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Intensities and Other Spectral Parameters of Infrared Amide Bands of Polypeptides in the α-Helical Form

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Synopsis

Intensities and other spectral parameters of infrared amide I and II bands of α-helical polypeptides in solutions have been determined for poly(γ-benzylglutamate), poly(γ-ethylglutamate), and polymethionine in chloroform, polylysine, poly(glutamic acid), and fibrillar protein tropomyosin from rabbit muscles in heavy water. The majority of spectral parameters are characteristic. The half-width of the amide I band was found to vary in the range of 15–40 cm^{-1} for different polypeptides in the different solutions. The correlation between this parameter of the amide I band and the stability of the α-helix was estimated. A new weak band near 1537 cm^{-1} of unknown origin was observed for the hydrogen form of polypeptides in the α-helical state.

INTRODUCTION

Infrared spectroscopy in the range of amide vibrations has been widely used for a qualitative evaluation of the character of the chain secondary structure in polypeptides and proteins. A theoretical elaboration of the nature of frequency splitting of amide bands[1–4] and the experimental determination of their intensities[5–7] made it possible to use infrared spectroscopy for a quantitative analysis. Determination of intensities and other spectral parameters of amide bands of polypeptides in the β form was performed by us in a previous communication.[6] The corresponding measurements for the α-helical form were done only for poly(γ-benzylglutamate) in some organic solvents.[5] This paper presents measurements of spectra of a number of α-helical polypeptides in organic solvents and heavy water.

EXPERIMENTAL

Poly(γ-benzylglutamate)* produced by Ferrac and poly(γ-ethylglutamate) produced by Courtaulds had a molecular weight of about 100,000. High-molecular-weight polymethionine, Pilot Chemical Company, Lot PC-M-1 had $\eta/c = 0.42$ in dichloroacetic acid at 25°C.

* All the studied polypeptides were in the L-form.

Poly(glutamic acid) (PGA) produced by Schüchardt had a molecular weight in the range of 40,000–100,000. The sample was converted to the sodium salt to study it in aqueous solution. The molecular weight of polylysine hydrobromide (Koch-Light Lab. Ltd.) was not lower than 50,000. We used this sample in the hydrochloride form.[8] Before preparation of the solutions, these polypeptides were dried under vacuum at 60°C for two days.

Tropomyosin was extracted from rabbit muscles by Bailey's method.[9]

The concentrations of water-soluble polypeptides and tropomyosin in D_2O were determined by the micro-Kjeldahl method with an accuracy of about 2–3%. The range of concentrations for the studied samples was 0.2–0.5%. For all other solutions the concentrations were determined after solution preparation by weighing the dry substance obtained from a 1–2-ml solution. The polypeptides in chloroform were centrifuged at 15,000 rpm at −10°C for 30 min. The PGA solution in D_2O–dioxane was also centrifuged at the same speed at +5°C for 30 min. To eliminate aggregation of the molecules in organic solvents, we used a low-solution concentration (0.08–0.3%); the relative error of the concentration measured was about 2%. The measurements of polypeptides in chloroform were performed at room temperature, the cells with water-soluble polypeptides were thermostated at a temperature of 22° ± 0.5°C.

The deuterated reagents were produced by Isotope (U.S.S.R.) and had a purity of 99.8% for D_2O and 99% for NaOD and DCl. Stabilized chloroform with 0.75% ethanol was purified by usual fractionation.

Infrared spectra in the region of 1400–1800 cm^{-1} were measured by the Perkin-Elmer 180 spectrophotometer. The spectral slit width was 1.5 cm^{-1} for narrow bands and 3 cm^{-1} for measurements in heavy water. We used an extended fivefold optical density scale, thus ensuring a measurement range of 0.3 optical density units. The photometric linearity was checked with rotating calibrated discs and was better than ±1%.

The cell thickness was 80 and 120 μm for measurements in water and 240 and 400 μm for measurements in chloroform. The technique of quantitative measurements of the absorption band intensities is described in Ref. 6.

