AB INITIO AND DFT SEARCH FOR CONFORMATIONAL TRANSITION STATES OF N-FORMYL-L-PROLINAMIDE

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Dedicated to Prof. Imre G. Csizmadia on the occasion of his 75th birthday

Abstract

The ω cis-trans isomers, backbone conformers (α-L, ε-L and γ-L) and syn-anty ring puckered structures of formyl L-prolinamide were studied at the RHF/3-21G, RHF/6-31G(d) and RB3LYP/6-31G(d) level of theory. In addition single point calculations using a more accurate and extended basis set (aug-cc-PVDZ) were carried out. The barrier heights for cis-trans isomerization fell in the range of 19.58 to 24.6 Kcal/mol and those for the interconversion between backbone conformations were in the range of 0.61-5.56 Kcal/mol. The barrier heights for syn-anty ring puckering were found within 1.79 to 7.46 Kcal/mol at the aug-cc-pVDZ//RHF/3-21G level of theory.

Resumen

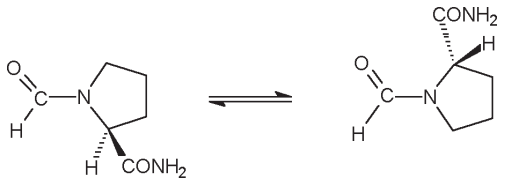
Empleando cálculos RHF/3-21G, RHF/6-31G(d) y RB3LYP/6-31G(d) se estudiaron los isómeros ω cis-trans, los conformeros de la cadena principal (α-L, ε-L y γ-L) y las estructuras plegadas syn-anty del anillo para la formil L-prolinamida.

Además se realizaron cálculos de punto único empleando un grupo de bases más flexible (aug-cc-PVDZ). Las alturas de las barreras para la isomerización cis-trans fueron encontradas en el rango de 0,61-5,56 Kcal/mol. El valor de la barrera para el arrugado del anillo syn-anty se encontró entre 1,79 to 7,46 Kcal/mol al nivel de teoría aug-cc-pVDZ// RHF/3-21G.

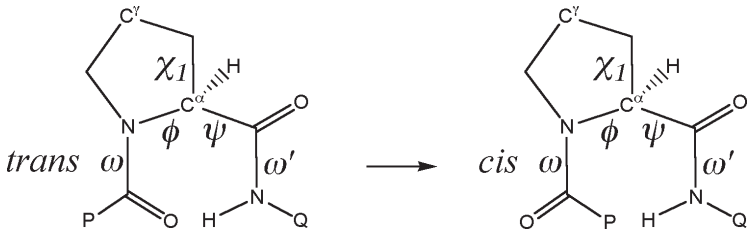
Introduction

The *cis-trans* isomerization of peptidyl-proline.

The evolution of the understanding of the *cis-trans* isomerization of a proline residue in a peptide chain has a long history [1]. Such a residue may well be modelled by **N**-formyl-L-Prolinamide (eq.1). The *cis-trans* isomerization of **N**-formyl-Prolinamide is expected to be an equilibrium between structures of similar stabilities since the $-CONH_2$ substituent, on the pyrrolidine ring, is expected to be a relatively minor perturbation on the equilibrium (eq. 2). With this in mind it is not surprising that more proline residues in proteins occupy the *cis* isomeric state than in the case of others naturally occurring amino acid [2].



In fact, in proteins, proline amides display a similar tendency, assuming both the *cis* and *trans* conformations [2] in a protein chain (eq. 3) :



Since only proline amides possess this conformational flexibility, it has been considered that the *cis-trans* proline isomerization plays many important biochemical roles. These include controlling the rate of protein folding [3], initiating receptor-mediated transmembrane signalling [4], recognition of peptide antigens [5], and regulating the activation as well as the breakdown of peptide hormones [6]. For this reason the *cis-trans* isomerization of the proline residue is of utmost importance.

Mechanistic background of the *cis-trans* isomerization

As early as 1958 it was proposed [7] that protonation of the amide nitrogen would change the hindered rotation due to the partial double bond character of the peptide bond to a nearly free rotation of the N-protoned peptide bond. Later the mechanism was extended to involve **O**-

protonation followed by **N**-deprotonation [8]. In the mean time it has been noticed that certain types of enzymes called rotamases can catalyse the *cis-trans* isomerization [9].

