Vol. 97, No. 2, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 28, 1980 Pages 751-758

^{1}H-^{1}H AND ^{13}C-^{13}C VICINAL COUPLING CONSTANTS AND AMINO ACID SIDE CHAIN CONFORMATION IN PEPTIDES

F. Toma, M. Monnot, F. Piriou, J. Savrda[1] and S. Fermandjian[+]
Service de Biochimie, Département de Biologie
CEN-Saclay, B.P. n° 2, 91190 Gif-sur-Yvette, France
and
[1]Institut Pasteur, 25 rue du Docteur Roux, 75015 Paris, France

Received October 7,1980

SUMMARY : The vicinal coupling constants $^{13}C'$-$^{13}C^{\gamma}$ were measured in aspartic acid and phenylalanine (85 % ^{13}C enrichment) as free amino acids and in the peptides Asp-Pro and Gly-Pro-Phe. These coupling constants used in connection with those between the α- and the β-protons provide the unambiguous assignment of rotamers I and II in the Asp and Phe side chains. The method is generally applicable to other amino acids and residues even in large peptides. A possible set of $J_g^{C,C}$ and $J_t^{C,C}$ values is proposed for the use of carbon 13-carbon 13 vicinal coupling constants in the side chain conformational studies of amino acid residues with a free carboxyl group.

INTRODUCTION :

The determination of peptides conformation constitues an essential step towards the understanding of their mode of action. The early stage of such an analysis was predominated by X-rays studies which yielded parameters which excelled by far those of solution. Nowadays, multinuclear magnetic resonance associated to a better chemistry allows for an improved approach of peptide conformation in solution, especially when isotopically labelled amino acids are inserted in peptides.

This report, following our previous studies on several 85 % ^{13}C enriched free amino acids and peptides [1-9], constitutes an application of the joint use of ^{13}C-^{13}C and ^{1}H-^{1}H vicinal coupling constants for evaluating the side chain arrangement of amino acids.

By choosing the compounds 85 % ^{13}C-aspartic acid, 85 % ^{13}C-phenylalanine and {85% ^{13}C-Asp}-Pro and Gly-Pro-{85% ^{13}C-Phe} our major objects were : (i) to

---

[+] to whom correspondence should be addressed.

0006-291X/80/220751-08$01.00/0

establish a set of parameters J_g and J_t for ^{13}C-^{13}C coupling constants over three bonds in side chains being as reliable as the one proposed by Pachler for ^1H-^1H coupling constants [10] ; (ii) to ascertain the assignment of the two protons attached to the β-carbon and then yield unambiguous the assignment of rotamers in side chains ; (iii) to show the specific steric effects of the proline residue, either cis or trans, on the immediately before and next residues in the sequence ; (iv) to learn about the factors responsible for the conformational changes of the side chains by varying the ionization state of the molecules.

MATERIALS AND METHODS :

The uniformly ^{13}C labelled amino acids Asp and Phe were obtained in our laboratory as previously reported [2]. The preparation of the ^{13}C enriched peptides {85% ^{13}C-Asp}-Pro and Gly-Pro-{85% ^{13}C-Phe} was tested and scaled down with natural abundance ^{13}C amino acids by liquid phase synthesis.
The ^{13}C NMR spectra were recorded at 22.63 MHz in the Fourier transform mode under broad band proton decoupling on a Bruker WH90 spectrometer equipped with a Aspect 2000 computer (16K data points) and a ^{13}C homodecoupler.
The concentration of the samples in ^2H_2O (99.8% ^2H, C.E.A.) was about 0.1 M.
The ^{13}C-^{13}C coupling measurements were performed with a digital resolution of 0.25 Hz or 0.125 Hz. ^{13}C-{^{13}C} decoupling was used to supress additional geminal couplings obscuring the vicinal coupling constants $^3J_{C'-C\gamma}$ (related to the X^1 dihedral angle) on the carbonyl carbon signals.
The ^1H NMR spectra were recorded at 250 MHz in the Fourier transform mode on a Cameca TSN 250 spectrometer equipped with a Nicolet 1080 computer (16K data points). Samples (0.05 M in ^2H_2O) were studied at a digital resolution of 0.37 Hz.
In the two peptides, Asp-Pro and Gly-Pro-Phe, the ratios of the cis-trans isomerism of proline were estimated from the ^{13}C and ^1H signal intensities.