The obtained results were averaged on six to eight different measurements. Determination accuracy for the frequency of the band maximum was ±1 cm^{-1}, ±0.5 cm^{-1} for the half-widths of narrow bands, and ±1 cm^{-1} for the broad bands. The mean-root-square error of measuring the intensity of the main strong bands was 6–8%. It includes the ambiguity in determination of the contour-shape parameter f_G, which was ±0.1. For some weak bands this parameter was chosen depending on the half-width.[6] Resolution of the complex spectrum into separate components was performed with a specially designed 5-channel spectral curve resolver[10] similar to the Curve Resolver 310 produced by the Du Pont Company. In contrast to the latter, our curve resolver not only allows one to change continuously the position, half-width, and the band contour maximum

value, but also to change discretely its Lorentz shape to the Gaussian one with steps of $f_G = 0.1$. The approximation of separate contours by our analyzer is 1% from the maximum amplitude.

In all the cases the α-helical content was checked either by the b_0 parameter of the optical rotatory dispersion or by circular dichroism with the JASCO ORD-UV-CD-5 instrument.

RESULTS

Poly(γ-Benzylglutamate)

High-molecular-weight poly(γ-benzylglutamate) is practically 100% α-helical in organic solvents of chloroform and dichloroethane. Figure 1 represents the molar absorption coefficient of one of the samples in chloroform. Three strong bands of about 1550, 1651, and 1732 cm^{-1} refer to the amide II and amide I vibrations and to the stretching vibration of the CO ester group. The amide II vibration is displayed by two components close to 1517 and 1550 cm^{-1} corresponding to the vibrations of the A and E_1 symmetry types of the helical molecule. The corresponding components of the amide I vibration have frequencies of about 1650 and 1652 cm^{-1} and can be distinguished only when measuring the spectra of oriented samples in polarized light.[1] As these components have very close frequencies, the amide I vibration in this and in all the other considered cases manifests itself as one practically symmetrical band. This approximation of the band is quite satisfactory. The measured intensities and other spectral parameters of amide bands are given in Table I. The

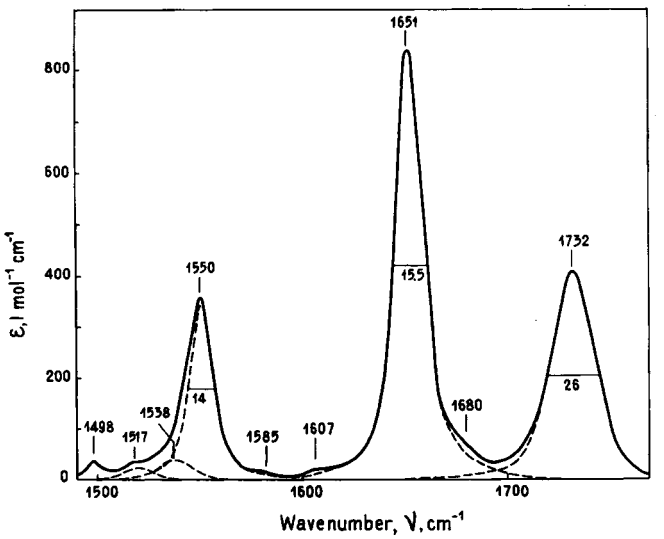

Fig. 1. Spectrum of poly(γ-benzylglutamate) in chloroform in the α-helical state. Concentration 2 mg/ml, $b_0 = -640$ deg $cm^2 dmol^{-1}$.

