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Intramolecular Distortion of the α-Helical Structure of Polypeptides

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Broadening of the infrared amide A, amide I and amide II bands of α-helical polypeptides has been observed for thermodynamically unstable α-helices. This spectroscopic fact can be explained now by the geometrical distortions of the backbone of the helical structure. Two models for distorted helices which include regular or irregular distortions of the angles of internal rotation of the main polypeptide chain have been considered. It is pointed out that the instability of α-helix is associated with irregular distortions of the polypeptide backbone.

1. Introduction

From the time a model of the α-helix was proposed by Pauling & Corey (1951) this type of polypeptide structure has been studied intensively. α-Helices have been discovered in polypeptides, fibrous proteins, and, as short fragments, in globular proteins. The flexibility and distortion of the helix backbone are conditioned mainly by the internal rotation of peptide groups about single bonds. This explains the presence of a set of helical structures similar in parameters and energies (Fraser & MacRae, 1973).

It is known that the half-widths of infrared amide bands depend strongly on the degree of order of the polypeptide structure (amide A band, Kobyakov, 1969; amide I and II bands, Chirgadze *et al.*, 1973). A study of the parameters of the amide I band of helical polypeptides in solution shows that a correlation exists between band half-width and stability of the α-helix, namely, the lower the stability, the larger the half-width (Chirgadze & Brazhnikov, 1974). The spectroscopic data allows to describe the helical state in terms of molecular geometry. It is possible to get such information on the basis of a general approach to the resonance coupling of transition moments (Chirgadze & Nevskaya, 1973). The experimental results in the present paper give the values for the half-widths of a few infrared amide bands for two extremely different helical states—stable and unstable—which are observed in polypeptides and apparently in some fibrous proteins. A theoretical consideration gives a description of the geometrical distortion of an unstable helical state in terms of the distribution of angles of internal rotation.

2. Materials and Methods

(a) *Materials*

Poly(glutamic acid) produced by Schüchardt had a molecular weight in the range of 40,000 to 100,000. The sample was studied also as the sodium salt. Before preparing the solutions this polypeptide was dried in a vacuum at 60°C for 2 days. Polylysine hydrobromide was obtained from Koch Light Lab. Ltd with a molecular weight of over 50,000. In this work we used it as the hydrochloride (Davidson & Fasman, 1967). Poly(γ-benzylglutamate) from Ferrac and poly(γ-ethylglutamate) from Courtaulds had molecular weights of about 100,000. The sample of polymethionine was used with a molecular weight of about 36,000, Lot PC-M-1, Pilot Chemical Co. Tropomyosin was isolated from rabbit muscle according to Bailey (1948). Silk fibroin from *Bombyx mori* was isolated from cocoons and prepared in an aqueous solution by the method of Iizuka & Yang (1968). Deuterated reagents from Isotop, U.S.S.R., had a purity of 99.8% for ^2H_2O and 99% for NaO^2H and ^2HCl. Stabilized chloroform with a 0·75% ethanol admixture and dioxane were purified by the usual fractionation procedure.

(b) *Methods*

(i) *Preparation of solutions*

The solutions of poly(glutamic acid) in water/dioxane mixtures were prepared by dissolving the samples at first in dioxane, and then water was added with pH (pD) about 7. Water solutions with NaPGA† and polylysine in an α-helical state for films were obtained by dialysis against water at the given pH to remove salts. The pH or pD was adjusted by 0·1 M-NaO^2H or ^2HCl. The solutions of PGA in water with dioxane were centrifuged at 15,000 revs/min at 5°C for 30 min. The chloroform solutions of polypeptides were centrifuged at the same speed at -10°C also for 30 min. The concentration of PGA was determined by the micro-method of Kjeldahl with an accuracy of about 3%. The infrared spectra of solutions were measured at a concentration in the range of 0·2 to 0·5%.

(ii) *Preparation of films*

Films were cast from solutions placed on horizontal calcium fluoride plates and evaporated. The PGA film was cast from the water/dioxane solution by drying over the same solvent saturated with $NaNO_2$, the other films were dried over silica gel. The plates with the films were then placed in an hermetic chamber and dried over P_2O_5 for about 2 h. The films of polypeptides and proteins were exposed in a heavy water atmosphere with a 65% relative humidity for 100 to 200 s to remove small interfering absorption bands of side groups and water (mainly in the region of the amide A band).

