^{13}C-n.m.r. studies of the conformational changes in proline oligomers brought about by lithium and calcium salts*

Ch. Pulla Rao, P. Balaram and C. N. R. Rao†

Solid State and Structural Chemistry Unit, Indian Institute of Science, Bangalore 560012, India

(Received 5 January 1983; revised 16 May 1983)

A detailed ^{13}C-n.m.r. investigation has been carried out on the conformational changes in proline oligomers brought about by interaction with lithium and calcium perchlorates. Interaction of lithium and calcium salts with Piv-$(Pro)_n$-OMe, n = 2, 4 and 5 results in trans–cis *isomerization. In the case of pentaproline, metal salts also give rise to other* trans*-isomers caused by the rotation about the C^{α}–C(=O) bond (Ψ,* cis′*). Calcium salts seem to stabilize* cis′*-isomers and produce effects somewhat different from those of lithium salts.*

Keywords: Peptides; proline oligomers; ^{13}C-n.m.r.; peptide conformation; salt effects

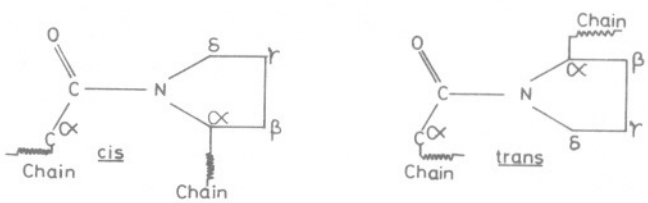
Introduction

Concentrated aqueous salt solutions of alkali and alkaline earth metals, particularly lithium and calcium, are known to disorder the poly(L-proline) chain structure[1–9]. Two possible mechanisms have been suggested to explain this phenomenon. One involves the formation of *cis*-imide bonds in an all-*trans* peptide chain[1–5] and the other depends on the increase in the freedom to rotate about the C^{α}–C(=O) bond[6–8]. Kurtz and Harrington[9] suggest that the metal salt binds at peptide linkages leading to a lower barrier to rotation of the ring about the peptide bond. O.r.d. studies of Schleich and von Hippel[7] suggest that the shortening of the (C′–N) bond is accompanied by a lengthening of the adjacent C^{α}–C(=O) bond which causes an increase in the accessible range of the angle Ψ. Deber *et al.*[10] monitored isomerization of poly(L-proline) I to poly(L-proline) II by n.m.r. spectroscopy and identified separate resonances due to *cis*-imide bonds (C^{α} proton in ^{1}H-n.m.r.; C^{β} and C^{γ} carbons in ^{13}C-n.m.r.). Bovey and coworkers[10–12] interpret the destruction of the homogeneous form of poly(L-proline) II as due to the random introduction of *cis*-imide bonds.

Johnston and Krimm[8] as well as Swenson[3] investigated the effect of aqueous solutions of salts on polyproline employing infrared spectroscopy. Johnston and Krimm explain their results on the basis of an increase in the accessible range of Ψ while Swenson proposes the formation of random sequences of *cis*- and *trans*-peptide bonds. Recently, Clark *et al.*[13] have suggested the presence of another type of *trans*-conformer (*cis′*- in addition to *cis* and *trans*) in the vicinity of $\Psi = -50°$ based on their studies of the ^{13}C-n.m.r. and characteristic ratio of the polymer. These results are consistent with conformational energy calculations[14] based on pyrrolidine ring geometry, relaxed from the restrictions imposed by crystallographic data.

It is clear from the above discussion that the mechanism of disordering of polyproline by salts is far from being clearly understood. We felt that it would be worthwhile to investigate the effect of lithium and calcium salts on proline oligomers employing high resolution ^{13}C-n.m.r. spectroscopy. Proton magnetic resonance studies of proline oligomers (t-Boc-$(Pro)_n$-OBz, $n = 2$, 3, 4, 5 and 6) by Deber *et al.*[10] have provided some information on the conformations of these peptides in solution. These workers found that when $n = 2$ both *cis*- and *trans*-isomers were present while $n = 3$ showed at least five out of eight possible conformers; the oligomers with $n = 4$ showed eight out of an expected sixteen conformers and when n was 5, the spectrum was dramatically simplified showing the resonance corresponding to only *trans*, suggesting that the chain had assumed a form II helical conformation. This behaviour is in contrast with linear sarcosine oligomers[15] which show no tendency to assume a regular structure as the chain is lengthened.

Recent studies of Chao and Bershon[16] on proline oligomers ($^{+}NH_2$Pro-$(Pro)_n$-COO^{-}, $n = 1$–3,5) employing a 25.03 MHz ^{13}C-n.m.r. spectrometer showed only broad C=O, C^{β} and C^{γ} resonances (unlike in the present study). High resolution ^{13}C-magnetic resonance spectra would be expected to show different ^{13}C-resonances due to *cis*- and *trans*-peptide bonds for C^{β} and C^{γ} and also for the carbonyl carbons. We have, therefore, investigated the effect of lithium and calcium salts on small proline oligomers, Piv-$(Pro)_n$-OMe(Piv=Pivaloyl) with $n = 1$, 2, 4 and 5, employing a 67.89 MHz ^{13}C-n.m.r. spectrometer to identify the possible isomers.