TABLE I
Spectral Parameters of the Amide I and II Bands of α-Helical Polypeptides in the Hydrogen Form

Vibration	Maximum Frequency ν (cm^{-1})	Half-Width of Band $\Delta\nu_{1/2}$ (cm^{-1})	Absorption at Maximum ϵ_0 (l. mol^{-1} cm^{-1})	Intensity $B \times 10^{-4}$ (l. mol^{-1} cm^{-2})	Contour Shape f_G
Poly(γ-Benzylglutamate) in Chloroform					
Amide I					
ν_A, ν_E	1651	15.5	840 ± 30	4.1 ± 0.2	0.4
Amide II					
ν_E	1550	14	310 ± 10	1.35 ± 0.1	0.4
ν_A	1517	14	~25	~0.1	0.4
Poly(γ-Ethylglutamate) in Chloroform					
Amide I					
ν_A, ν_E	1651	16	750 ± 40	4.0 ± 0.2	0.2
Amide II					
ν_E	1550	14	280 ± 10	1.2 ± 0.05	0.4
ν_A	1517	14	~30	~0.1	0.4
Polymethionine in Chloroform					
Amide I					
ν_A, ν_E	1651	16.5	765 ± 15	4.2 ± 0.2	0.2
Amide II					
ν_E	1548	15	290 ± 10	1.45 ± 0.1	0.4
ν_A	1517	15	~20	~0.1	0.4
Poly(Glutamic Acid) in Heavy Water–Dioxane, 1:1					
Amide I					
ν_A, ν_E	1651	16.5	820 ± 40	4.3 ± 0.2	0.4
Amide II					
ν_E	1551	14	280 ± 20	1.2 ± 0.1	0.4
ν_A	1517	15	~20	~0.1	0.4

intensity of the ester band is 2.7×10^4 l. mol^{-1} cm^{-2}. All these data agree with our earlier publication[5] within the limits of experimental errors.

The nature of the weak band with a maximum at about 1538 cm^{-1} and a half-width of 14 cm^{-1} remains obscure. In the polarized spectrum[1] this band is also displayed as an asymmetry of the main component of the amide II band at 1546 cm^{-1}.

Poly(γ-Ethylglutamate)

In its properties this polypeptide is very similar to poly(γ-benzylglutamate). For our sample in chloroform the b_0 parameter of the optical rotatory dispersion is −560 deg cm^2dmol^{-1}. The ir spectrum is presented in Figure 2 and differs from the preceding polypeptide spectrum by the absence of weak bands near 1498, 1585, 1607, and 1680 cm^{-1} stipulated by the stretching vibrations of the benzene ring. Spectral parameters of amide bands of poly(γ-ethylglutamate) are given in Table I; the intensity of the ester band is 2.8×10^4 l. mol^{-1} cm^{-2}.

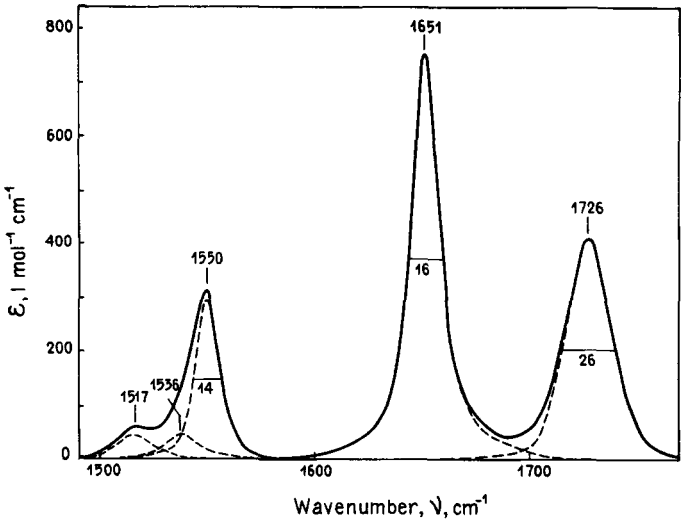

Fig. 2. Spectrum of poly(γ-ethylglutamate) in chloroform in the α-helical state. Concentration 1.4 mg/ml, $b_0 = -565$ deg cm^{2}dmol^{-1}.