Maigret, Perahia and Pullman [10] pioneered the computational study of the *cis-trans* isomerization of prolyl residues in 1970. The theoretical work implies by definition the study of a gas-phase process. Thus any energetics (thermodynamic or kinetic), that may be obtained, represent intrinsic properties without the influence of any environmental factors. These authors were the first to present the conformations for the *cis* and *trans*-isomers of **N-** and **C-**protected proline in terms of potential energy curves:

$$E = f(\psi) \tag{4}$$

And the *cis-trans* isomerization as a potential energy surface (PES):

$$E = F(\psi, \omega) \tag{5}$$

Subsequently, Farmer and Hopfinger [11] also presented the *cis-trans* isomerization in terms of a PES.

Karplus and coworkers [12] pointed out how important is the pyramidalization of the amide nitrogen to the process of *cis-trans* isomerization. He also emphasised that while the barrier to unimolecular isomerization, in solution, may be in the order of 19-20 Kcal/mol rotamerase enzymes can reduce such a barrier to 5-6 Kcal/mol. More recently Kang [13] reported that the calculated rotational barriers for the *trans*-to-*cis* and *cis*-to *trans* isomerization are in the order of 19.0 and 18.8 Kcal/mol at the RHF/6-31G(d) level in water.

Conformational Analysis of the Proline Residue

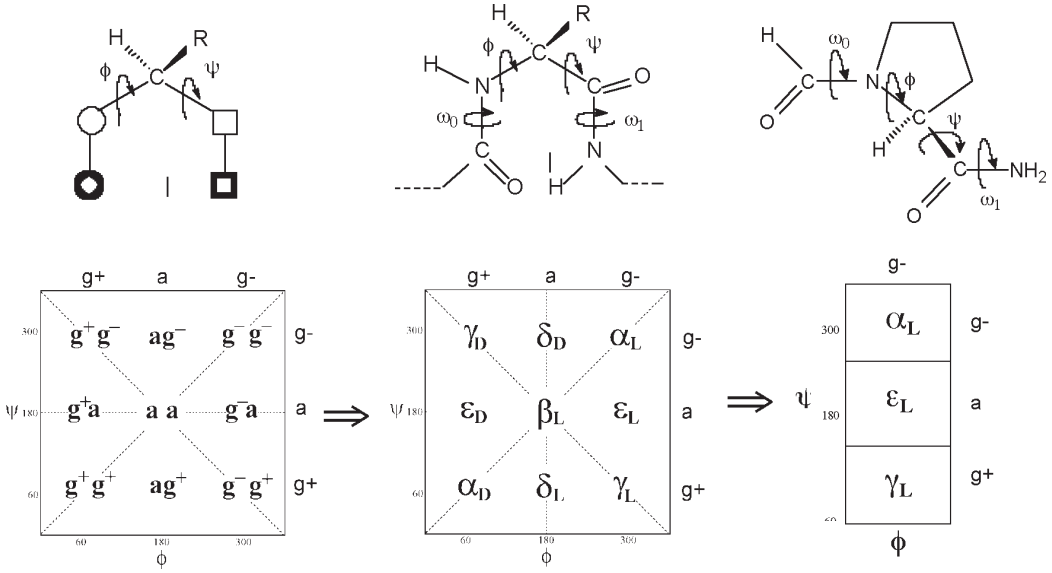


Figure 1. *Conformational characteristics of double rotors*
A. *Conformational PES of a general double rotor,*
B. *Conformational PES of an amino acid diamide as specific example for a double rotor*
C. *Conformational PES for a proline diamide as specific example for a restricted double rotor. (Note: restriction on ϕ is due to ring closure).*

Proline differs from all naturally occurring amino acids in the sense that it does not have an N-H bond but instead the side chain of proline forms a five-member ring with the nitrogen. One of the consequences of the ring closure is that the pyrrolidine ring will permit only one ϕ value in the vicinity of the ***g-*** (i.e. -60º or 300º). Of course ψ may assume three different discrete values ***g+*** (i.e. +60º), ***a*** (i.e. 180º) and ***g-*** (-60º or 300º). As a result of this limitation, instead of nine, only three discrete conformers may be expected as illustrated in Figure 1**.**