* Contribution No. 157 from the Solid State Structural Chemistry Unit.

† To whom correspondence should be addressed.

0141–8130/83/050289–07$03.00

Table 1 Effect of calcium perchlorate on the ^{13}C-chemical shifts of Piv-(Pro)-OMe in acetonitrile

Resonance	Chemical shifts (δ) at various concentrations of salt		
	0.0 M	0.6 M	1.2 M
Ester *C*=O	177.0	179.5	179.8
Piv *C*=O	174.2	174.8	175.1
Pro-C^{α}	61.8	62.4	62.4
Ester-CH_3	52.1	53.3	53.6
Pro-C^{δ}	49.0	49.7	49.7
Piv-C^{α}	39.2	39.7	39.5
Pro-C^{β}	28.6	28.2	28.0
Piv methyl carbons	27.6	27.5	27.3
Pro-C^{γ}	26.5	26.2	26.1

Experimental

All the proline oligomers, a study of which is described in this paper, were synthesized by Dr Y. V. Venkatachalapathi of the Molecular Biophysics Unit of this Institute. 67.89 MHz ^{13}C-n.m.r. spectra were recorded with a Bruker W. H. 270 *FT* n.m.r. instrument with a repetition time of 1 and/or 1.5 s. Peak positions are referenced to TMS, taken as internal standard. Errors in chemical shift measurements are of the order of 0.05 ppm and do not exceed 0.1 ppm in any case. Acetonitrile was used as solvent as both the peptide and the salt are considerably soluble in it. Acetonitrile-d_3 provided the deuterium lock signal. The spectra for all the oligomers were measured with different concentrations of lithium and calcium perchlorate salts. The concentrations of peptide employed were: Piv-Pro-OMe, 0.31 M; Piv(Pro)$_2$-OMe 0.22 M; Piv-(Pro)$_4$-OMe, 0.13 M and Piv-(Pro)$_5$-OMe, 0.11 M. A large number of transients were collected in order to get a reasonably good spectrum and this number ranged from 2000 to 8000. The effect of water on Piv-(Pro)$_2$-OMe was also measured. The population of the *cis*-isomer was estimated from the areas of *cis*- and *trans*-resonances of the C^{β} and C^{γ} carbons. The percentages of *cis*-isomers listed in the table are averages of estimated values.

Results and discussion

Assignment of ^{13}C-resonances in proline oligomers

^{13}C-N.m.r. spectra of proline oligomers studied by us exhibit well separated resonances for C=O, C^{α}, C^{δ}, ester CH_3 and Piv C^{α} as well as C^{β}, C^{γ}, and Piv methyl carbons. The signals were assigned based on the values reported by Dorman and Bovey[12] for various proline oligomers (^{13}C-chemical shifts for typical proline residues in the *trans*- and *cis*-geometries are as follows. *C*=O: 175.4–179.9, 175.2–179.2; C^{α}: 57.9–62.9, 60.3–62.6; C^{β}: 27.9–31.0, 30.2–32.6; C^{γ}: 24.6–25.5, 23.1–23.5; C^{δ}: 47.1–49.4, 45.2–48.2) and also by comparison with the spectra of compounds containing a small number of proline residues obtained in the present studies. The signals in the carbonyl region of the spectrum were assigned to ester carbonyl (downfield signal), pivaloyl carbonyl (high-field signal) and peptide carbonyl carbons. On interaction with lithium and calcium salts, the C^{β} and C^{γ} resonances corresponding to the *cis*-isomer (separated from those due to the *trans*-isomer) are readily delineated. The spectra obtained in the presence of salts were analysed by taking the *trans*-isomer as the predominant species. Values of Ψ were calculated based on the separation between C^{β} and C^{γ} resonances ($\Delta\delta_{\beta\gamma}$) using the following expressions given by Siemion *et al.*[17]

$$\Delta\delta_{\beta\gamma} = 0.036\ |\theta| + 0.73 \quad \ldots \text{ for } \textit{trans}\text{-series}$$

$$\Delta\delta_{\beta\gamma} = 0.081\ |\theta| + 2.47 \quad \ldots \text{ for } \textit{cis}\text{-series}$$

where $|\theta| = \Psi - 60°$.

Effect of calcium perchlorate on Piv-Pro-OMe

Interaction of calcium perchlorate with Piv-Pro-OMe was studied in order to establish trends in the chemical shifts of various carbon nuclei and also to examine whether the pivaloyl group locks the N-terminal peptide unit (Piv-Pro) in the *trans*-geometry. ^{13}C-n.m.r. spectra of this peptide were recorded with various concentrations of calcium perchlorate and the spectral data are listed in the *Table 1*. We see that the ester carbonyl carbon shows the largest shift in the spectrum suggesting direct interaction of the metal ion with carbonyl group. Direct binding of the metal ion with the ester carbonyl appears to be preferred over the pivaloyl carbonyl group. Changes in the chemical shifts of the other carbons are due mainly to the extended effect of direct interaction of the metal ion with the carbonyl group.