Polymethionine

The measurements were performed at a concentration of 0.08–0.12% and showed that the parameter $b_0 = -600$ deg cm^{2}dmol^{-1}. This agrees with literature data.[11] Thus, our sample in chloroform practically has a completely helical form. In contrast to the spectra of the preceding polypeptides, all the strong bands in the polymethionine spectrum refer only to peptide group vibrations (Figure 3). As in the previous cases, how-

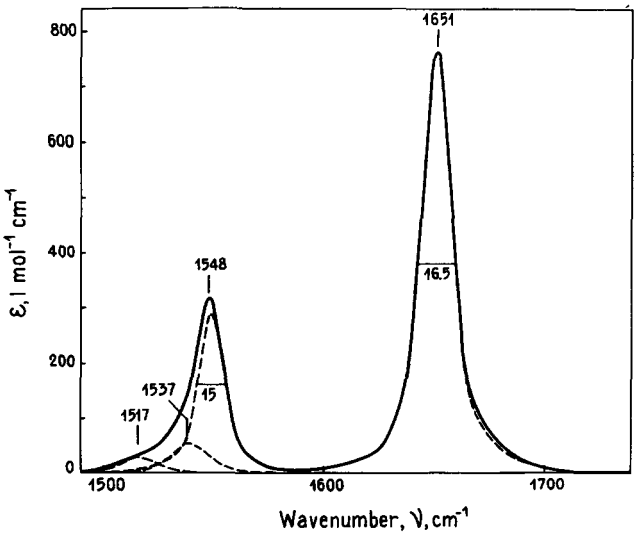

Fig. 3. Spectrum of polymethionine in chloroform in the α-helical state. Concentration 1 mg/ml, $b_0 = -600$ deg cm^{2}dmol^{-1}.

ever, the new band is observed at about 1537 cm^{-1}. It is probably one of the overtones of low-frequency amide vibrations.

Poly(Glutamic Acid)

Poly(glutamic acid) and polylysine are classic objects for studying the secondary structure of the polypeptide chain in aqueous solutions. The parameters of the amide I band for the random state of these polypeptides in heavy water were studied by us in Ref. 6. The degree of helicity for the samples studied was checked by circular dichroism[12] at 222 nm and by the b_0 parameter of rotatory dispersion.[13] In our case it was about 85–90%.

It was reported[14,15] that the α-helical form of poly(glutamic acid) and polylysine in water with organic solvents becomes more stable than in pure water. We studied the PGA in a 1:1 mixture of heavy water and dioxane and at pD 4.4–4.8. In these conditions the rate of hydrogen–deuterium exchange is very low.[16] Our samples with a concentration of 0.1–0.5% were practically unexchangeable during 5–7 days at room temperature. Thus, we can measure the hydrogen form of α-helix PGA (Figure 4). Spectral parameters of amide I and II bands are given in Table I. These parameters are exactly the same as those for other polypeptides in chloroform. This especially applies to very low values of half-widths of amide bands. In Figure 4 it can be seen that the new weak band at about 1537 cm^{-1} is also displayed. The side carboxyl groups are apparently displayed as two overlapping bands near 1714 and 1725 cm^{-1}.

We can obtain the completely deuterated form of the α-helix in heavy water–dioxane after heating the solution at 60°C for 12 hours. The parameters of the amide I bands are given in Table II. It was found that there was only a shift of the maximum position to 1641 cm^{-1}.

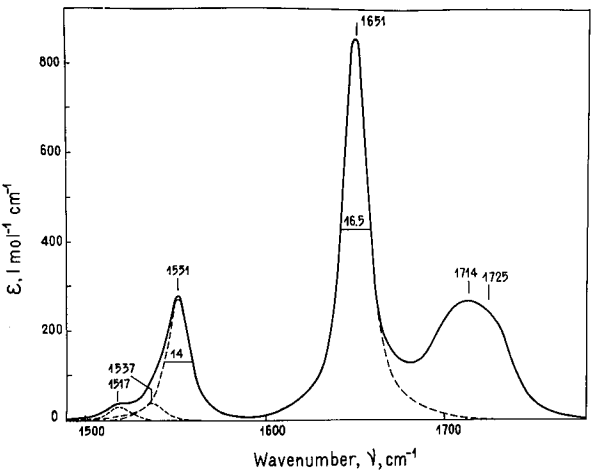

Fig. 4. Spectrum of poly(glutamic acid) in heavy water–dioxane mixture, 1:1, pD = 4.8, in the α-helical state. Concentration 3 mg/ml, temperature 22°C, $b_0 = -635$ deg $cm^2 dmol^{-1}$. The spectrum shows an unexchangeable hydrogen form of molecules.