(iii) *Measurement of the infrared spectra*

All infrared spectra were recorded with the Perkin–Elmer 180 spectrophotometer. For film measurements the spectral resolution was about 1·5 cm^{-1} in the region of 1400 to 1800 cm^{-1}, and 3 cm^{-1} in the region of 2700 to 3700 cm^{-1}. For heavy water solutions the resolution used was about 3 cm^{-1}. If not mentioned specially the accuracy of measurement of the half-widths of the adsorption bands was about ± 1 cm^{-1} for narrow bands, and ± 3 cm^{-1} for broad bands. A 5 or 10-fold expanded scale of optical density was usually used to measure spectra of solutions. The cell thicknesses were 80 and 120 μm for water solutions, and 240 and 400 μm for chloroform solutions. The technique of intensity measurements, decomposition of overlapping bands and other spectroscopic details have been described in previous papers by Chirgadze *et al.* (1973) and Chirgadze & Brazhnikov (1974).

(iv) *Measurement of circular dichroism and optical rotatory dispersion*

The α-helix content was checked by circular dichroism and optical rotatory dispersion. Circular dichroism spectra were run on the JASCO ORD/UV/CD-5. The "b_0" parameter of optical rotatory dispersion was obtained with the Perkin–Elmer 141 M spectropolarimeter.

† Abbreviation used: PGA, poly(glutamic acid).

(v) *Calculation of amide I band contours*

It has been shown that splitting of amide vibrations in the peptide structure can be quantitatively explained by the theory of resonance interaction of transition moments in a dipole–dipole approximation (Chirgadze & Nevskaya, 1973). This method was applied to α-helical structures in the paper by Nevskaya & Chirgadze (1976). The transition moment of the amide I vibration was assumed to equal 0·30 Debyes and to be located in the plane of the peptide group at a point 0·4 Å away from the oxygen atom in the O → N direction and inclined at 17° to the C'O bond within the angle OC'C^{α}. The frequency of the unperturbed oscillator, ν_0, was assumed to be 1663 cm^{-1}. Every transition moment was allowed to interact with every other, independent of the distances between them. To compare more easily the theoretical and experimental results, each individual normal vibration was assumed to appear in the infrared spectra as a single band with a Lorentzian shape and a half-width of 9 cm^{-1} (Chirgadze *et al.*, 1973).

The geometry of the peptide group was taken from Corey & Pauling (1953), and the angle C'C^{α}N was assumed to be 110°. All our α-helical models have been generated by a variation of internal rotation angles ϕ and ψ using a method like the transformation procedure by Ooi *et al.* (1967). The helices with regular distortions were created by changing the angles of internal rotation in the region of the energy minimum on the conformational peptide map. A variation of the peptide hydrogen-bond length from 2·6 to 3·2 Å and the angle N—H . . . O from 0 to 30° was allowed. The helices with irregular distortions were self-generated by an arbitrary choice of internal rotation angles. The possibility of the choice of these angles, with a step of 5°, has been narrowed by the above mentioned limits of the hydrogen bonds and by the van der Waals' distances between all the contacting atoms. The distance values were taken to be 0·2 Å smaller than those reported by Ramachandran & Sasisekharan (1968). In order to restrict the helix bending its radius (C^{α}-atoms) was allowed to be between 2·20 and 2·45 Å.

3. Results

(a) *Infrared amide I band of α-helical poly(glutamic acid) in solution*

In aqueous solution at neutral or basic pH the PGA molecule is in a charged state. It has been pointed out that charged poly(glutamic acid) as well as other similar polypeptides like polylysine has a coiled but partially ordered form close to the extended helical conformation (Tiffany & Krimm, 1968). The parameter b_0 of optical rotatory dispersion of such solutions is about zero. The sharp transition from the coiled form to the α-helical state occurs in water solution, for example in water with 0·2 M-NaCl at pH 4·8 and pD 5·4 (Appel & Yang, 1965). A thermodynamically more stable α-helical form of PGA exists in water/dioxane solution (Bychkova *et al.*, 1971).

We carried out measurement of the half-width of the amide I band in heavy water solution. The conditions were chosen such that all labile hydrogen atoms of peptide and side groups would be replaced by deuterium. In this case the amide II band shifts to about 1450 cm^{-1} and is not observed in the region studied, while the amide I band shifts by only about −10 cm^{-1}. The band-width of amide I remains practically the same. The change of geometry of the α-helix on deuteration is negligible (Tomita *et al.*, 1962).

