ -helix proposed by Pauling and Corey<sup>7</sup> has been considered. The unit cell contains 18 peptide groups included in five helical turns. This means that the angle of twist of the peptide groups is equal to  $\theta = (2\pi/18) \times 5 = 100^\circ$ . The length of the unit cell in the  $z$  direction along the helix axis is equal to 27 Å and the pitch of the helix is equal to 5.4 Å. In the  $\alpha$ -helix, each peptide transition is split into 18 resonance exciton modes: symmetrical A, antisymmetrical B, and eight doubly degenerate of the E type. In infrared spectra of infinite  $\alpha$ -helix, only vibrations of the A and E<sub>1</sub> types are active. Symmetry coefficients for these vibrations are:<sup>8</sup>

$$\begin{aligned}
 C_{Ai} &= \sqrt{\frac{1}{18}} \\
 C_{E'_{1i}} &= \sqrt{\frac{2}{18}} \cos(i-1)\theta \\
 C_{E''_{1i}} &= \sqrt{\frac{2}{18}} \sin(i-1)\theta
 \end{aligned} \quad (1)$$

where  $i = 1, 2, \dots, 18$  = number of peptide group in the unit cell.

Equations for resonance frequencies of active infrared spectra vibrations can be written:<sup>5,9</sup>

$$\nu^A = \nu_0 + \frac{1}{h} \frac{1}{18} \sum_{i,j=1}^{18} L_{ij} = \nu_0 + \frac{1}{h} \sum_{i=1}^{18} L_{i1} \quad (2)$$

$$\nu^{E_1} = \nu_0 + \frac{1}{h} \frac{2}{18} \sum_{i,j=1}^{18} L_{ij} \cos(i-1)\theta \cos(j-1)\theta \quad (3)$$

$$\nu^{E''_1} = \nu_0 + \frac{1}{h} \frac{2}{18} \sum_{i,j=1}^{18} L_{ij} \sin(i-1)\theta \sin(j-1)\theta \quad (4)$$

where

$i, j$  = number of groups in the unit cell;

$L_{ij}$  = matrix element of resonance interaction;

$\nu_0$  = unperturbed frequency of the peptide oscillator.

For infinite helix the frequencies of doubly degenerate transitions are equal; i.e.,  $\nu^{E_1} = \nu^{E'_1} = \nu^{E''_1}$ .

The matrix elements of resonance interaction are written as:

$$L_{ij} = \sum_n' V_{mj,ni} \quad (5)$$

where

$n, m = 1, 2, \dots, M$ , numbers of unit cells;

$V_{mj,ni}$  = matrix element of excitation transfer from the group  $ni$  onto the group  $mj$ ;

$\Sigma'$  = the sum lacks the term with both indices  $m = n, i = j$  simultaneously.

In the nearest neighbor approximation with a radius of the sphere of interaction  $R_0$  of about 5 Å the three neighbor groups along the chain in both directions should be taken into account. In this case we obtained the following equations for resonance frequencies:

$$\nu^A = \nu_0 + \frac{2}{h} (V_{11,12} + V_{11,13} + V_{11,14}) \quad (6)$$

$$\nu^{E_1} = \nu_0 + \frac{2}{h} (V_{11,12} \cos \theta + V_{11,13} \cos 2\theta + V_{11,14} \cos 3\theta) \quad (7)$$

These equations can readily be compared with the equations suggested earlier<sup>1</sup> by introducing the generalized coefficients of interaction:

$$\nu^A = \nu_0 + D_1 + D_2 + D_3 \quad (8)$$

$$\nu^{E_1} = \nu_0 + D_1 \cos \theta + D_2 \cos 2\theta + D_3 \cos 3\theta \quad (9)$$

where  $D_i = (1/h) 2V_{11,1i}$  with  $i = 1, 2, 3$ .