RESULTS AND DISCUSSION :

A - Spectral analysis :

a) Proton NMR spectra of the unlabelled compounds

At all the pH examined, the $C^{\beta}H_2$ resonances of the two amino acids and residues are well resolved in a typical ABX system the analysis of which provides the two vicinal coupling constants $H^{\alpha X}$-$H^{\beta A}$ and $H^{\alpha X}$-$H^{\beta B}$ related to the X^1 dihedral angle. Iteration of these values was performed by the ITRCAL programme (Nicolet 1080 package). However, the uncertainty in the assignment of the two β-resonances to their respective protons prohibits the unambiguous attribution of the fractions of rotamers I and II (Fig. 1).

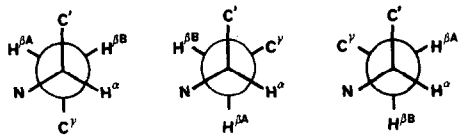

Figure 1. Staggered rotamers around C^{α}-C^{β} : R_I (χ^1:-60°), R_{II} (χ^1:180°), R_{III} (χ^1:60°). $^3J_{H^{\alpha}-H^{\beta A}}$ and $^3J_{H^{\alpha}-H^{\beta B}}$ are related to R_I and R_{II}. $^3J_{C'-C^{\gamma}}$ is related to R_I.

b) Carbon 13 NMR spectra of the labelled compounds

Analysis of the ^{13}C spectrum of Gly-Pro-{85% ^{13}C-Phe} (Fig. 2) shows the C' signals of the phenylalanine residue enriched with ^{13}C at 85 % in the tripeptide at acidic pH. A straightforward analysis of the spectrum gives us (i) the chemical shifts of the C' atom of Phe in the cis and trans conformers from the center of the two triplets ($\delta_{C'}$ = 175.49 ppm trans form ; $\delta_{C'}$ = 175.26 ppm cis form) ; (ii) the fractions of cis and trans proline isomers from the ratio of the intensities of the two triplets (trans : 80 % ; cis : 20 %) ; (iii) the C'-C^{α} (one bond) coupling constant ($\sim$ 60 Hz) in Phe ; (iv) the C'-C^{γ} (three bonds) coupling constant in Phe for the trans (2.9 Hz) and the cis (3.8 Hz) conformers.

B - Coupling constants and fraction of rotamers in the free amino acids Asp and Phe in the three ionization states :

^1H-^1H and ^{13}C-^{13}C coupling constants as well as fraction of rotamers for Asp

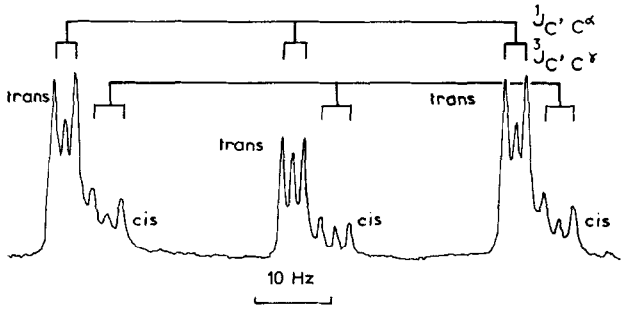

Figure 2. High resolution ^{13}C NMR spectrum of Gly-Pro-{85% ^{13}C-Phe} in the C' region showing the signals of the cis and trans conformers.