Figure 1 represents the spectra of poly(glutamic acid) in three states: the stable α-helix in a mixture of water/dioxane, pD 4·4; the unstable α-helix in water, 0·2 M-NaCl. pD 4·9; and the charged state in water, 0·2 M-NaCl, pD 7. The stable α-helix displays an amide I band at 1641 cm^{-1} with the half-width of its main component equal to about 18 cm^{-1}. This value is comparable with the value of 16·5 cm^{-1} for this polypeptide under the same conditions in the hydrogen form, as well as with values of about 16 cm^{-1} for poly(γ-benzylglutamate) and other polypeptides in dioxane and chloroform solutions (Chirgadze & Rashevskaya, 1969; Chirgadze &

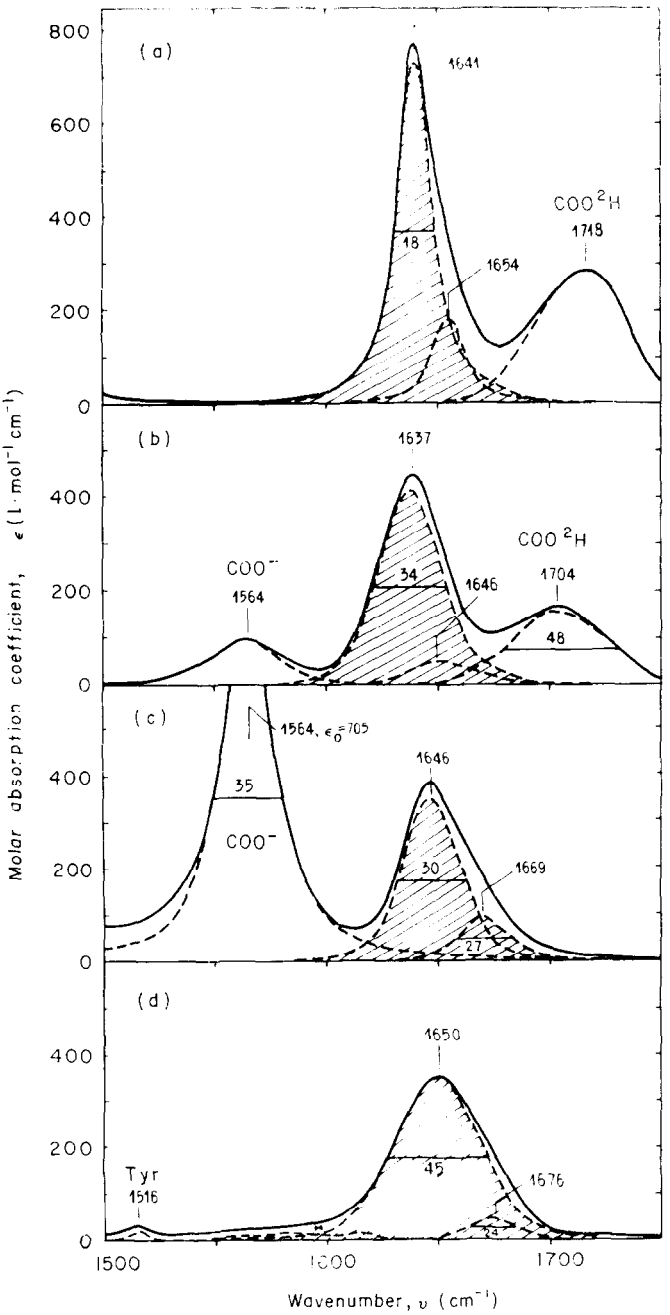

FIG. 1. Infrared spectra of deuterated poly(glutamic acid) and silk fibroin from *B. mori* in solution. Concentrations, 0·2 to 0·5%. Amide I band contours are hatched.

(a) Stable α-helix, PGA in a 1:1 mixture of heavy water/dioxane, pD 4·4; $b_0 = -640$.

(b) Unstable α-helix, PGA in ^2H_2O, 0·2 M-NaCl, pD 4·9; $b_0 = -620$.

(c) Charged state, NaPGA in ^2H_2O, 0·2 M-NaCl, pD 7; $b_0 = +20$.

(d) Random coil, silk fibroin in ^2H_2O, pD 7.

Brazhnikov, 1974). In aqueous solution when the α-helix of poly(glutamic acid) has a lower stability, the maximum of the amide I band shifts to 1637 cm^{-1} and its half-width increases to 34 cm^{-1}. In all these states the α-helix content was about 80 to 90% as shown by b_0 and $[\theta]_{222}$. For example, for PGA these values were equal to −640 and −41,000 deg cm^2 $dmol^{-1}$ for water/dioxane solutions, and about −620 and −38,000 for water solutions. The small portion of the unordered charged state of the amide I band near 1646 cm^{-1} was subtracted according to Chirgadze & Brazhnikov (1974). Thus we see that the broad amide I band appears for the unstable α-helical structure.