Coefficients of interaction  $V_{mj,ni}$  have been calculated in dipole-dipole approximation taking into account the transition moment coupling of peptide oscillators associated with the amide-I and amide-II vibrations. Details of the method of calculation are described in Ref. 5. The magnitude of the amide-I transition dipole is assumed to be equal to 0.30 D, which is derived from the experimental value of intensity  $4.15 \times 10^4$  l. mol<sup>-1</sup> cm<sup>-2</sup>.<sup>3</sup> The transition dipole vector is in the plane of a peptide group and its deviation angle from the CO bond to the CC bond is 17°. <sup>10</sup> The center of the transition dipole was localized at a point 0.4 Å away from the oxygen atom in the direction O → N. The value of the amide-II transition dipole is assumed to be equal to 0.18 D, which is derived from the observed value of the intensity  $1.4 \times 10^4$  l. mol<sup>-1</sup> cm<sup>-2</sup>.<sup>3</sup> The deviation angle of the amide-II transition dipole from the CO bond toward the CC bond was observed to be about 80°. <sup>10</sup> We chose this angle to be 65° in order to give better agreement between theoretical and observed values of the ratio of intensities of two components of the amide-II vibration in the infrared spectra. The point of location of this transition moment was chosen at the midpoint of the CN bond.<sup>4</sup> The shift of this point in the plane of the peptide group up to ±0.5 Å in any direction does not affect the results of these calculations.

## RESULTS AND DISCUSSION

### Infinite $\alpha$ -Helix

The resonance interactions of each peptide group of a single  $\alpha$ -helix with all others contained within a sphere of radius  $R_0$  ranging from 5 to 30 Å have been considered.

TABLE I  
Calculated Resonance Frequency Shifts of the A and E<sub>1</sub> Components of Amide-I Vibration for the Infinite  $\alpha$ -Helix

Radius of Sphere of Interaction $R_0$ (Å)	Symmetry- Independent Shift $\Delta\nu_{\text{res}}^c$ (cm <sup>-1</sup> )	Symmetry-Dependent Shift $\Delta\nu_{\text{res}}^f$ (cm <sup>-1</sup> )	
		A	E <sub>1</sub>
5	0	+1.34	-4.67
10	0	-6.10	-4.93
15	0	-8.35	-5.51
20	0	-9.47	-5.55
30	-0.06	-9.96	-5.58

Total resonance frequency shift is equal to  $\Delta\nu_{\text{res}} = \Delta\nu_{\text{res}}^c + \Delta\nu_{\text{res}}^f$ .<sup>5</sup>



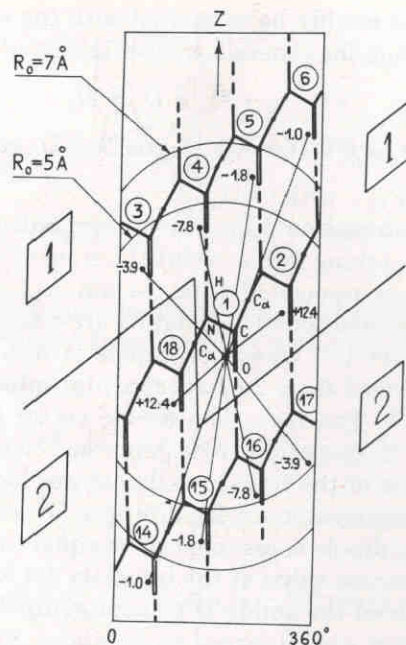


Fig. 1. A map of resonance interactions for the amide-I vibration of the  $\alpha$ -helix. Interaction coefficients  $V_{11,ni}$  are given in  $\text{cm}^{-1}$ . The structure of the  $\alpha$ -helix is presented in a radial projection. Figures in circles give the peptide groups in a unit cell; figures in squares give the cells. The circles with radii  $R_0 = 5$  and  $7 \text{ \AA}$  show the first and second coordination spheres relative to peptide group 1 of the first unit cell.

For the amide-I vibration, the calculated resonance frequency shifts of A and  $E_1$  types are presented in Table I. A negligible value of the symmetry-independent shift is explained by the large  $z$  dimension of the unit cell of the  $\alpha$ -helix, which is equal to  $27 \text{ \AA}$ . Saturation of the total frequency shifts and frequency splitting occur at about  $R_0 = 10 \text{ \AA}$ . The coefficients  $V_{11,ni}$  of interactions of peptide group 1 of the first unit cell with the neighbor groups is presented in Figure 1. Interactions

TABLE II  
Calculated Resonance Frequency Shifts of the A and  $E_1$  Components of Amide-II Vibration for the Infinite  $\alpha$ -Helix

Radius of Sphere of Interaction $R_0 (\text{\AA})$	Symmetry- Independent Shift $\Delta\nu_{\text{res}}^c (\text{cm}^{-1})$	Symmetry-Dependent Shift $\Delta\nu_{\text{res}}^f (\text{cm}^{-1})$	
		A	$E_1$
5	0	-12.81	+4.74
10	0	-12.41	+6.11
15	0	-12.29	+6.45
20	0	-12.30	+6.56
30	-0.01	-12.29	+6.62

