An Infrared Study of Unordered Poly-L-Proline in CaCl$_2$ Solutions

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Synopsis

Infrared spectra have been obtained of poly-L-proline in aqueous CaCl$_2$ solutions. As the salt concentration is increased, the C=O stretching band develops a component at the frequency found in the solid state while the CH$_2$ bending band broadens to higher frequency. Since circular dichroism spectra indicate progressive disordering of the chain with increasing salt concentration, we associate the infrared spectral changes with the same phenomenon. Our interpretation of these changes, particularly in the CH$_2$ bending modes, is that disordering is associated primarily with an increase in the range of accessible C$^\alpha$—C' (=O) rotation angles rather than with the random introduction of *cis* imide bonds in the chain.

INTRODUCTION

Poly-L-proline (PP) is known to occur in two ordered helical conformations: form I, a right-handed helix of *cis* imide bonds with 10 residues in 3 turns and a residue repeat of 1.9 Å, and form II, a left-handed helix of *trans* imide bonds with 3 residues per turn and a residue repeat of 3.1 Å.[1] Form I is only slightly soluble in salt-free water. Form II is soluble at low temperatures (0–5°C), but precipitates when the temperature is raised to 67°C (or somewhat lower if the temperature is raised slowly enough).[2] While PP I in water transforms in time to PP II, in organic solvents a II → I transformation can be induced.[3]

Early studies of the properties of PP in aqueous salt solutions[4,5] suggested that the chain conformation is collapsed under these conditions. A circular dichroism study of PP in concentrated CaCl$_2$ solutions by Tiffany and Krimm[6] showed that the spectrum differed from those of forms I and II, and in fact corresponded to the circular dichroism spectra of polypeptides in unordered conformations.[7] This collapse in structure was subsequently supported by intrinsic viscosity studies of PP in CaCl$_2$ solutions.[8]

The mechanism of such disordering of chain conformation in salt solutions is of considerable interest since it can reveal the nature of the interaction of salts with polypeptides and proteins. Disordering in PP can occur only via a *cis–trans* isomerization, an increased freedom of rotation about the C$^\alpha$—C' (=O) bond, or a combination of both processes. Since the N—C$^\alpha$

rotation is essentially completely restricted, PP provides a good model system for studying some of the disordering effects of salts.

In assessing the contributions of the above two factors to chain disordering, many workers[5,6,10] have concluded that increased freedom of rotation about C^α—C' (=O) is the predominant process occurring. This received support from circular dichroism studies of dilution of salt solutions of PP I,[6] which indicated that the system returned to a state of *cis–trans* isomerization of roughly the same extent as if it had been in water for a comparable time. Yet the initial conversion to the unordered state in concentrated salt solution was much more rapid than the isomerization process. This suggested[6] that the disordering process and the *cis–trans* isomerization were proceeding independently.

In order to elucidate further the nature of this process, we have studied the infrared spectra of PP in aqueous CaCl$_2$ solutions. We believe that these studies provide additional support for the C^α—C' (=O) rotation being the main contributor to the disordering of the chain.

EXPERIMENTAL

Poly-L-proline was obtained in form I from Sigma Chemical Company, with a molecular weight of 7500, and from Mann Research Laboratories as form II, with molecular weights of 25,000 and 62,500. Samples of different molecular weights gave the same solution spectra.

Solutions of 1–2.5% (w/v) were prepared by weighing dry PP II and dissolving it in distilled water or deuterium oxide at 0°C. The exact concentration of polymer did not affect the positions of the peaks.

Infrared spectra were recorded on a Perkin-Elmer model 421 spectrophotometer; band positions are accurate to ± 2 cm^{-1}. Solid samples were studied as KBr pellets. Solutions were studied in a CaF$_2$ cell of 0.05-mm thickness, with a variable-thickness BaF$_2$ cell containing H$_2$O or D$_2$O in the reference beam. Whenever possible D$_2$O was used; the only region requiring H$_2$O was that near 1450 cm^{-1}, where there is interference from an HDO peak.