It would be interesting to compare the spectra of α-helices with those of the unordered form. The unstable α-helix of NaPGA is destroyed when the polypeptide passes to the charged state. The main component of the amide I band has a frequency near 1646 cm^{-1} and a half-width of about 30 cm^{-1}. Unfortunately this state is not a good model of the unordered form. As an example of such a model silk fibroin from *B. mori* is preferable. We have taken the spectrum of silk fibroin from our previous paper (Chirgadze *et al.*, 1973). The half-width of the amide I band of this sample is about 45 cm^{-1} (see Fig. 1(d)). We can conclude now that the stable α-helix of PGA has an amide I band half-width of about 18 cm^{-1}, and the unstable one 34 cm^{-1}, which is closer to the value of 45 cm^{-1} for the random coil state of silk fibroin. It should be mentioned here that the α-helix of PGA with an intermediate stability in water/dioxane (2:1), 0·2 M-NaCl, pD 5·2, has an intermediate value of the amide I band half-width equal to 28 cm^{-1} (Chirgadze & Brazhnikov, 1974).

(b) *Infrared amide A, amide I and amide II bands of some polypeptides in α-helical states*

In addition to the amide I band other amide bands are of considerable interest. In general the amide A and amide II must display the same features as the amide I band. The broadening of the amide A band also provides information on the distribution of peptide hydrogen bonds as the frequency of NH stretching vibration depends greatly on the length of the hydrogen bond (Nakamoto *et al.*, 1955; Pimentel & Sederholm, 1956). These bands also are important for studies of deuteroexchange in proteins by infrared spectroscopy (Blout *et al.*, 1961). Since measurements in ordinary water solution are difficult, we studied infrared spectra of samples in the solid state.

Figure 2 shows spectra of films taken from solutions of poly(glutamic acid) and silk fibroin in the same conditions as indicated in Figure 1. The PGA film taken from a water/dioxane solution displays a spectrum with very narrow amide bands as would be expected for a stable α-helix. The half-widths of the amide A, amide I and amide II bands are equal to 60, 18 and 17 cm^{-1}; they are very close to values of 62 16·5 and 14 cm^{-1} in solution. The latter measurements were carried out for PGA, in a hydrogen form in a solution of water/dioxane using the fact that deuteroexchange in the stable α-helix proceeds very slowly with a time of half-exchange of over 30 days; see also Chirgadze & Brazhnikov (1974). These observations imply that an interaction of α-helices side by side does not have any considerable influence on the width of amide bands.

The unstable PGA α-helix in water at pD 4·9 exchanges hydrogen atoms for deuterium quickly, with a time of half-exchange of about an hour (Welch & Fasman, 1974) and thus we could study only solid state films (Fig. 2(b)). To prepare these films