TABLE II
Spectral Parameters of the Amide I Band of α-Helical Polypeptides in the Deuterated Form

Vibration	Maximum Frequency ν (cm^{-1})	Half-Width of Band $\Delta\nu_{1/2}$ (cm^{-1})	Absorption at Maximum ϵ_0 (l. mol^{-1} cm^{-2})	Intensity $B \times 10^{-4}$ (l. mol^{-1} cm^{-2})	Contour Shape f_G
Poly(Glutamic Acid) in Heavy Water–Dioxane, 1:1					
Amide I					
ν_A, ν_E	1641	17	800 ± 40	4.3 ± 0.2	0.4
Poly(Glutamic Acid) in Heavy Water					
Amide I					
ν_A, ν_E	1637	34	485 ± 20	4.6 ± 0.2	0.7
Polylysine Deuterochloride in Heavy Water					
Amide I					
ν_A, ν_E	1633	38	450 ± 20	4.6 ± 0.2	0.8
Tropomyosin From Rabbit Muscle in Heavy Water					
Amide I					
ν_A, ν_E	1637	38	440 ± 20	4.5 ± 0.2	0.8

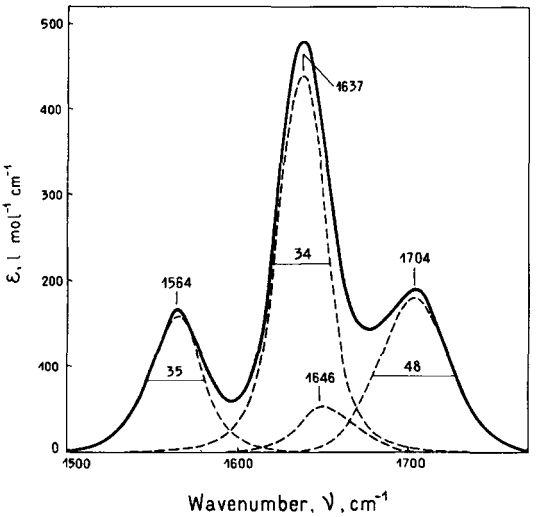

Fig. 5. Spectrum of poly(glutamic acid) mainly in the α-helical state in heavy water, pD 5.0, 0.2 *M* NaCl. Concentration 4 mg/ml, temperature 22°C; degree of helicity 85%, degree of ionization of side groups 23%.

The Na–PGA solutions in heavy water in an α-helix state were prepared by titrating with 0.1 *M* DCl to pD 5. Thus, we have the spectrum of a fully deuterated sample (Figure 5). We can see carboxyl group bands in the ionized form near 1564 cm^{-1} and in the nonionized form with the maximum near 1704 cm^{-1}. The degree of ionization of side groups was about 20% and was determined with a high accuracy by using the intensity of the fully ionized state.

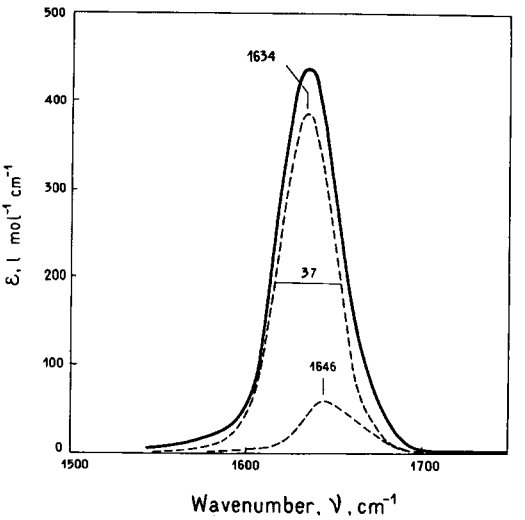

Fig. 6. Spectrum of polylysine deuterochloride mainly in the helical state in heavy water, pD 11.2, 0.2 *M* NaCl. Concentration 4 mg/ml, temperature 22°C, $b_0 = -520$ deg cm²dmol⁻¹.