Another stereochemical consequence is that the pyrrolidin ring is not planar but puckered. Thus, the CH_2 opposite to the N-C^{α} bond (denoted by C^{γ} in eq. 3) may be 'UP' or 'DOWN'. Puckering may be characterized by the sign of torsional angle χ_1 (defined as N-C^{α}-C^{β}-C^{γ}). When $\chi_1>0$, C^{γ} is in *syn*-relation with the adjacent –CNCOP moiety, while $\chi_1<0$ signals an *anti*-relationship relative to the same amide group.

The set ω, ϕ, and ψ of the backbone torsional angles (see eq.3) is more convenient for conformational studies than the set ϕ, ψ, and ω'. Considering that the pyrrolidine ring may also have two different puckers, a four-character code seems appropriate to label a proline residue. The first letter is *c* if the peptide bond preceding the residue is *cis* (i.e.$\omega \approx 0^{\circ}$) or *t* if the same bond is *trans* (i.e.$\omega \approx 180^{\circ}$). The subscripted Greek letter characterizes the backbone conformation of the residue: γ_L, ε_L or α_L signal $\psi \approx 60^{\circ}$, 180° or -60°. The last character of the conformational code is either '–'or '+' opposing the sign of the phase angle *P* of the ring (calculated as described in reference [14]) and thus reflecting the sign of χ_1.

Potential energy curves of the type $E=f(\psi)$ and frequencies calculations revealed [15] that the α_L and ε_L conformations correspond to very shallow minima. It is not surprising therefore that at a higher level of theory they have disappeared due to the fact that these levels of theory usually provide smoother potential energy surfaces.

Methods of calculation

Ab initio Hartree-Fock and density functional geometry optimizations have been carried out using the Gaussian 03 program system [16]. Two basis sets 3-21G and 6-31G(d) were employed at the Hartree Fock (HF) level of theory and the B3LYP types of DFT procedure were applied using the larger basis set 6-31G(d) only. The energy-optimised outputs obtained with RHF/3-21G calculations were used as the input geometries for aug-cc-pVDZ single point calculations. This most reliable and flexible basis set was used to better evaluate the energies and energy gap among the critical points.

The relative energies (ΔE_{rel}) were calculated with respect to the γ_L *syn* ring puckering *trans* backbone conformations.

The statistical distribution analysis of a total of 7466 Pro residues were collected from 1135 non-homogenous protein [17,18]. All entries included in this study have high-resolution X-ray structures taken from the 1996 issue of the Brookhaven Protein DataBase (PDB) [19,20].

Results and Discussion

As it has been pointed out previously [14] the *syn* puckered form of proline diamide (c.f. Figure 2) is more stable than the *anti* puckered (down) structure. For this reason we carried out a 1D-scan, varying ψ, at the HF/3-21G level of theory, on both *cis*- and *trans*- **N**-formyl-L-prolinamide. The two potential curves of the type:

$$E = E(\psi)$$

for the *cis*- and *trans* isomers with *syn* puckering are presented in Figure 3.