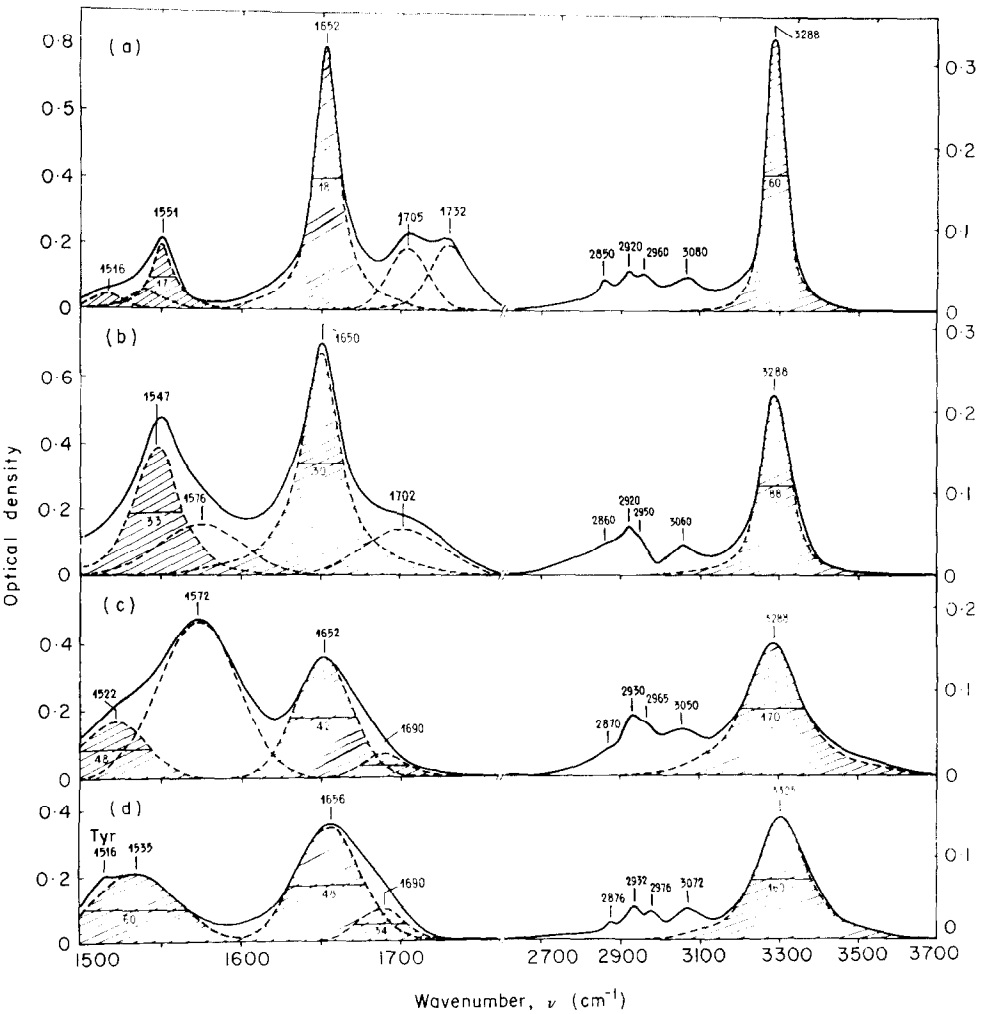

FIG. 2. Infrared spectra of poly(glutamic acid) and silk fibroin from *B. mori* in the solid state. All films were deuterated for about 100 s at a 65% relative humidity and then placed in a dry atmosphere over P_2O_5. Amide band contours are hatched.

(a) Stable α-helix, PGA, film cast from solution in a 1:1 mixture of water/dioxane, pH 4·4.

(b) Unstable α-helix, PGA, film cast from water solution, pH 5·6.

(c) Charged state (close to random coil), NaPGA, film cast from water solution, pH 7.

(d) Random coil, silk fibroin, film cast from water solution, pH 7.

we used a solution with pH 5·6, as the mid-point of helix-coil transition in salt-free solutions of ordinary water shifts to higher pH values (Iizuka & Yang, 1965). The half-widths of the amide bands increase and become equal to 88, 30 and 33 cm^{-1}. The half-width of the amide II band in this spectrum (the same should be noted for the next spectrum in Figure 2(c)) has been obtained with less accuracy (about ± 5 cm^{-1}) due to overlapping by the strong carboxyl band near 1575 cm^{-1}. For this α-helical state the broad amide bands have also been observed by Lenormant *et al.* (1958), Kobyakov (1969) and Bohm *et al.* (1974). A greater broadening of amide bands was observed for the random coil of silk fibroin and the unordered form of NaPGA (Fig. 2). An increase in the half-width of the amide I band of NaPGA in the solid

state of up to 42 cm^{-1} has been assigned by us to the transformation of the extended helical form to the true random coil state.

We also observed the different types of α-helical structures in a number of other polypeptides and some fibrous proteins (Table 1). Stable α-helices were observed for poly(γ-ethylglutamate), poly(γ-benzylglutamate) and polymethionine in chloroform or in the solid state. Unstable α-helices were observed also for polylysine and apparently for rabbit muscle tropomyosin in a water solution or in the solid state. The half-widths of the amide I and II bands for the unstable α-helix in all cases are about twice as large as for the stable helix, being close to values for the random coil. The half-width of the amide A band for the unstable helix is about 1·5 times larger than for the stable α-helix, and still approximately twice as small as for the random form. This important fact testifies to a relatively narrow variation in the parameters of peptide hydrogen bonds as compared with the random coil state.