Total resonance frequency shift is equal to  $\Delta\nu_{\text{res}} = \Delta\nu_{\text{res}}^c + \Delta\nu_{\text{res}}^f$ .<sup>5</sup>

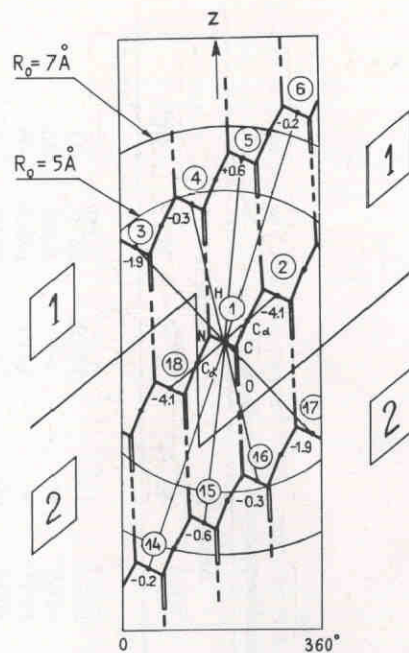


Fig. 2. A map of resonance interactions for the amide-II vibration of the  $\alpha$ -helix. Designations are the same as in Fig. 1.

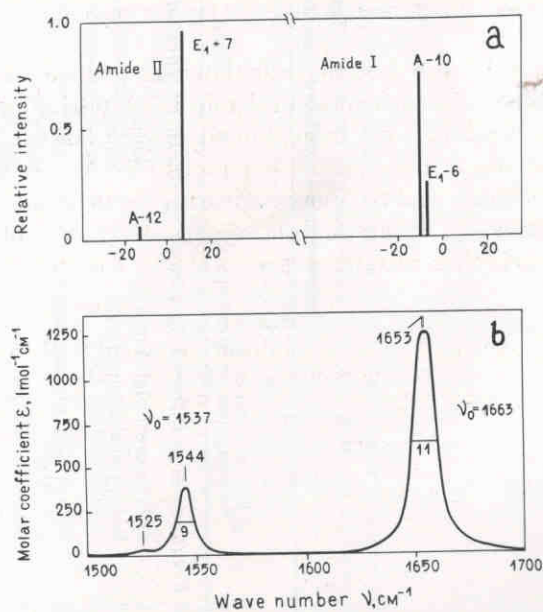


Fig. 3. The calculated infrared spectra of the amide-I and II vibrations of the infinite  $\alpha$ -helix. The shape of the absorption contour for the single transition is assumed to be Lorentzian with a half-width of  $9 \text{ cm}^{-1}$ .

TABLE III  
Observed and Calculated Amide-I and II Band Frequencies for  $\alpha$ -Helix

No.	Polypeptide or Structure	State	Amide I		Amide II		Ref.
			A	E <sub>1</sub>	A	E <sub>1</sub>	
			<i>Observed Frequencies in Infrared Spectra of Synthetic Polypeptides</i>				
1	Poly( $\gamma$ -methylglutamate)	Solid	1654	1659	1519	1550	11
2	Poly( $\gamma$ -ethylglutamate)	Solid	1658	—	—	1550	11
3	Poly( $\gamma$ -benzylglutamate)	Solution	1651	—	1517	1550	3
		Solid	1650	1652	1516	1546	1
		Solid	1652	1655	1518	1549	10
		Solid	1650	1653	1515	1545	12
		Solution	1651	—	1517	1550	3
4	Polymethionine	Solution	1651	—	1517	1548	3
5	Poly(L-Met-D-Met-L-Met)	Solid	1657	—	—	1555	13
6	Polyalanine	Solid	1659	—	—	1545	14
7	Poly( $\alpha$ -amino- <i>n</i> -butyric acid)	Solid	1656	—	—	1545	14
8	Polynorvaline	Solid	1659	—	—	1549	14