The contour of the helical part of the amide I band can be obtained by subtracting the spectrum of the random form, the content of which is known. We assume that the sum of optical properties of different forms is additive. We used the contour parameters of the random part from Ref. 6. The possible errors in estimation of parameters of the amide I band in the α-helix must be negligible because the helical content is very high. The helical part of the amide I band is always sufficiently symmetrical and its half-width is about 34 cm^{-1} (Table II).

The half-width of the amide I band in this case is twice as high as that of the PGA amide I band in heavy water–dioxane, which is also in the deuterated form. We assume that the reason for this is the consequence of a different stability of polypeptides in different solvents.

Polylysine

In salt aqueous solutions this polypeptide undergoes coil–helix transition[17] near pD 11. We studied six newly prepared samples in 0.2 *M* NaCl at pD 10.8–11.9. The degree of helicity was 60–90%. Figure 6 shows the spectrum of one of the samples with a helical content of about 85%. The evaluation of the random form content was carried out as in the case of poly(glutamic acid). The half-width is very large and it varies from 37 to 40 cm^{-1} for different samples.

Tropomyosin

Tropomyosin is a fibrillar muscle protein with an irregular sequence of amino acid residues in the polypeptide chain. The best studied tropomyosin is from rabbit skeletal muscles. In 0.3 *M* KCl at neutral pH the pro-

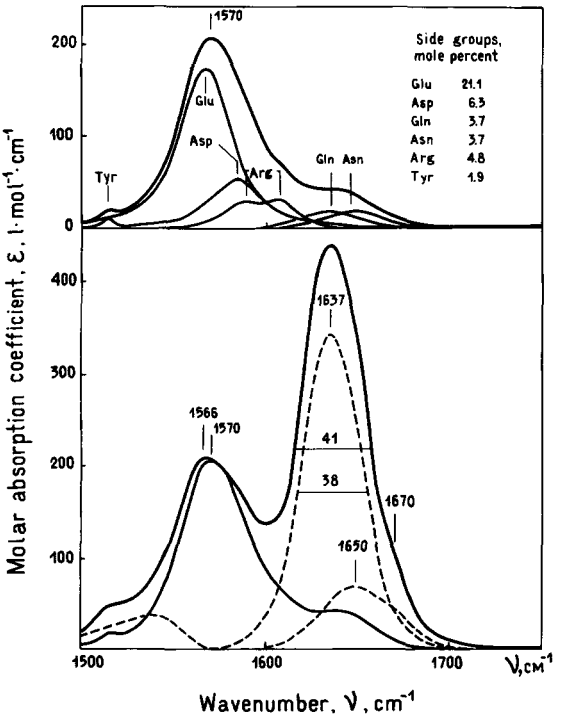

Fig. 7. Spectrum of tropomyosin from rabbit muscle in heavy water, pD 7.0, 0.1 *M* phosphate buffer, 0.3 *M* KCl. Concentration 3 mg/ml, $b_0 = -600$ deg cm^{2}dmol^{-1}. Upper part of the figure shows absorption of side groups on the basis of Ref. 19.

tein is practically in the helical state. Circular dichroism spectra of tropomyosin correspond to the spectra of α-helical polypeptides.[18] For our preparation $b_0 = -600$, $[\theta]_{222} = 37{,}000$ deg cm^{2}dmol^{-1}.