TABLE 1

Half-widths of amide bands of polypeptides and some proteins in the α-helical and random coil states

Compound	Band half-width (cm^{-1}) Amide A	Amide I	Amide II
Stable α-helix, solutions (see text)			
Poly(γ-ethylglutamate), chloroform	56	16	14
Poly(γ-benzylglutamate), chloroform	56	15·5	14
Polymethionine, chloroform	59	16·5	15
Poly(glutamic acid), water/dioxane (1:1), pD 4·4	62	16·5	14
Unstable α-helix, films			
Poly(glutamic acid)	88	30	33
Polylysine	90	33	32
Tropomyosin from rabbit muscle	90	30	32
Random coil state, films			
Poly(glutamic acid)	170	42	48
Polylysine	155	38	50
Silk fibroin of *B. mori*	160	48	60

1. The optical activity parameters of solutions are given in Chirgadze & Brazhnikov (1974).
2. Films of polylysine were cast: for the distorted α-helix from water solution (pH 9·7) and for the random coil from pH 4·4. The film of tropomyosin was cast from pH 7 water solution. All the films were deuterated for about 100 s at a 65% relative humidity. Due to overlapping bands in these samples the accuracy of amide II half-width was ±5 cm^{-1}.
3. When amide components overlapped, the half-width of the main one is given.

(c) *Interpretation of the half-width of amide bands and the models of the distorted α-helices*

The amide bands arise from vibrations which are localized within the peptide group (Miyazawa *et al.*, 1958; Chirgadze, 1962). In compounds with one peptide group in dilute non-polar solution the half-width of the amide I band is about 15 cm^{-1}. In such circumstances there is a lack of significant interactions between peptide groups

or with the molecules of the solvent. For example, for dilute solutions in carbon tetrachloride we found this value equal to 18 cm^{-1} for *N*-methylacetamide and 11 cm^{-1} for dimethylformamide. The amide band half-width increases with an increase of the perturbing effect of the molecular environment, for example, with increase of the dipole moments of the solvent molecules. In polypeptides and proteins the local medium around a given peptide group consists entirely of neighbouring peptide groups and side chain groups. In the case of the α-helix, the solvent effect on the spectral parameters of amide bands can be counted as negligible since the molecules of the solvent cannot approach within a distance less than 4 Å of the peptide groups. Neither does the nature of the side groups exert any effect on the band half-widths of α-helices as is seen from the data of Table 1. This fact means that the broadening of amide bands is connected with a property of the main chain and apparently does not depend on the amino acid sequence.

The amide band half-widths for the random coil state are observed to be about two or three times larger than for regular polypeptide structures such as the stable α-helix or the β-sheet. Since the amide band half-widths of the unstable α-helix have intermediate values compared with those for the stable α-helix and random coil, it can be proposed that the broadening of amide bands of the unstable α-helix is evoked by some disorientation of peptide groups and the spread of the parameters of hydrogen bonds. More detailed information on the possible distortions in this α-helical state can be obtained by a comparison of experimental and calculated values of amide band half-widths for a number of distorted helical models.

Regular α-helical structures are characterized by constant values of the ϕ and ψ angles and, consequently, by the constant parameters of the hydrogen bond. Figure 3 gives a schematic representation of hydrogen bonds in an ideal α-helix (α_0), in an α-helix with regular uniform distortions (α_u), and in an α-helix with irregular distortions (α_s). A theoretical calculation of amide I and II band contours carried out for

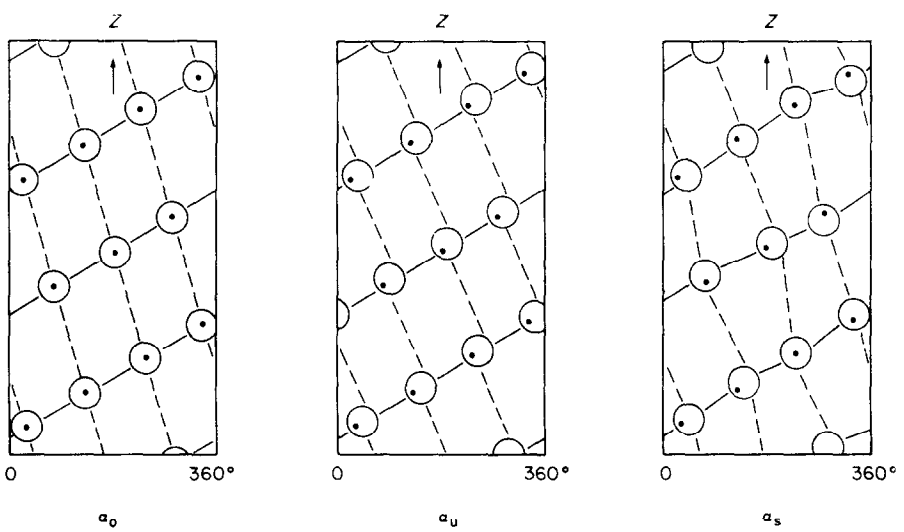

FIG. 3. Schematic representation of distortions of the α-helical structure in a radial projection. Peptide groups are shown by circles and hydrogen bonds by broken lines.
α_0, a Pauling & Corey "ideal" α-helix;
α_u, the α-helix with regular distortions;
α_s, the α-helix with irregular distortions.