RESULTS

Solid State

Our infrared spectra of solid PP I and PP II in KBr pellets are similar to those obtained by previous authors.[11,12] The frequencies found in the regions of the C=O stretching and pyrrolidine ring CH$_2$ bending vibrations are given in Table I. (The PP I was identified by its circular dichroism spectrum and, in the infrared, by its characteristic strong bands at 960 and 1349 cm^{-1}.) The C=O stretching vibration in form II appears at 1643 cm^{-1}, compared to values found by other authors at 1639 cm^{-1},[12] 1643 cm^{-1},[13] and 1650 cm^{-1}.[11] In agreement with two previous authors,[11,12] we find the C=O stretching vibration in form I to be at the same frequency

TABLE I
Infrared Bands of Poly-L-Proline

	Solid state		Solution	
Assignment	Form I	Form II	Aqueous	6M CaCl₂
C=O stretch	1643(vs)	1643(vs)		1641(ms)
			1619(vs)	1621(s)
CH₂ bend	1447(vw)	1447(vw)		~1472(m)
	1421(s)	1421(s)	1458(ms)	1458(m)

Key: s = strong, m = medium, w = weak, v = very.

as that in form II; another author[13] reports coincidence only at higher moisture contents. The strong band which we find at 1421 cm⁻¹ has been reported previously at 1426 cm⁻¹ [13] and 1427 cm⁻¹.[12]

Aqueous Solution

In aqueous solution the C=O stretching frequency is shifted to 1619 cm⁻¹ and the CH₂ bending mode now appears as a singlet at 1458 cm⁻¹. This
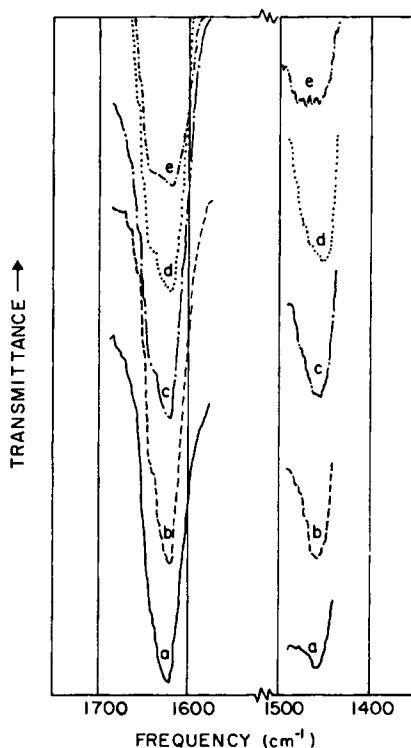

Fig. 1. Infrared spectra of poly-L-proline in aqueous and aqueous CaCl₂ solutions. In the carbonyl (1600–1700 cm⁻¹) region, measured in D₂O: (a) no salt present, (b) 1.6M, (c) 3.5M, (d) 4.5M, (e) 5.8M. In the CH₂ bending (1400–1500 cm⁻¹) region, measured in H₂O: (a) no salt present, (b) 1.5M, (c) 3.0M, (d) 4.5M, (e) 6.0M.

is illustrated in Figure 1, and the bands are listed in Table I. Similar shifts were observed previously,[14] although the frequencies were given as 1624 and 1456 cm^{-1}, respectively. In the latter work[14] it was observed that these bands weakened when the solution was heated, being replaced by bands of increasing intensity at 1640 and 1427 cm^{-1}. At 65°C the former bands were almost completely absent.

CaCl$_2$ Solution

In CaCl$_2$ solution a new band appears in the C=O stretching region at 1641 cm^{-1}, increasing in intensity with CaCl$_2$ concentration until at 6.0M CaCl$_2$ its intensity is almost equal to that of the band at about 1620 cm^{-1}. This is shown in Figure 1, and the frequencies are listed in Table I. A broadening occurs in the CH$_2$ bands, out to a value of about 1472 cm^{-1}, increasing in intensity with CaCl$_2$ concentration.