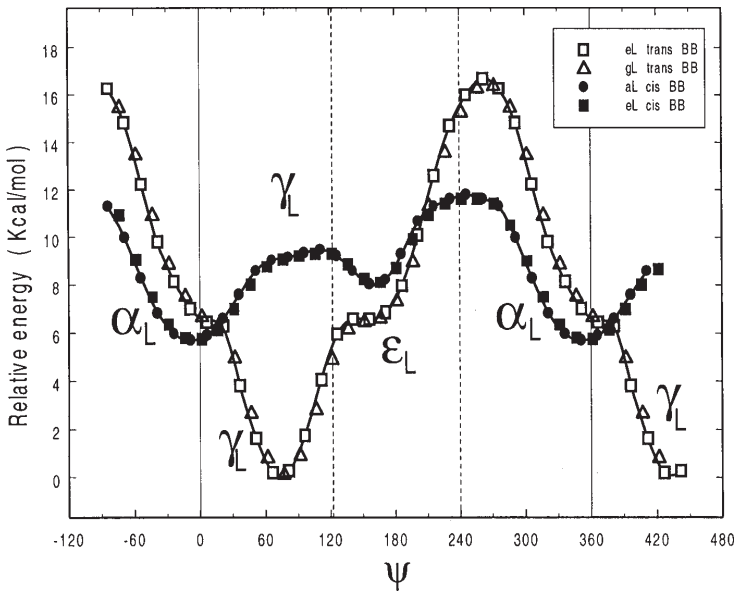


Figure 2. *Potential energy curve of the type E = E(ψ) for N-formyl prolinamide with syn (up) ring puckered form. Solid symbols obtained for cis-peptide bond while open symbols computed for trans- peptide bond.*

From these two potential energy curves it is clear that the *trans* peptide bond containing formyl-L-proline amide has the γ_L conformation as its global minimum and a very shallow local minimum at the ε_L conformation. In contrast, for the *cis*-isomer the γ_L conformation is completely annihilated and the *cis*-form has α_L as its global minimum and ε_L as its local minimum. All of these results are for the *syn* puckered form.

Observing the *cis* and *trans* isomers with *anti* (-) puckering, the global minimum for ω c-*trans* is the γ_L, conformation and α_L is a local minimum. In the case of ω c*is*, there are three low-energy conformers: γ_L, α_L and ε_L, the α_L form being the global minimum.

Potential energy surfaces, involving the ring puckering coordinate and ψ, were generated for each of the *cis* and *trans* backbones. The energy contour diagrams are shown in Figures 4 and 5 and the PES landscape representations are given in Figures 6 and **7** for the *trans* and *cis* isomers respectively.

These surfaces reconfirm the findings provided by the E = E(ψ) potential energy curves, namely, there are two minima for *cis*- and two minima for *trans*- peptide in the *syn* (UP) ring puckered form of formylprolinamide. However, the surfaces also show that there are 2 plus 2 minima for the *anti* puckered form.

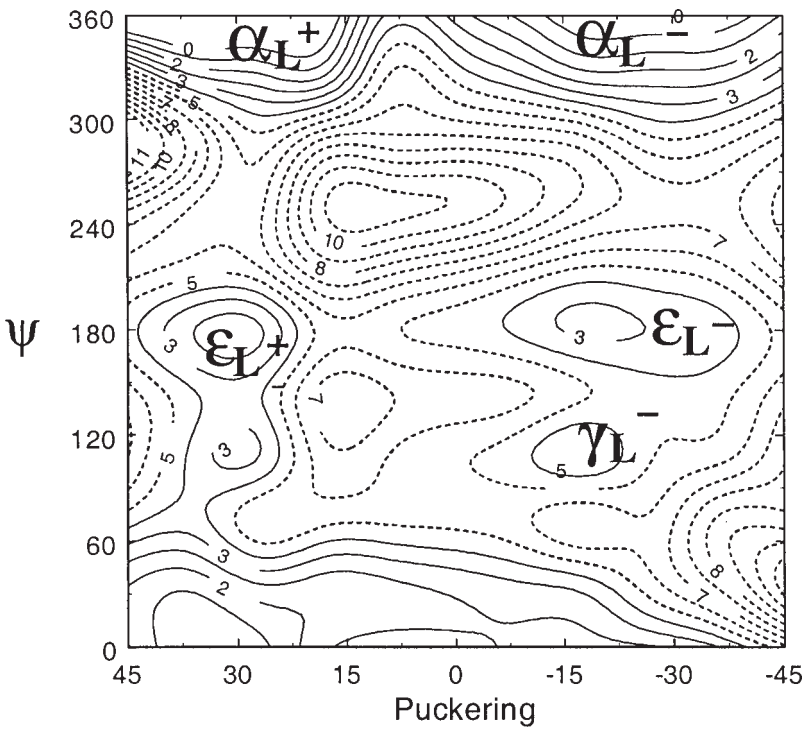


Figure 3. *Energy contour diagram (HF/3-21G) of* ***For-L-Pro-NH$_2$*** *as a function of ψ and ring puckering with trans backbone. Contour lines up to 10 Kcal/mol are solid lines, above 10 Kcal/mol broken lines. A positive and negative puckering means syn and anti arrangement respectively.*

The other important surface representations of the *cis- trans* isomerization is

$$E = f(\psi, \omega)$$

This surface is shown in energy contour representation in Figure 8 for *syn* ring puckering and Figure 9 for *anti* ring puckering. The potential energy landscape is given in Figure 10 and Figure 11 for *syn* and *anti* puckering respectively.