From *Table 1* we see that chemical shifts of C^{α} and C^{δ} move downfield whereas those of C^{β} and C^{γ} move upfield on adding the metal salt. This might be due to the flexibility of the pyrrolidine ring in accommodating the structural changes due to metal ion interaction. This observation is consistent with the semiquantitative analysis[18] of C^{β}, C^{γ} and C^{δ} carbon correlation times of the pyrrolidine ring, which suggest that rapid ring motion (or ring flexibility) involves primarily C^{β} and C^{γ} atoms. Addition of even large amounts (1.24 M) of calcium perchlorate does not produce any extra lines corresponding to the *cis*-isomer. This observation clearly rules out the possibility of *cis–trans* isomerization about the Piv-Pro peptide bond due to steric interactions between the Pro(1) carbonyl and methyl groups of the bulky pivaloyl residue as mentioned in the literature[19–21].

Effect of metal salts on Piv-(Pro)$_2$-OMe

Since the salts used in the present investigation tend to contain some amount of water, we performed a few control experiments with this diproline (up to a peptide: water mole ratio of 1:270) in order to understand the effect of water on the proline oligomers. Addition of water causes a larger shift of the ester carbonyl carbon ($\sim$1.7 ppm) than of the pivaloyl carbonyl ($\sim$0.8 ppm) or peptide carbonyl ($\sim$0.8 ppm) of this dipeptide. Small changes (0.1–0.4 ppm) observed in C^{α}, C^{β}, C^{γ} and C^{δ} resonances show the flexibility of the pyrrolidine ring in aqueous solution. Interaction of water with the peptide (through hydrogen bonding[22]) does not show any evidence of *cis–trans* isomerism. The shifts in various carbon resonances induced by water are generally much smaller than those induced by metal ions, suggesting that direct interaction of metal ions with the carbonyl group is stronger compared to that of water, an observation supported by earlier studies on model amides[23].

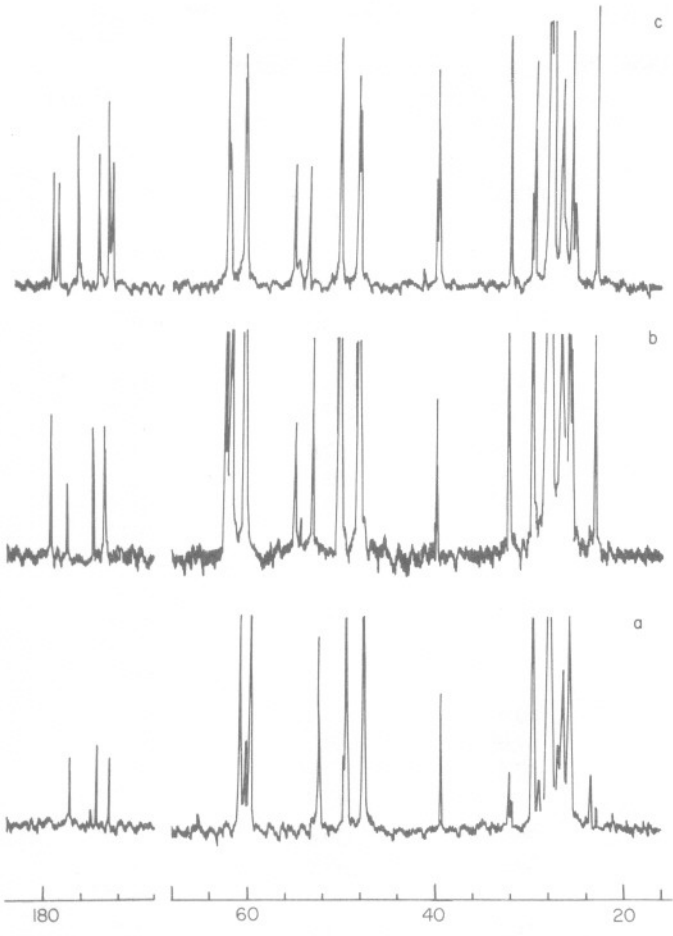


Figure 1 ^{13}C-n.m.r. spectra showing the effect of $LiClO_4$ on Piv-$(Pro)_2$-OMe: (a) no salt; (b) 0.5 M; (c) 1.5 M. (Full heights of all the peaks are not shown for convenience in this and other figures giving the spectra)