9	Polynorleucine	Solid	1654	—	—	1548	14
10	Polyvaline	Solid	1655	1650	1520	1535	15
11	Polyleucine	Solid	1656	—	—	1545	14
12	Poly(lysine·HCl)	Solid	—	—	—	—	—
	$\alpha'$ -form	Humidity 50%	1655	—	—	—	16
	$\alpha''$ -form	Humidity 100%	1645	—	~1515	1550	16
13	Sodium poly(glutamate)	Solid	—	—	—	—	—
	$\alpha'$ -form	Humidity <10%	1645	—	~1515	1545	17
	$\alpha''$ -form	Humidity 90%	1637	—	—	1550	17
14	Poly(glutamic acid)	Solid, humidity 10-90%	1650	—	~1515	1550	17
		Solution, water-dioxane	1651	—	1517	1551	17
		Mean Values:	1652	1654	1517	1548	—
<i>Calculated Frequencies of Right-Handed <math>\alpha</math>-Helix</i>							
Right-handed $\alpha$ -helix <sup>7</sup>							
$\nu_0 = 1663 \text{ cm}^{-1}$ , amide I							
$\nu_0' = 1537 \text{ cm}^{-1}$ , amide II							
			1653	1657	1525	1544	This work

In the majority of cases, the two components of the amide-I transition are not resolved. The observed frequency of the maximum was attributed to the A component which is always stronger than the E<sub>1</sub> component.



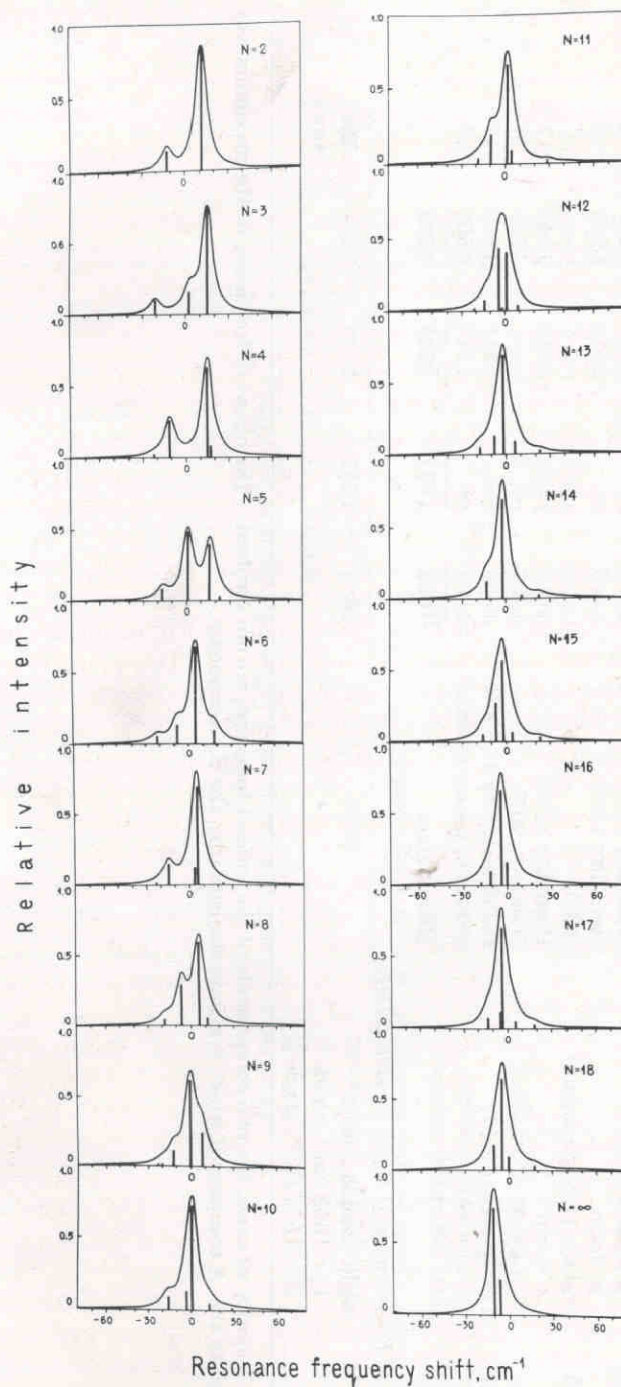


Fig. 4. The calculated infrared spectra of the amide-I vibration of the finite  $\alpha$ -helical fragments.  $N$  is the number of peptide groups.

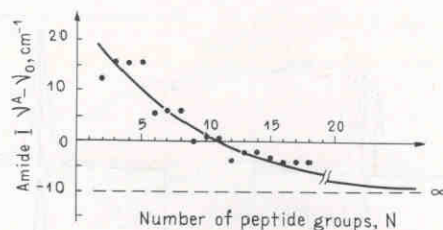


Fig. 5. Dependence of the resonance frequency shift of the amide-I main infrared transition of A type on the length of the  $\alpha$ -helical fragment.

with the three neighboring groups along the chain are most essential. It is noted that the third neighbor along the chain is linked through a hydrogen bond with the group considered.