Table 1 shows the optimised structures at various levels of theory. It should be noted that only a minute deviation was found between the torsion angle values at RHF/6-31G(d) and RB3LYP/6-31G(d) when compared to those found at RHF/3-21G level. On the basis of these results it appears that *ab initio* calculations using a modest basis set (RHF/3-21G) are enough for an exploratory and preliminary conformational analysis. A series of single-point energy calculations using the RHF/3-21G geometries were performed for the low-energy conformations, to investigate the effects of the basis set (Table 2). With the aid of these results the 3D-potential energy hypersurface may be constructed showing the conformational topomerization of **N**-formyl prolinamide (Figure 12).

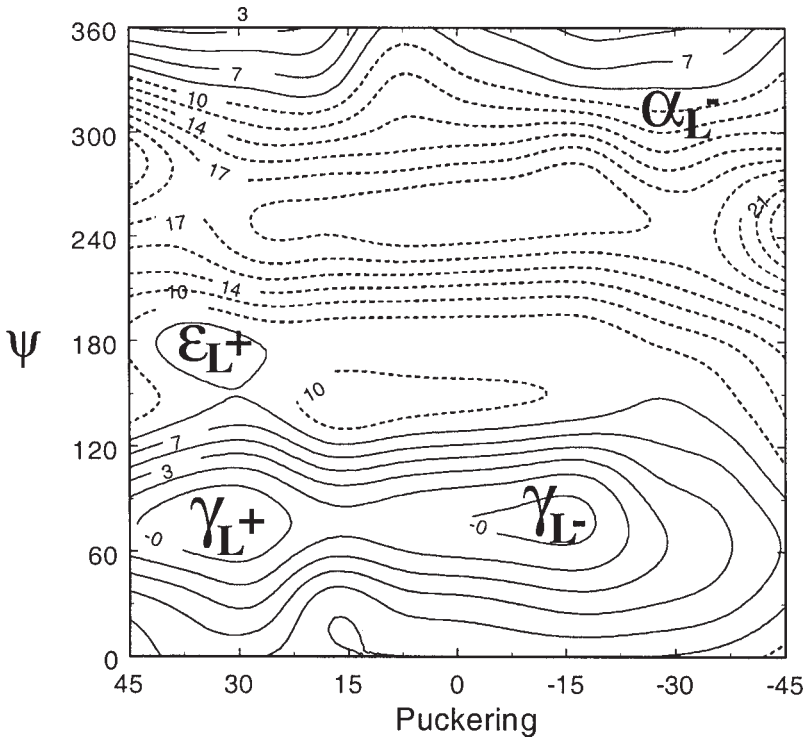


Figure 4. *Energy contour diagram (HF/3-21G) of* ***For-L-Pro-NH$_2$*** *as a function of ψ and ring puckering with cis backbone. Contour lines up to 5 Kcal/mol are solid lines, above 5 Kcal/mol broken lines. A positive and negative puckering means syn and anti arrangement respectively.*

According to the pattern given in Figure 12 we have twelve different transition states (TS) which may be grouped into three categories:

3 TS (TS_1, TS_{11} and TS_{12}) between the conformers of the *trans*-isomers

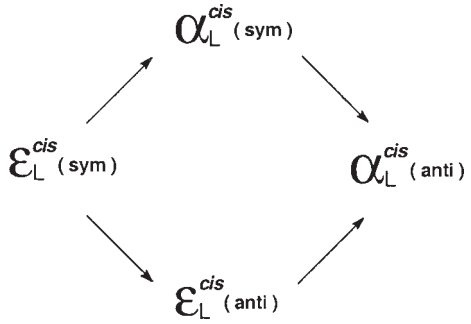
6 TS (TS_3-TS_7, TS_9) between the conformers of the *cis*-isomers

3 TS (TS_2, TS_8 and TS_{10}) associated with the *cis-trans* isomerization

process.