Table 1. Vicinal coupling constants and rotamers of aspartic acid and phenylalanine side chains in the free amino acids at different ionization states

	Ionic State	^3J (Hz) H^{α}-$H^{\beta A}$	^3J (Hz) H^{α}-$H^{\beta B}$	^3J (Hz) C'-C^{γ}	Rotamer Populations I	II	III	ΔR_I
ASPARTIC ACID	+	6.4	4.2	2.5	0.35 / 0.42	0.15	0.50	+0.07
	±	8.7	3.8	3.2	0.56 / 0.57	0.11	0.33	+0.01
	-	9.9	4.0	3.9	0.67 / 0.73	0.13	0.20	+0.06
PHENYLALANINE	+	7.6	5.7	2.4	0.46 / 0.40	0.28	0.26	-0.06
	±	7.9	5.2	2.7	0.48 / 0.47	0.24	0.28	-0.01
	-	7.3	5.6	2.4	0.43 / 0.40	0.27	0.20	-0.03

ΔR_I : difference of rotamer I populations as obtained by carbon-13 carbon 13 and proton-proton vicinal coupling constants, respectively.
Rotamer populations were calculated using the sets : J_g = 2.6 Hz, J_t = 13.6 Hz (proton-proton); J_g = 0.6 Hz, J_t = 5.1 Hz (carbon-carbon).

and Phe are listed in Table 1. Assignment of $H^{\beta A}$ and $H^{\beta B}$ and fraction of rotamers are similar to those found for these two amino acids respectively by Hansen et al. [11], who used the vicinal coupling constants between the carbonyl carbon and the β-protons jointly to the H^{α}-$H^{\beta A,B}$ coupling constants, and by Kainosho and Ajisaka [12] on the basis of stereoselective deuteration in the $C^{\beta}H_2$ fragment. The ^{13}C-^{13}C set of values J_t = 5.1 Hz and J_g = 0.6 Hz has been established by considering experimental and calculated values on carboxylic acids [13,14] and other free amino acids [3,7,8] to take into account the substituent effects of the carboxylic group. However, no serious attempt of estimating the absolute magnitudes of J_g and J_t for $^3J_{C'-C^{\gamma}}$ has been done and the validity of the set partially reposes on the comparison between numerous experimental $^3J_{H^{\alpha}-H^{\beta A}}$ and $^3J_{C'-C^{\gamma}}$ values as well as on the limits of confidence of the values J_t = 13.6 Hz and J_g = 2.6 Hz proposed by Pachler for H^{α}-H^{β} coupling constants [10]. Yet, Table 1 shows that the two coupling

constants $^{3}J_{H^{\alpha}-H^{\beta A}}$ and $^{3}J_{C'-C^{\gamma}}$, both directly related to the fraction of rotamer I, vary in the same direction when the pH is changed and that at the three ionization states the agreement between the fraction of rotamer I determined from ^{1}H-^{1}H and ^{13}C-^{13}C coupling constants is good for the two amino acids, aromatic and carboxylic.

It is fair to mention that London et al. [15] proposed on theoretical grounds the set $J_{g}^{C,C}$ = 1.2 Hz and $J_{t}^{C,C}$ = 4.5 Hz for Asp. Several values of rotamer fractions in Asp and Phe determined from this set are in rather good agreement with our values. However, this does not seem to fit very well to the peptides studied in this work.

C - Coupling constants in the Asp and Phe residues for the cis and trans conformers of proline containing peptides :

The problem was then to verify whether the ^{13}C set estimated for the amino acids was suitable for the Asp residue at the N-terminal position (free carboxylic group in the side chain) and for Phe at the C-end position (free carboxylic group).

Data for Asp and Phe, respectively in the dipeptide Asp-Pro and the tripeptide Gly-Pro-Phe, are reported in Tables 2 and 3. We find again that the variations of the C'-C^{γ} coupling constants parallel those of H^{α}-$H^{\beta A}$ and that the agreement between the fraction of rotamer I determined by the two techniques is good. We can already conclude on the validity of using jointly the two types of couplings for discriminating rotamer I and II in such peptides.