The tropomyosin spectrum in heavy water is shown in Figure 7. A sufficiently strong absorption of side residues is displayed in the spectrum alongside with the amide I band. This absorption is taken into account on the basis of data in Ref. 19. The total content of the amide residues asparaginyl and glutaminyl (about 7.4%) was taken from Ref. 20. The data on the content of other residues were taken from Ref. 21. A small side-chain absorption in the region of 1500–1550 cm^{-1} is evidently related to the unexchanged hydrogen part of peptide groups. The contour of the amide I band of the deuterated protein has a small shoulder on the high-frequency side of the spectrum. Assuming that the helical contour of the amide I band is symmetrical and the contour of the random part is close to that of silk fibroin,[6] we can resolve the complex contour of the tropomyosin amide I band. In this case the content of the random part will be about 15%. The spectral parameters of the amide I band recalculated per 100% of the α-helical structure and averaged by four measurements of newly prepared solutions are given in Table II. They agree with the spectral parameters for polypeptides.

DISCUSSION

In the hydrogen form we can study α-helical polypeptides in different solutions. The amide I band vibration of the α-helix has two components: the symmetrical type A and the doubly degenerated type E_1. The ratio of the intensities of these components polarized parallel and perpendicular to the helix axis was found to be approximately 5:1.[1] This corresponds to a 30° angle between the transition moment and the helix axis. The frequency splitting ν_E-ν_A is very small and is equal to about 2–4 cm^{-1}. Deuteration does not lead to any change in the value.[22,23] This is why the amide I band is well described by the individual symmetrical contour. The intensity and half-width of the amide I band of the α-helix are very close to those of the β form.

In contrast, the intensity of the amide II band of the α-helix is about two times less that of the β form. The reason for this is the corresponding decrease of the half-width. We can see that α-helix splitting ν_E-ν_A of the amide II band vibration is very constant and is about 31–34 cm^{-1}. In all the polypeptides studied we observe the new weak band near 1537 cm^{-1} with $\Delta\nu_{1/2}$ = 14 cm^{-1}, ϵ_C = 40 l. mol^{-1} cm^{-1}, and $B = 0.2 \times 10^4$ l. mol^{-1} cm^{-2}. We assume that the band arises from one of the α-helix vibrations in line with the observation in Ref. 24 that it may be an overtone of the amide V band.

The amide I band of the α-helix was examined in a very wide range of conditions. In contrast with the β form, we found that its half-width can vary from 15 to 40 cm^{-1}. This very surprising result may be understood after comparing it with the data available on the stability of the α-helix

TABLE III
Correlation Between the Half-Width of the Infrared Amide I Band and the Stability of the α-Helix in Solution

Sample and Solution	Half-Width of Amide I $\Delta\nu_{1/2}$ (cm^{-1})	$-\Delta F_0$ (cal mol^{-1})	Ref. for ΔF_0
Polylysine			
D_2O, 0.2 *M* NaCl, pD 11	38	90	15
Poly(glutamic acid)			
D_2O, 0.2 *M* NaCl, pD 4.8	34	180	14
Poly(glutamic acid)			
D_2O:dioxane, 2:1, 0.2 *M* NaCl, pD 5.2	28	300	14
Poly(glutamic acid)			
D_2O:dioxane, 1:1, pD 4.8	17	>300	—
Polymethionine	16.5	>300	25
Poly(γ-ethylglutamate)	16		
Poly(γ-benzylglutamate)	15.5		
(All in chloroform)			

ΔF_0 = change of free energy of helix–coil transition per monomer unit. All measurements of ΔF_0 in water solutions were carried out for solvents with ordinary water.

in corresponding solutions (Table III). We also measured the half-width of the poly(glutamic acid) amide I band in a 2:1 heavy water–dioxane mixture. There is an obvious correlation between the half-width of the amide I band and the stability of the α-helical structure. It may be concluded that the regular space-expanded polypeptide chain of the α (and also the β) structure displays a very narrow infrared amide I band with a half-width of about 15 cm^{-1}. The broadening of the band by more than double cannot be explained by the direct influence of a solvent since the accessibility of solvent molecules to the peptide group at a distance smaller than 4 Å is impossible in the static α-helix structure. In this case the peptide groups are shielded by the side chains. The latter do not cause a special broadening of amide bands (Table I). The observed broadening of amide I bands of the α-helix seems to be connected with geometrical distortions of helical structure in water and other destabilizing solvents. Such a deformation of the peptide backbone really exists as a result of conformational mobility of the structure.

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