DISCUSSION

Poly-L-proline in Aqueous Solution

In order to interpret the changes taking place in CaCl$_2$ solution, it is necessary first to understand the spectral results in aqueous solution. In the latter case, in which the imide bonds are all in the *trans* form, we have seen that the C=O stretching frequency is shifted down by 24 cm^{-1} from its value in the solid state. The strong CH$_2$ bending mode has shifted up by 37 cm^{-1} from its solid-state value. Furthermore, upon heating of the solution, these bands are increasingly replaced by bands at the solid-state frequencies.[14]

These results strongly suggest that the spectral changes occurring upon solution are due primarily to hydrogen bonding between the C=O groups on the polymer and water molecules. This has been suggested before,[14] at which time it was pointed out that the C=O frequency shifts are consistent with those seen for small-molecule lactams upon hydrogen bonding. A similar shift is seen in the Raman spectrum of PP in aqueous solution.[15] In addition, the C=O frequency in the infrared is found to be downshifted by about 20 cm^{-1} when trifluoroethanol binds to solid films.[16] In the latter case the band is shifted by the same amount for PP I and for PP II, showing that the C=O groups in the two structures are equally accessible for hydrogen bonding to solvent molecules. The shift in the CH$_2$ bending mode is not directly related to the hydrogen bonding of H$_2$O to the C=O group, nor to a solvent effect.[14] It has been thought to arise from a change in the ring geometry[15] or from a conformational change about the C$^\alpha$—C' (=O) bond.[14] Conformational energy calculations[17] indicate, however, that the latter change is the major result of hydrogen bonding to water.

The spectral changes which were noted above to occur upon heating of PP solutions[14] are also consistent with the suggested hydrogen bonding. Heated solutions of PP are known to give precipitates of PP II,[2,14] and it is likely that this is preceded by the formation of aggregates of molecules in
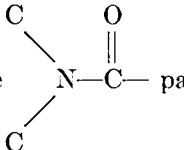
solution. Such aggregates would form by excluding water, and therefore their C=O frequencies would correspond to that of the non-hydrogen-bonded groups in the solid state. The CH_2 modes would of course also take on the values found in the solid.

The spectral effects observed in aqueous solutions of PP are thus completely consistent with the results expected for hydrogen bonding of water to the C=O groups.

Poly-L-proline in $CaCl_2$ Solution

In $CaCl_2$ solutions PP is known from circular dichroism studies[6] to be converted to an unordered form.[7] The extent of this conversion is greater at higher $CaCl_2$ concentrations.[6] We find that in the infrared spectra of PP in $CaCl_2$ solutions a new band appears at a frequency corresponding to the C=O stretching vibration in the solid state. The intensity of this band increases with increasing $CaCl_2$ concentration. The question is whether this helps to reveal if disordering occurs via cis–trans isomerization or by rotational changes around the C^{α}—C' (=O) bond.

Our own, as well as previous,[11,12] infrared studies indicate that the C=O stretching vibration occurs at the same frequency in solid PP I and PP II. This has also been found to be true in the Raman spectra.[18] While the frequency is lowered by hydrogen bonding to alcohols, it is again the same for PP I and PP II.[16] It is therefore reasonable to infer that it is essentially impossible to distinguish the cis from the trans imide bond by means of the C=O frequency. This is plausible when one considers that the

vibration is localized in the

$$\begin{array}{ccc} C & O & \\ \diagdown & \| & \\ N{-}C{-} \\ \diagup & & \\ C & & \end{array}$$

part of the molecule,[19] which is

essentially the same for these two structures. Therefore, the observation in $CaCl_2$ solutions of a C=O vibration which is close to that of the solid-state frequency must be interpreted as arising from a loss of hydrogen bonding to solvent for some C=O groups. This is not surprising since it has been pointed out by many workers[1,9,10] that cation binding or complexing to the C=O group is indicated in salt solutions. We therefore suggest that the upward shift in the C=O frequency is due to the replacement of hydrogen bonding to water by a complexing to the Ca^{++} ion. The development of a higher frequency CH_2 bending vibration could be a result of changes in ring geometry and/or in C^{α}—C' (=O) conformation induced by the ion binding. These changes of course need not resemble those involved in the transformation to solid PP, and therefore although the C=O frequency corresponds to that of the solid state structure this need not be true of the ring modes.