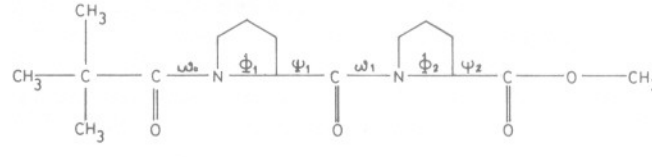
Lithium perchlorate. The effect of lithium perchlorate on the ^{13}C-n.m.r. spectra of Piv$(Pro)_2$-OMe was studied up to 2.0 M concentration. Typical spectra are shown in *Figure 1* and the results are summarized in *Table 2*. The spectrum recorded with 0.5 M lithium perchlorate (*Figure 1b*) shows marked changes. Thus, we see signals for C^β and C^γ of the *cis*-species. Two signals, 1.9 ppm apart, are observed for ester methyl carbon. A downfield signal is also observed at 176.7 ppm due to the *cis*-peptide bond. The C^α and C^δ resonances of both the proline residues show splitting to different extents, C^α of Pro(2) and C^δ of Pro(1) being larger. When the salt concentration is increased to 1.0 M, upfield resonances for the ester and pivaloyl carbonyl carbons are noticed. Piv C^α (tert-carbon) and Piv methyl carbon signals split into two lines. The *trans*-C^β and C^γ resonances also exhibit splitting. Similar spectral features are seen even when the concentration of the salt is 1.5 M. When the concentration is raised to 2.0 M, most of the lines in the spectrum become broad, perhaps due to the increase in viscosity of the solution.

At 0.5 M salt concentration, there are only four signals in the carbonyl region: three of them are as in the parent compound and the fourth one is due to the metal ion interaction. The number of signals in the carbonyl region is increased to six at higher concentrations. It is known from the literature that the carbonyl resonances also show splitting under high resolution due to *cis–trans* isomerism[12]. We suggest that the spectral changes observed by us are due to the formation of *cis*-isomers. Thus we find *cis*-C^β and *cis*-C^γ resonances with the concomitant changes in the carbonyl region. The presence of two signals for each of the ester, pivaloyl methyl and pivaloyl C^α carbons, also support this observation. It appears that lithium ion disrupts the polyproline II type structure by introducing *cis*-imide bonds in the peptide chain. The percentage of *cis*-isomer calculated from the *cis*-C^β and C^γ resonances are shown in *Table 2*. There seems to be an increase in the percentage of *cis*-species with increases in salt concentration, reaching 45% at $\sim$1.5 M $LiClO_4$.

Calcium perchlorate. The effect of calcium perchlorate on Piv(Pro_2)-OMe was studied up to a concentration of 1.9 M. Typical ^{13}C-n.m.r. spectra are shown in *Figure 2*. When the concentration of the salt is small (0.03 M), minor changes are observed in the chemical shifts of various carbons. The spectrum shows marked changes when the concentration of the salt is raised to 0.06 M. The changes observed in the spectrum are similar to those found with 1.0 M $LiClO_4$; thus we see *cis*-resonances of C^β and C^γ as well as a few extra resonances in the carbonyl region. The ester methyl carbon shows two distinct lines separated by 2.6 ppm, the pivaloyl methyl groups also show two signals. The spectrum obtained with 0.7 M $Ca(ClO_4)_2$ (see *Figure 2b*) is comparable to that obtained with 1.5 M $LiClO_4$. We see a splitting of resonances due to pivaloyl carbonyl, pivaloyl C^α, *trans*-C^β and *trans*-C^γ similar to that observed in the case of lithium perchlorate. C^α of Pro(1) shows two lines separated by $\sim$0.7 ppm, but the pivaloyl methyl groups show at least three signals, indicating the formation of one or more species in addition to *cis*-isomers. The spectrum at 1.3 M $Ca(ClO_4)_2$ shows distinct carbonyl resonances (*Table 2*) and the *cis*-peptide carbonyl and C^α of Pro(1) carbon resonances undergo further splitting. An essential feature in this spectrum is the appearance of a third resonance due to the ester methyl and pivaloyl C^α carbons. At 1.9 M of the salt, we found an additional line associated with each of the carbonyl resonances; the ester methyl carbon resonances due to the *trans*-species also splits into two. The estimated values of the percentage of *cis*-isomers are 16, 37, 40, 42 and 46 at salt concentrations of 0.03, 0.06, 0.7, 1.3 and 1.9 M respectively.

It appears that the interaction of calcium perchlorate with Piv-$(Pro)_2$-OMe produces three resonances for each of the carbonyl carbons, thereby showing nine lines in the carbonyl region. The Pro(1) resonances are affected in the presence of the calcium salt. The weaker resonances of C^α of Pro(1) and C^γ occur at higher field than their stronger signals. On the other hand, the weaker resonances of C^δ of Pro(1) and C^β occur downfield compared to their stronger ones. Such a behaviour has been explained[24] on the basis of C^α–C' rotational isomers.