However, the results allow several comments concerning the conformational properties of the peptides. First of all, we note that in the two peptides the trans conformer prevails over the cis conformer in almost all the cases. In these two conformers the arrangement of the side chain of the residues under study is not the same : this underlines the specific effects of cis and trans proline in peptides. In addition, we find that the strongest stabilization of rotamer I in Asp and Phe does not occur in the same conformer since for Asp (dipeptide) the rotamer I is the most populated in the trans conformer

Table 2 - Vicinal coupling constants and rotamers of the aspartic acid side chain in the dipeptide Asp-Pro.

	Form %	Ionic state	^3J (Hz) H^{α}-$H^{\beta A}$	H^{α}-$H^{\beta B}$	C'-C^{γ}	Rotamer populations I	II	III	ΔR_I
TRANS	83	+	9.0	4.0	3.7	0.59 / 0.67	0.13	0.28	+0.08
	58	±	11.2	2.9	4.5	0.79 / 0.87	0.03	0.18	+0.08
	50	-	10.6	3.4	4.0	0.73 / 0.75	0.08	0.19	+0.02
CIS	17	+	8.0	5.6	3.0	0.50 / 0.53	0.28	0.22	+0.03
	42	±	8.8	5.2	3.0	0.57 / 0.53	0.24	0.19	-0.04
	50	-	6.4	8.0	1.9	0.35 / 0.29	0.50	0.15	-0.06

ΔR_I : difference of rotamer I populations as obtained by carbon 13-carbon 13 and proton-proton vicinal coupling constants, respectively. Rotamer populations were calculated using the sets of Table 1.

and for Phe (tripeptide) the rotamer I is now the most populated in the cis conformer. Such effects may be reasonably explained by the difference of the

Table 3. Vicinal coupling constants and rotamers of the phenylalanine side chain in the tripeptide Gly-Pro-Phe.

	Form %	Ionic state	^3J (Hz) H^{α}-$H^{\beta A}$	H^{α}-$H^{\beta B}$	C'-C^{γ}	Rotamer Populations I	II	III	ΔR_I
TRANS	80	+	8.6	6.0	2.9	0.55 / 0.51	0.31	0.14	-0.04
	75	±	7.2	5.5	2.3	0.42 / 0.37	0.27	0.31	-0.05
	65	-	7.4	5.5	2.3	0.44 / 0.37	0.27	0.29	-0.07
CIS	20	+	11.2	5.0	3.8	0.79 / 0.71	0.21	0.00	-0.08
	25	±	10.0	4.6	3.3	0.67 / 0.60	0.19	0.14	-0.07
	35	-	10.2	4.6	3.3	0.70 / 0.60	0.19	0.11	-0.10

ΔR_I : difference of rotamer I populations as obtained by carbon-13 carbon 13 and proton-proton vicinal coupling constants, respectively. Rotamer populations were calculated using the sets of Table 1.

steric effects exerced by cis and trans proline towards the side chains of the adjacent residues. Yet, the nature of the side chains of Asp and Phe as well as the size of the peptides must be considered. In this respect charge effects are significant since noticeable modifications occur in the molecules when the pH is changed. These concern both the proportions of cis and trans conformers and side chain rotamers. In the dipeptide the effects are the most important very likely for the presence of the ionizable carboxylic group in the side chain of Asp and also for the close proximity of the N- and C- terminal groups. The trends in the repartitions of rotamers in the Asp residue, as far as the trans conformer is concerned, mimic well however those found in the free amino acids, with rotamers I and III prevailing over rotamer II in the three ionization states. The low fraction of rotamer II is explained by the geometrical impossibility in this rotamer of interactions between the side chain and the nitrogen of the amino group. In contrast, both $^{3}J_{H^{\alpha}-H^{\beta A}}$ and $^{3}J_{C'-C^{\gamma}}$ in the cis conformer indicate that in the anionic state rotamer II becomes preponderant over the other two rotamers. The increase of rotamer II should be explained by interactions occuring between the two terminal groups in the cis conformer [4] preventing the interaction between the carboxylate group of the Asp side chain and the amino group. As the proportions of cis conformer and rotamer II increase concomitantly (both are at their highest level in the anionic state) this gives additional support to the idea of interactive forces between the N- and C- terminal groups in the cis conformer.