The above arguments do not reveal whether the 1641 cm^{-1} band in $CaCl_2$ solutions arises from cis or trans imide bonds. As we noted, this is not feasible from considerations of the C=O frequency alone. We believe,

however, that a distinction is possible from consideration of the CH_2 bending modes. Although it has been suggested[15] that frequency differences between this vibration in the solid state and in aqueous solution might arise from changes in ring geometry, we think that the experimental evidence does not favor this explanation. Rather we believe that it supports the alternative (which is also consistent with theoretical studies[17]), viz., that change in the C^{α}—C' (=O) rotation angle (ψ) accounts for the frequency shift. Our reasoning is as follows. In solid PP I and PP II the ψ angles given by x-ray diffraction studies are 158° and 146°, respectively.[20] (While the structure of PP II has been refined,[21] that of PP I has not, so it is still possible that the ψ values in the two solid state structures may be more nearly equal, as is indicated by conformational energy calculations of individual chains.[17,22]) The differences in ring geometries for these two structures are large.[17] Yet the CH_2 bending modes, both in our as well as in other[12,13] infrared spectra and in the Raman spectra,[18] are found at the same frequencies. The 12° (or smaller) difference in ψ either makes only a small difference or compensates the differences due to changes in ring geometry. However, when solid PP II is put into aqueous solution this is accompanied by no change in ring geometry but by an increase of about 20° in the ψ rotation angle.[17] Yet now the CH_2 vibration increases some 37 cm^{-1} in frequency. Thus, the value of ψ appears to have the predominant effect on the CH_2 bending frequency. The further upshift in frequency of about 14 cm^{-1} in $CaCl_2$ solution would therefore represent an extension of the allowed range of ψ under the influence of ion complexing to the C=O group. This might be associated with an extension of the previously allowed range of ψ or with the energetic accessibility of the region near $\psi = -35°$.[23] (We have built a CPK model with all *trans* imide bonds in which $\psi = -35°$ alternates with $\psi = 145°$. This structure with alternating ψ brings several C=O groups into the same region in such a way that complexing to a Ca^{++} ion could occur. This form of the chain is much more flexible than forms I or II and collapses easily.)

The important result to be derived from the above arguments is that the $\sim$50 cm^{-1} range of frequencies found for the CH_2 bending mode is most consistent with a large range of permissible values of ψ. Now it is well known[22-24] that for an isolated chain a larger range in ψ is feasible for *trans* imide bonds than for *cis* bonds. This is also true when water molecules are hydrogen bonded to the C=O groups,[17] and we assume that it holds as well in the presence of salt. The spectroscopic data on PP in $CaCl_2$ solutions therefore suggest that most imide bonds are in the *trans* form and that, as a result of complexing, a large range of ψ rotation angles is present in the chain.

This conclusion runs counter to that recently suggested by Torchia and Bovey[25] from NMR studies, but we believe that there is reason to question the certainty of the deductions from these studies. The NMR results were taken to imply that disordering of PP in $CaCl_2$ (as well as other salt) solutions is a result primarily of the random introduction of *cis* bonds in the

chain. Yet the NMR peak which was associated with the *cis* bond does not increase in area with increasing $CaCl_2$ concentration; if anything its area seems to decrease relative to that of the *trans* peak. The most characteristic feature of the disordering effect of $CaCl_2$ is that circular dichroism and infrared spectra both show enhanced effects with increasing salt concentration. We would therefore expect to see a comparable change in the NMR spectrum. Indeed there is one, viz., the gradual replacement of the narrow *trans* resonance at τ ca. 5.3 with a broad resonance at τ ca. 5.2–5.3. We believe that this broad resonance could arise from *trans* bonds associated with a wider range of ψ values. This wider range would not produce a narrowing of the line, as the authors claim,[25] if complexing with the salt were to result in a larger range of relatively immobile structures rather than the increased mobility which the authors apparently envision. Certainly if complexing of more than one C=O group with a Ca^{++} ion occurred, it would be expected to lead to smaller not greater dynamic flexibility about the C^{α}—C' (=O) bond. Until it can be shown that the area of the *cis* peak does in fact increase with increasing $CaCl_2$ concentration, we believe that the invoking of *cis* bonds as the primary cause of chain disordering must be held in question.