In order to understand the spectral changes properly, it is important to examine various possible conformations. The parameters that determine the conformations in Piv-$(Pro)_2$-OMe are shown below:



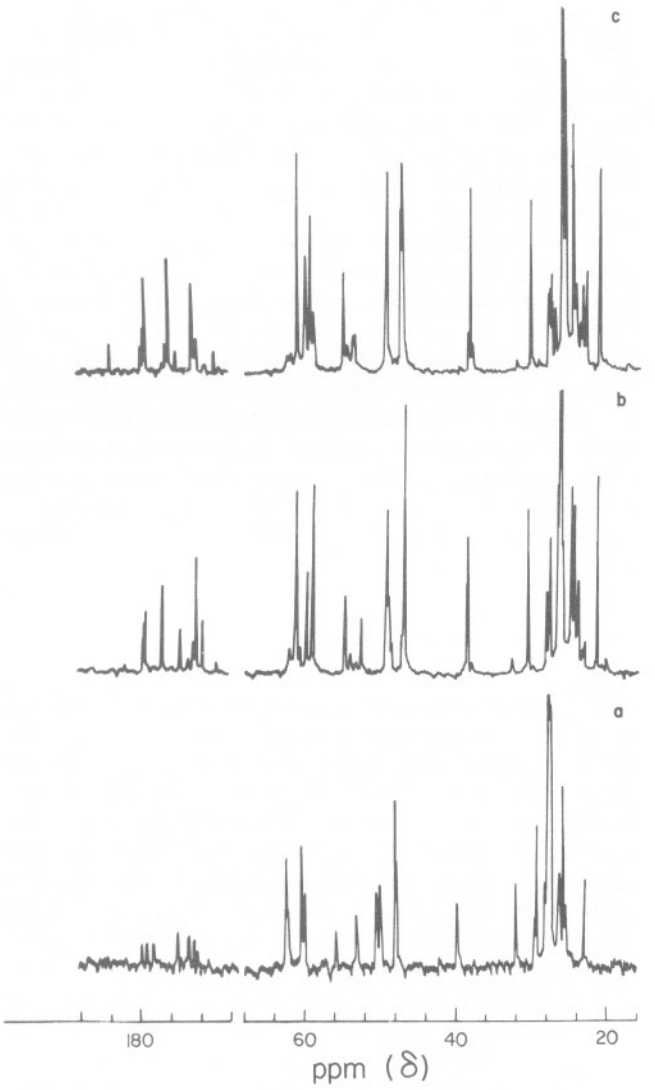


Figure 2 ^{13}C-n.m.r. spectra showing the effect of $Ca(ClO_4)_2$ on Piv-$(Pro)_2$-OMe: (a) 0.03 M; (b) 0.70 M; (c) 1.90 M

either the *cis'* or *trans'*-conformers, but the C^{α}-C(=) rotation was predicted on the basis of molecular dynamics. X-ray crystallographic studies of relatively inflexible peptides have shown *cis-trans'* and *trans-cis'* conformations in compounds like Boc-$(Pro)_4$-OBz[30], Boc-Pro-Pro-Gly-NH_2[31] etc. *Trans-cis'* alone is observed in Z-Aib-Pro-NHMe[32], Z-Aib-Pro-Aib-Ala-OMe[33], Piv-D-Pro-L-Pro-LAla-NHMe[34] and Boc-Leu-Aib-Pro-Val-Aib-OMe[35]. It is expected that at least in the presence of metal ions, these conformers should show separate resonances.

Following the equations given by Siemion *et al.*[17], we obtained Ψ values of $-59°$ or $179°$ [$\Delta\delta_{\beta\gamma} = 5.01$ ppm] based on the new resonances produced in the *trans*-C^{β} and C^{γ} region at 1.3 M $Ca(ClO_4)_2$. These values suggest that *cis'*-species may be present at higher calcium ion concentrations. It appears that in the presence of calcium ions, these species interconvert slowly on the n.m.r. timescale. The present studies reveal that the calcium salt stabilizes those isomers which are not found in the presence of the lithium salt. The shifts in values of various carbons are also more pronounced in the case of the calcium salt than with the lithium salt.'

Effect of metal salts on Piv-$(Pro)_4$-OMe

Lithium perchlorate. The spectrum of this peptide (*Figure 4*) shows three sets of resonances in the C^{α}, C^{δ} regions: one due to Pro(1), the second due to Pro(4) and the remaining due to Pro(2, 3). More specific assignment of these resonances is, however, not feasible. Measurable shifts in the various resonances (though small) are often observed due to interaction with lithium perchlorate (see *Figure 4*). When the concentration is in the range 0.10–0.25 M, we see two lines each for ester methyl, Piv C^{α}, *cis*-C^{β}, *cis*-C^{γ} and pivaloyl methyl carbons. The C^{α} and C^{δ} signals of N- and C-terminal Pro(1,4) residues do not seem to be very much affected. This implies that the interaction of the metal salt with the peptide favours the formation of conformers having *cis*- and/or *trans*-peptide bonds. At higher concentrations of the salt, the lines become broader due to increased viscosity of the solution, making it difficult to identify distinct resonances. An increase in viscosity due to metal ion interaction with amides has been explained in the literature as indicative of aggregation[36,37].