CONCLUSION :

In conclusion, the results indicate that the ^{13}C-^{13}C three bonds coupling constants are helpful for the conformational analysis of side chains in free amino acids and residues.

When used jointly, $^{1}H^{\alpha}$-$^{1}H^{\beta}$ and $^{13}C'$-$^{13}C^{\gamma}$ coupling constants discriminate between rotamer I and rotamer II even in difficult cases such as the conformational mixtures exhibited by the proline containing peptides. Undoubtely, ^{13}C-^{13}C coupling constants offer an alternative way for studies of side chain

conformations in peptides having overcrowded ^1H NMR spectra. However, the $J_g^{C,C}$ and $J_t^{C,C}$ values proposed in this study apply exclusively to residues having a free carboxylic group. For other residues and especially those in non terminal position another set must be considered [8,9].

REFERENCES :

1. Piriou,F.,Lintner,K.,Fermandjian,S.,Fromageot,P.,Khosla,M.C.,Smeby,R.R.,and Bumpus,F.M. (1980) Proc.Nat.Acad.Sci.USA 77,82-86.
2. Tran-Dinh,S.,Fermandjian,S.,Sala,E.,Mermet-Bouvier,R.,Cohen,M. and Fromageot, P. (1974) J.Amer.Chem.Soc. 96,1484-1493.
3. Tran-Dinh,S.,Fermandjian,S.,Sala,E.,Mermet-Bouvier,R. and Fromageot,P. (1975) J.Amer.Chem.Soc. 97,1267-1269.
4. Fermandjian,S.,Tran-Dinh,S.,Savrda,J.,Sala,E.,Mermet-Bouvier,R.,Bricas,E., and Fromageot,P. (1975) Biochim.Biophys.Acta 399,313-338.
5. Haar,W.,Fermandjian,S.,Vicar,J.,Blaha,K.,and Fromageot,P. (1975) Proc.Nat. Acad.Sci.USA 72,4948-4952.
6. Vicar,J.,Abillon,E.,Toma,F.,Piriou,F.,Lintner,K.,Blaha,K.,Fromageot,P.,and Fermandjian,S. (1979) FEBS Lett. 97,275-278.
7. Piriou,F.,Toma,F.,Savrda,J.,and Fermandjian,S. (1979) Tetrahedron 35,441-446.
8. Fermandjian,S.,Piriou,F.,Toma,F.,Lam-Thanh,H.,Lintner,K.,Vicar,J., and Fromageot,P. (1978) in : "Proc.European Conference on NMR of Macromolecules", (Conti,F.,ed.) Lerici,Sassari-Sardinia,pp.229-242.
9. Fermandjian,S.,Piriou,F.,Lintner,K.,Toma,F.,Lam-Thanh,H.,and Fromageot,P. (1979) in :"Peptides : Structure and Biological Function", Proc. Sixth American Peptide Symp.,(Gross,E.,and Meienhofer,J.,eds.), Pierce Chem.Co., Rockford,Illinois,pp.205-208.
10. Pachler,K.G.R. (1964) Spectrochim.Acta 20,581-587.
11. Hansen,P.E.,Feeney,J.,and Roberts,G.C.K. (1975) J.Magn.Resonance 17, 249-261.
12. Kainosho,M.,and Ajisaka,K. (1975) J.Amer.Chem.Soc. 97,5630-5631.
13. Marshall,J.L.,and Miller,D.E. (1973) J.Amer.Chem.Soc. 95,8305-8308.
14. Barfield,M.,Burfitt,I.,and Doddrell,D.(1975) J. Amer.Chem.Soc. 97, 2631-2634.
15. London,R.E.,Walker,T.E.,Kollman,V.H.,and Matwiyoff,N.A. (1978) J.Amer. Chem.Soc. 100,3723-3729.