regular α-helices gives contour half-widths of 11 and 9 cm^{-1}, respectively (Nevskaya & Chirgadze, 1976), which are close to the observed values in stable α-helices. We have found that a change of the ϕ and ψ angles leads only to a shift of the maximum frequency within the limits of a few cm^{-1} while the half-width of the contour does not change. As an example Figure 4(a) shows an amide I band spectrum for the Pauling & Corey (1951) infinite "ideal" α-helix with angles $\phi = -57°$ and $\psi = -47°$. In α-helical structures a disruption of the regularity of the ϕ and ψ angles is possible (Skvortsov *et al.*, 1971). Consider two models of distorted helical structures related to distortions of either α_u or α_s types.

The first model represents a set of regular α-helices differing from each other by their ϕ and ψ angle values. A calculation was made of the sum of 13 α_u-helices with 39 groups in each with angles ϕ and ψ satisfying conditions imposed on the hydrogen-bond parameters. The chosen angle values are indicated in the upper Figure 4(b). To form a set of these helices we took into account the probabilities of angles ϕ and ψ in the helical region of map. These were obtained from their energy values on the peptide map and the contributions of given pairs of angles were designated by the

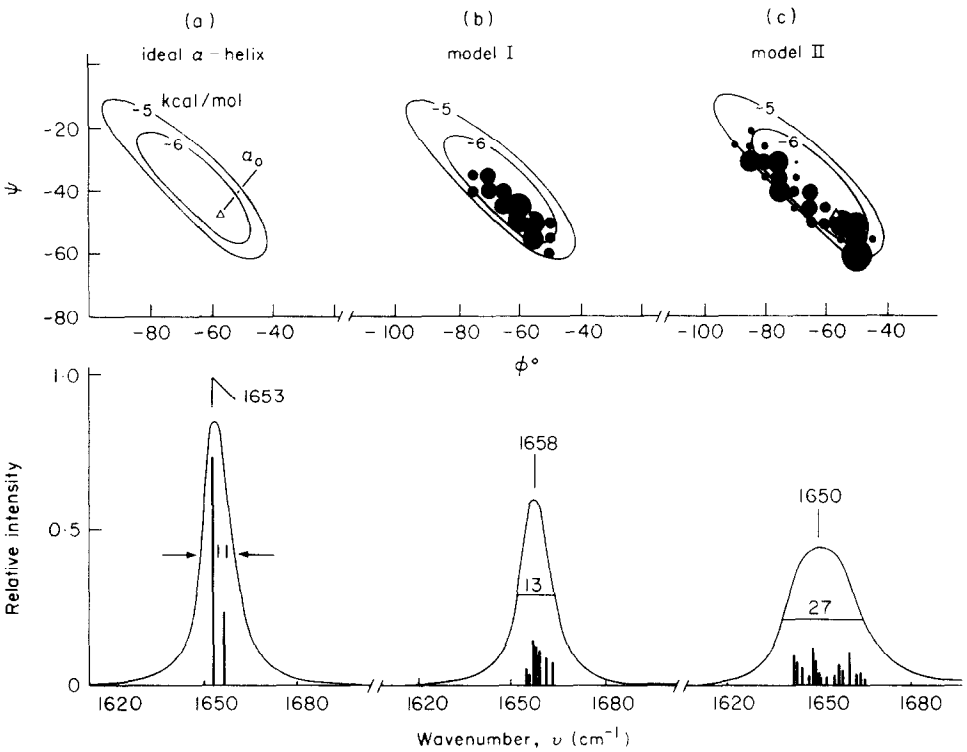

FIG. 4. Lower Fig.: calculated contours of the infrared amide I band for ideal and distorted α-helices. Active infrared spectra modes are represented by vertical lines. The intensity of the single unperturbed oscillator was assumed to be unity. Other details are given in the text. Upper Fig.: energy permitted regions of angles of internal rotation are shown. The size of the circles corresponds to the share of definite α-helices in the common helical set (b) and to the occurrence frequency of given angles ϕ and ψ in the distorted α-helix (c).