For the amide-II vibration, the calculated resonance frequency shifts are presented in Table II and the map of resonance interactions in Figure 2. Saturation of the total frequency shifts occurs at  $R_0 = 5 \text{ \AA}$ ; this means that the nearest neighbor approximation will give almost the same results as approximation including all peptide groups. The interactions with only two neighbor groups along the chain are significant, while the interaction through the hydrogen bond is no longer essential.

A theoretical infrared spectrum of the infinite  $\alpha$ -helix is presented in Figure 3a. Figure 3b shows the same data but in a form more convenient for comparison with the experimental data, when a Lorentzian contour with a half-width of  $9 \text{ cm}^{-1}$  was chosen for a single transition.<sup>5</sup> At present many examples of infrared spectra of  $\alpha$ -helical polypeptides can be found in the literature.<sup>1,3,10-18</sup> As can be seen from Table III, the observed frequencies of the amide-I and amide-II components are, in general, conservative. They are practically independent of such factors as the type of side-chain residues or the physical state of the polypeptide. The mean values have root-mean-square deviations of only a few  $\text{cm}^{-1}$ . However, in the case of hydrophilic polylysine and sodium polyglutamate a dependence of the frequencies of the maxima on the humidity of the surrounding atmosphere has been observed.<sup>16,17</sup> These two polypeptides also display exceptionally low frequencies of their amide-I bands, which lie near  $1645 \text{ cm}^{-1}$  and  $1637 \text{ cm}^{-1}$ , respectively, while the mean value is equal to about  $1652 \text{ cm}^{-1}$ . The special optical properties of these polypeptides seem to be evoked by the strong interaction between charged side chains within a single  $\alpha$ -helix.

Absolute theoretical frequencies of the components of the amide-I and amide-II vibrations of  $\alpha$ -helix are in good agreement with the experimental ones, if we choose the following frequencies of unperturbed oscillators:

$$\text{amide-I } \nu_0 = 1663 \text{ cm}^{-1}$$

$$\text{amide-II } \nu_0 = 1537 \text{ cm}^{-1}$$

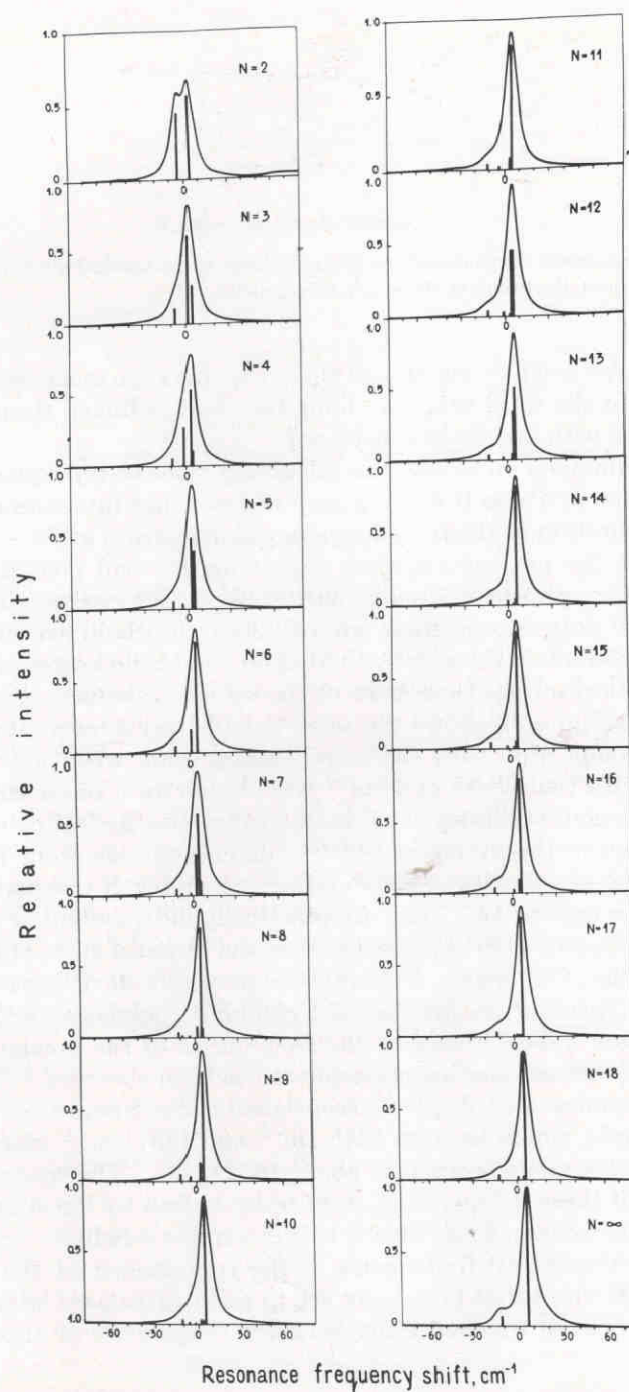


Fig. 6. The calculated infrared spectra of the amide-II vibration of the finite  $\alpha$ -helical fragments.  $N$  is the number of peptide groups.