There is an additional problem with the NMR interpretation, viz., the assignment[25] of the τ ca. 5.8 peak to *cis* imide bonds within the chain. Not only is this peak position higher than the *cis* peak of PP I (a shift which the authors attribute to solvent effects), but it is not clear how an assignment to bonds at the ends of the chain (which may be *cis*) can be eliminated. Such an assignment of higher field peaks was found to be plausible for oligomers,[26,27] and in fact the area of the τ ca. 5.8 peak in comparison to that of the τ ca. 5.2–5.3 peak for the highest concentration ($4M$) $CaCl_2$ used[25] would be consistent with an assignment to end groups in the DP ca. 25 PP sample used. In other words, the complexing of salt with the end residues could perhaps make the terminal (and possibly near-terminal) bonds *cis*, and this would have nothing to do with the disordering process in the main part of the chain. Until it can be shown that an end-group origin for the τ ca 5.8 peak is eliminated, e.g., by studying higher-molecular-weight samples, we believe that caution should be exercised in assigning to *cis* bonds the primary cause of the disordering in the body of the chain. This is particularly true at the high concentrations of $CaCl_2$, where the τ ca. 5.7 contribution seems to be absent[25] and yet where the circular dichroism[6] and infrared effects are maximal (and observed, it should be noted, on high-molecular-weight samples). In addition, it is puzzling that in the analysis of the NMR spectrum of the dimer[27] only the *cis* or *trans* state of the imide bond was considered, thus giving four isomers, when it is known[23,24] that two conformational states in ψ are available to the molecule. It would appear that this would be important in the analysis of this spectrum, which is relevant to the interpretation of the polymer spectrum.

Finally, we wish to note that although a significant *cis* peak is seen in the NMR spectrum of PP in $5M$ KI solution,[25] the situation may not be the

same as in $CaCl_2$ solution. We have obtained the infrared spectrum of PP in 5M KI and find that, although the $C=O$ stretching band has developed a component at 1641 cm^{-1} of greater intensity than that in $4M$ $CaCl_2$, the CH_2 bending band shows essentially no broadening. This could indicate that the increased flexibility in ψ, with which we have associated the broadening, occurs only with multivalent cations which can complex to more than one $C=O$ group. Monovalent cations, on the other hand, may only be able to complex to one $C=O$ group (thus still displacing H_2O and leading to a higher $C=O$ frequency), and as a result do not induce the chain distortion which leads to a larger range in ψ.

We conclude, therefore, that the NMR studies of the effects of high concentrations of $CaCl_2$ on the disordering of PP do not show that *cis* bonds are introduced into the main body of the chain, and that in fact the progressive changes in the *trans* peak with salt concentration indicate more convincingly that the disordering process is associated primarily with the altered environment of this bond. Our infrared studies indicate that complexing with $CaCl_2$ increases the range of ψ rotation angles accessible to the chain, and this is also most consistent with the imide bond being in the *trans* form. The complex is not likely to be a single rigid structure which is identical in all parts of the chain, but rather a relatively restricted structure which can be formed with a range of ψ values. Hence we view the resultant chain to have lost the specific order which led to the circular dichroism spectra of PP I or PP II and to have adopted a range of local conformations which makes its circular dichroism spectrum strongly resemble spectra of unordered polypeptides and proteins.[7] We therefore associate the disordering of PP in concentrated $CaCl_2$ primarily with a larger range of accessible rotation angles about the C^α—C' ($=O$) bond.

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