(a) Pauling & Corey ideal α-helix.

(b) Model I of distorted α-helices, set of helices of the α_u-type with regular distortions in each.

(c) Model II of distorted α-helices, set of helices of the α_s-type with irregular distortions in each.

sizes of the corresponding circles. In the case of this model the half-width of the calculated amide I contour is equal to about 13 cm^{-1}.

In *the second model* ϕ and ψ angles were varied within one α_s-helix. The co-ordinates of each successive peptide group were determined by a pair of angles such that the hydrogen bond parameters and the van der Waals' distances between the atoms would be within definite limits (see Materials and Methods, section (b)). The contour of the amide I band for the set of four such helices each consisting of 25 groups is given in the lower Figure 4(c). The positions of circles on the map correspond to the chosen values of the ϕ and ψ angles and the dimensions of the circles indicate the number of occurrences of the given pair of angles. In the case of this model the amide I band broadens by more than a factor of two. The same result of band broadening has been also obtained for the amide II vibration, the half-width of which increases more than 1·5 times. Hence, only this model of the distorted helix allows one to explain the amide band broadening for the unstable α-helix.

4. Discussion

We can conclude that the half-widths of the main infrared amide bands seem to be directly connected with the degree of geometric distortion of the helical structure. Thus, for the two different states of the α-helix, stable and unstable, the experimentally observed optical, hydrogen exchange kinetic and thermodynamic properties can

TABLE 2

Properties of the α-helix in the ordered and distorted states

Property	Ordered α_0-helix	Distorted α_s-helix	Reference
Geometry of the molecule†			
Residue projection on the helix axis, Å	1·50	1·45 ± 0·09	Pauling
Helix radius (C^α atom), Å	2·28	2·32 ± 0·07	&
Hydrogen bond length, Å	2·89	2·93 ± 0·19	Corey
NH···O angle, deg.	13·3	19·5 ± 7·7	(1951)
Angles of internal rotation, deg.			
ϕ(N—C^α)	−57	−65 ± 13	this
ψ(C^α—C′)	−47	44 ± 11	paper
Optical properties			
Half-width of infrared amide bands, cm^{-1}††			
amide A	58	89	this
amide I	16	31	paper
amide II	14	32	
Kinetic properties			
Half-time of hydrogen–deuterium exchange, h	>1000	about 1	Welch & Fasman (1974); this paper
Thermodynamic properties			
Free energy of helix–coil transition:			Birshtein & Ptitsyn (1969); Bychkova *et al.* (1971)
$-\Delta F$, cal mol^{-1}	>300	about 100	

† For a distorted helix the mean parameters and their root-mean-deviations are given.

†† Averaged data of Table 1.

be compared with the geometric properties of ordered and distorted helical states (Table 2). The rate of hydrogen–deuterium exchange for poly(glutamic acid) is several orders higher in the distorted α-helix compared with the ordered helical structure. This means that the mobility of the distorted helix is considerably higher. It is known that the mobility of peptide structures is increased in the presence of water molecules (Chirgadze & Ovsepyan, 1972). On the contrary, a hydrophobic medium decreases the mobility and a more compact packing is more often attained in this medium. We could observe the ordered helical structure only in hydrophobic solvents. Certainly, this does not mean that hydration leads always to emergence of the ordered α-helix.

The observed order–disorder transition in the α-helix of polypeptides is very similar to the λ-transition in molecular crystals. The difference is that the first occurs inside one polymer molecule.

The real distortions in the long α-helix seem to be given by the model ($\alpha_s + \alpha_u$) which includes mainly irregular as well as partially regular distortions, i.e. in one part of the α-helix distortion of the α_s-type occurs, while in some other region the α_u-type exists. The same situation seems to occur in the short α-helical fragments of molecules of globular proteins. Indeed an extensive set of helical amide A band half-widths from 80 to 120 cm^{-1} has been observed in the infrared spectrum of sperm whale myoglobin (Chirgadze, 1972). Finally, it should be noted that the root-mean-square deviations of the shift of atomic co-ordinates in the distorted α-helix in respect to the ideal helix are evaluated as 0·73, 0·34, 0·44 and 0·60 Å for atoms C^{α}, C′, N and O, correspondingly. Since such values are usually close to the limit of accuracy of X-ray diffraction analysis of globular proteins the data obtained by this method cannot in practice be used to estimate the intramolecular distortion of α-helical structures inside these molecules.

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