Resonances corresponding to *cis*-C^{β} and *Cis*-C^{γ} show at least two lines. This can be explained on the basis of the different extents of isomerization of the oligomer chains (*cis–trans* isomerism) of the tetraproline; this is not possible in the case of Piv-$(Pro)_2$-OMe. The resonances of Piv-$(Pro)_2$-OMe remain unsplit even at high concentrations of calcium salt. Conformers with different extents

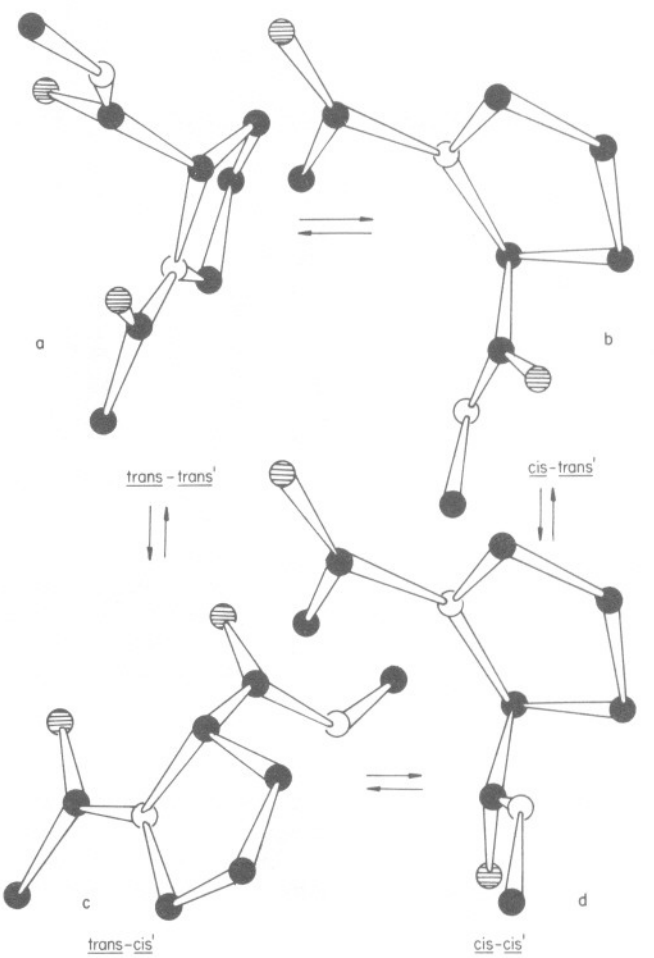


Figure 3 Perspective views of the four possible conformations of Ac-Pro-NHMe

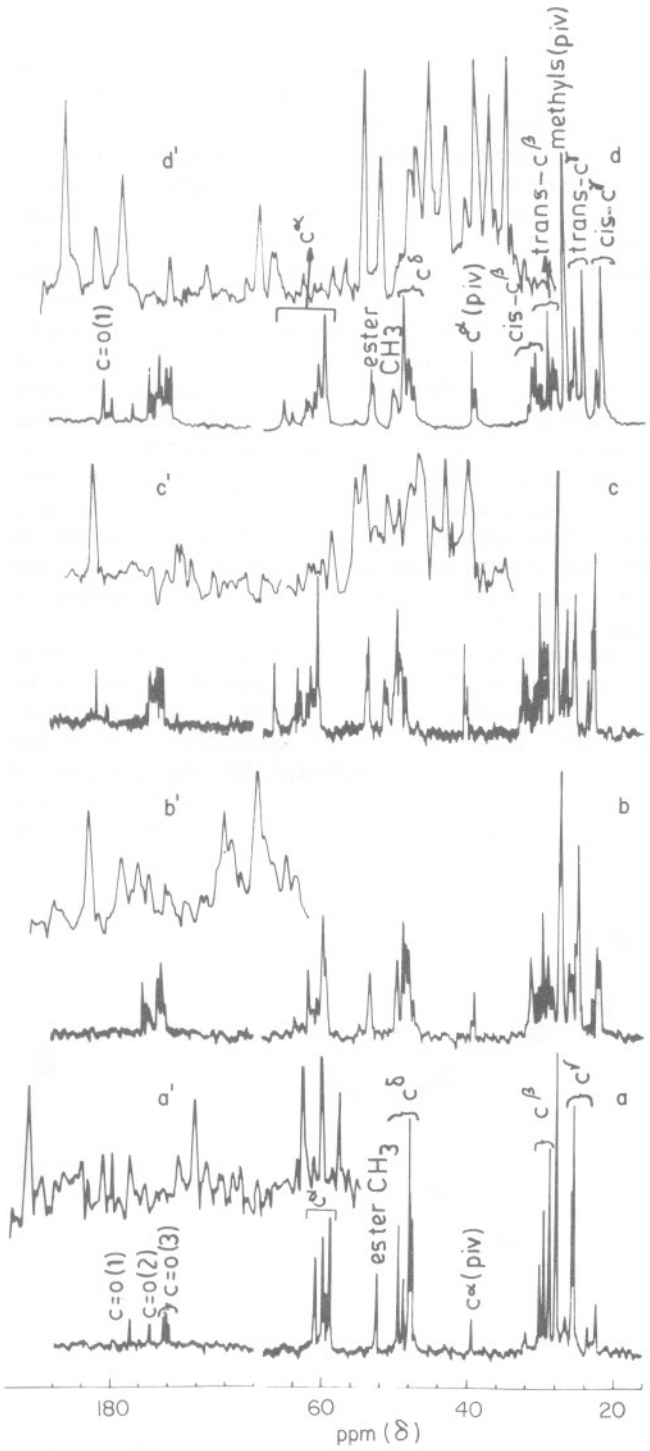


Figure 4 ^{13}C-n.m.r. spectra showing the effect of various concentrations of $LiClO_4$ and $Ca(ClO_4)_2$ on Piv-$(Pro)_4$-OMe: (a) no salt; (b) 2.0 M $LiClO_4$; (c) 0.37 M $Ca(ClO_4)_2$; (d) 1.30 M $Ca(ClO_4)_2$; insertions (a′, b′, c′ and d′) correspond to the expanded carbonyl regions of the connecting spectra. C=O (1), (2) and (3) refer to ester, Piv and peptide carbonyls, respectively. α, β, γ and δ carbons are those in proline

of *cis-trans* isomerization about the Pro-Sar bond have indeed been suggested in the case of cycle(Pro-Sar)$_3$ and cyclo(Pro-Sar)$_4$ based on ^{13}C-n.m.r. studies[38]. The population of *cis*-isomers estimated remains around 40%, independent of the salt concentration.

Calcium perchlorate. Typical ^{13}C-n.m.r. spectra showing the effect of calcium perchlorate on Piv-$(Pro)_4$-OMe are shown in *Figure 4*. When the concentration of the calcium salt is 0.2 M we see at least three species each for *cis*-C^β, *cis*-C^γ, pivaloyl C^α and pivaloyl methyl carbons, and at higher concentrations of the metal salt, the lines become broader. It is interesting that interaction with this salt gives rise to a larger number of resonances than with lithium perchlorate. We are, however, able to understand the major changes observed in the spectra in the light of *cis–trans* isomerization. Multiple resonance lines corresponding to *cis*-C^β and *cis*-C^γ are observed in the presence of calcium salt as well. There seem to be a set of three lines in each case, indicating the presence of oligomers possessing different numbers of *cis*-Pro-Pro bonds. A Ψ value of $-55°$ was estimated from the *trans*-C^β and *trans*-C^γ resonance lines. The changes observed in the C^β, C^γ resonances of various proline residues and also in the C^α of Pro(1) to Pro(3) suggest the presence of other conformers involving C^α-C′ rotation. The population of *cis*-isomer is around 45% in the presence of the calcium salt.