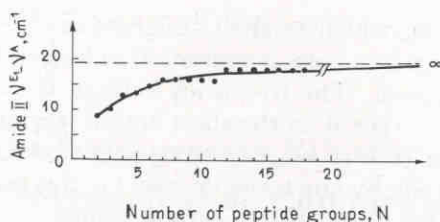


Fig. 7. Dependence of the resonance splitting of the  $E_1$  and A components of the amide-II band in the infrared spectra on the length of the  $\alpha$ -helical fragments.

The calculated splitting  $\nu^{E_1} - \nu^A$  for the amide-II transition is equal to  $19 \text{ cm}^{-1}$ , while the experimental value is near  $31 \text{ cm}^{-1}$ . The difference is evidently due to the vibrational interaction of adjacent peptide groups along the chain through the valence force field.

### Finite Fragments of the $\alpha$ -Helix

For the amide-I vibration, the theoretical infrared spectra of finite fragments are rather complex, if the number  $N$  of peptide groups in an  $\alpha$ -helix is small ( $N \leq 11$ ). With an increase in the number of groups in a helix, the amide-I spectrum appears as one narrow band. The difference between the spectra of finite helices and the spectrum of an infinite helix consists in a deletion of frequency degeneration for the transition  $E_1$ . Instead of one, two separate transitions  $E_1'$  and  $E_1''$  appear. In addition, degenerate vibrations of E types of a higher order become active. The main symmetrical transition A is the most intense (except for  $N = 5$ ), and it determines the shape of the infrared spectrum (Fig. 4). Within the first three turns of the helix, the amide-I band includes several transitions with a distribution of peak frequency shifts in the interval of  $-20$  up to  $+20 \text{ cm}^{-1}$ . With an increase in the length of the helix, this interval decreases to several  $\text{cm}^{-1}$ , and the maximum of the band shifts gradually towards negative values up to the value of  $-10 \text{ cm}^{-1}$  for the infinite helix. The dependence of the frequency shift of the main vibration of A type on the helix length is the same (Fig. 5). This must be reflected in the infrared spectra of polypeptides (the infinite helix) and globular proteins (finite helices). It is interesting to note the jump-like decrease in the frequency of this vibration (and, consequently, the frequency of the amide-I main maximum) with the start of each new turn of the helix, i.e., after  $N = 5, 8, 11$  and  $14$ .

In the case of the amide-II vibration, the infrared spectrum characteristic of the infinite  $\alpha$ -helix appears, beginning at about  $N = 6$  though the frequency splitting between the low-frequency transition A and the intensive main band of the E-type transitions is still moderate (Fig. 6). With an increase in the length of the helix, the shape of the spectrum changes gradually, and the frequency splitting increases. The depen-



dence of this splitting, which we shall designate as  $\nu^{E_1} - \nu^A$ , on the number of peptide groups in a helix is presented in Figure 7. It can be used for analytical purposes. The frequency shift of the main-band maximum (about  $+5 \text{ cm}^{-1}$ ) for all of the short helical fragments remains constant from  $N = 3$  up to  $N = \infty$ . Beginning with  $N = 8$ , the main band is determined practically by one transition  $E_1$ , i.e. this band must show no broadening due to the finite chain length of helices.

### CONCLUSION

In the approximation of transition dipole interactions, all the principle regularities of infrared spectra of the amide-I and II vibrations of infinite  $\alpha$ -helices can be well explained. The shape of the spectrum, which is characteristic for the infinite helix, in the case of finite  $\alpha$ -helices appears for the amide-I band beginning with approximately ten residues in a helix (about three complete turns of the helix) and for the amide-II band approximately with six residues (about 1.5 turns of the helix). The parameters sensitive to the length of the helix are the frequency of the main intensity maximum for the amide-I band and the frequency splitting between the two main transitions of the  $E_1$  and A type for the amide-II band.

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