Effect of metal salts on Piv-$(Pro)_5$-OMe

Effect of $LiClO_4$ on Piv-$(Pro)_5$-OMe was studied up to a salt concentration of 1.75 M and the effect of $Ca(ClO_4)_2$ up to 1.6 M. Salt-induced spectral changes observed with this pentapeptide were similar to those obtained with Piv-$(Pro)_4$-OMe. The C^β and C^γ carbons of both the *cis*- and *trans*-imide bonds show multiple resonance lines. Changes corresponding to *cis*-C^β and *cis*-C^γ observed in this case also suggest the occurrence of various oligomer chains with random *cis–trans* isomerization upon metal salt interaction. The new lines found in the case of *trans*-C^β and *trans*-C^γ are populated up to $\sim$15%. The C^α and C^δ carbon resonances of various proline residues (except for the C-terminal Pro residue) also show appreciable splitting. These changes seem to reflect the presence of the C^α-C′ rotational isomers. The percentage of *cis*-isomers of the pentamer in the presence of the salts is $\sim$40.

Conclusions

Various conformations having different degrees of *cis–trans* isomerization about the Pro-Pro bond are produced by the interaction of lithium and calcium salts with proline oligomers. Comparison of the results obtained with the tetra and pentapeptides suggest the presence of appreciable proportions of conformers with all their Pro–Pro bonds in *cis*-conformation [just like in poly(L-proline I)], as a result of lithium or calcium ion binding. Boc-$(Pro)_n$-OMe with $n=3$, 4 and 5 are indeed known to form polyproline II-type helices[10,30,39]. Interaction with lithium or calcium perchlorate significantly alters the peptide backbone conformation. The total percentage of *cis*-species formed as a result of the metal ion binding with the peptide group never exceeds 50, even at relatively high salt concentrations. It looks as though the polyproline II chain cannot accommodate more than 50% *cis*-peptide units. Mandelkern *et al.*[27] suggest that only about 25% of *cis*-peptide bonds are formed in polyproline upon addition of Li and Ca salts in aqueous solution. It appears that the percentage of *cis*-isomers may decrease as the chain length of the oligomer increases and give rise to the formation of other isomers (due to Ψ rotation).

Acknowledgement

The authors thank the US National Institutes of Health for support of this research (Grant No. 01-136-N).

References

1 Torchia, D. A. and Bovey, F. A. *Macromolecules* 1971, **4**, 246
2 Dorman, D. E., Torchia, D. A. and Bovey, F. A. *Macromolecules* 1973, **6**, 80
3 Swenson, C. A. *Biopolymers* 1971, **10**, 2591
4 Harrington, W. F. and Sela, M. *Biochim. Biophys. Acta* 1958, **27**, 24
5 Mattice, W. L. and Mandelkern, L. *J. Am. Chem. Soc.* 1971, **93**, 1769
6 Steinberbg, I. Z., Harrington, W. F., Berger, A., Sela, M. and Katchalski, E. *J. Am. Chem. Soc.* 1960, **82**, 5263
7 Schleich, T. and von Hippel, P. H. *Biopolymers* 1969, **7**, 861
8 Johnston, N. and Krimm, S. *Biopolymers* 1971, **10**, 2897
9 Kurtz, J. and Harrington, W. F. *J. Mol. Biol.* 1966, **17**, 440
10 Deber, C. M., Bovey, F. A., Carver, J. P. and Blout, E. R. *J. Am. Chem. Soc.* 1970, **92**, 6191
11 Bovey, F. A., Ryan, J. J. and Hood, F. P. *Macromolecules* 1968, **1**, 305
12 Dorman, D. C. and Bovey, F. A. *J. Org. Chem.* 1973, **38**, 2379
13 Clark, D. S., Detcher, J. J. and Mandelkern, L. *Macromolecules* 1979, **12**, 626
14 Madison, V. *Biopolymers* 1977, **16**, 2671
15 Toniolo, C., Bonora, G. M., Schilling, F. C. and Bovey, F. A. *Macromolecules* 1980, **13**, 1381
16 Chao, Y. Y. and Bershon, R. *Biopolymers* 1978, **17**, 2761
17 Siemion, I. Z., Wieland, Th. and Pook, K. H. *Angew. Chem., Int. Ed. Engl.* 1975, **14**, 702
18 Torchia, D. A. and Lyerla, J., Jr. *Biopolymers* 1974, **13**, 97
19 Nishihara, H., Nishihara, K., Uefuji, T. and Sakota, N. *Bull. Chem. Soc. Jpn* 1975, **46**, 553
20 Aubry, A. *Ph.D. Thesis* University of Nancy, France, (1976)
21 Venkatachalapathi, Y. V. and Balaram, P. *Nature* 1979, **281**, 83
22 Bonner, O. D. and Choi, Y. S. *Spectrochim. Acta* 1975, **31A**, 1975
23 Pulla Rao, Ch., Balaram, P. and Rao, C. N. R. *J. Chem. Soc. Faraday Trans* 1980, **76**, 1008
24 Han, S. L., Stimson, E. R., Maxfield, F. R. and Scheraga, H. A. *Int. J. Pept. Protein Res.* 1981, **17**, 297
25 Madison, V. *Biopolymers* 1979, **18**, 1563
26 Venkatachalapathi, Y. V. *Ph.D. Thesis* Indian Institute of Science, Bangalore, India, 1980
27 Mandelkern, L., Clark, D. S. and Detcher, J. J. *Macromolecules* 1980, **13**, 533
28 Deber, C. M., Fossel, E. T. and Blout, E. R. *J. Am. Chem. Soc.* 1974, **96**, 4055
29 Nagaraj, R., Venkatachalapathi, Y. V. and Balaram, P. *Int. J. Pept. Protein Res.* 1980, **16**, 291
30 Matsuzaki, T. *Acta Crystallogr.* (*B*) 1974, **30**, 1029
31 Tanaka, I., Ashida, T., Shimanishi, Y. and Kakudo, M. *Acta Crystallogr.* (*B*) 1979, **35**, 110
32 Prasad, B. V. V., Shamala, N., Nagaraj, R., Chandrasekaran, R. and Balaram, P. *Biopolymers* 1979, **18**, 1635
33 Shamala, N., Nagaraj, R. and Balaram, P. *Biochem. Biophys. Res. Commun.* 1977, **79**, 292
34 Nair, C. M. K., Vijayan, M., Venkatachalapathi, Y. V. and Balaram, P. *J. Chem. Soc. Chem. Commun.* 1979, 1183
35 Pulla Rao, Ch. *Ph.D. Thesis* Indian Institute of Science, Bangalore, India, 1981
36 Bello, J. Hass, D. and Bello, H. R. *Biochemistry* 1966, **5**, 2539
37 Bonner, O. D. and Jordan, C. F. *Physiol. Chem. Phys.* 1976, **8**, 293
38 Shimizu, T. and Fijishige, S. *Biopolymers* 1980, **19**, 2247
39 Kartha, G., Ashida, T. and Kakudo, M. *Acta Crystallogr.* (*B*) 1974, **30**, 1861