The computed and relative energies of these twelve TS are summarised in Table 2. These TS were obtained from single-point calculations using more accurate extended basis set (aug-cc-pVDZ).

Figure 13 shows the topomerization energy profile as going from γ_L^{trans}(*syn*) all the way around and back to γ_L^{trans}(*syn*). For the *trans*-level there is only one mechanism but for the *cis*-level there are a pair of competing mechanism as illustrated below



The energy profile given in Figure 13 clearly distinguishes these two alternate paths.

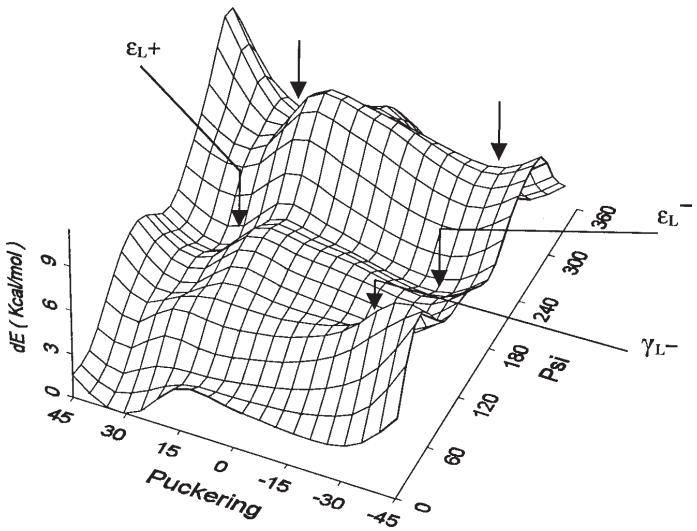


Figure 5. *Potential energy landscape of* ***For-L-Pro-NH$_2$*** *as a function of ψ and ring puckering with trans backbone computed by HF/3-21G.*

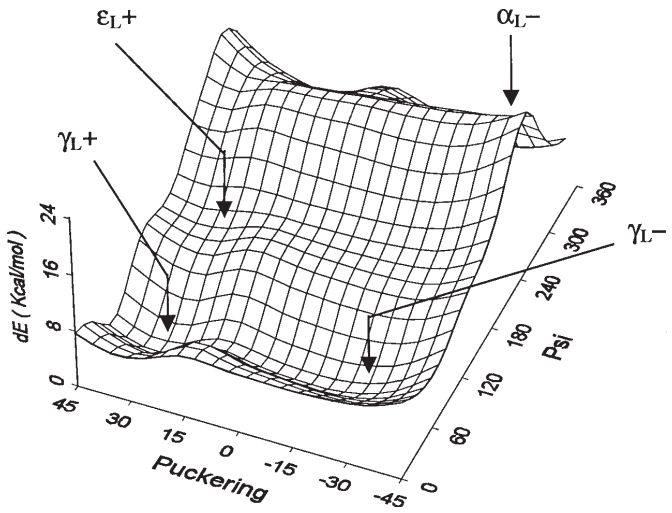


Figure 6. *Potential energy landscape of* ***For-L-Pro-NH$_2$*** *as a function of ψ and ring puckering with cis backbone computed by HF/3-21G.*

The identification of conformations of single amino acid residues is becoming increasingly used in studies on the tertiary structures on peptides. The validity of this type of calculations may be assessed by comparing the experimental data with those derived from theoretical calculations. Using a X-ray and NMR-determined protein data set of non homologous proteins we generated a population distribution map ploting ϕ against ψ. The statistical distribution of proteins as illustrated for *trans*- and *cis*-proline residues in proline are shown in Figures 13 and 14 respectively. While clearly the occurrence of the *trans*-isomer is overwhelming, nevertheless, the presence of the *cis*-isomer is significant. The calculated rotational barrier of 19.58 Kcal/mol for the *cis—trans* isomerization at the aug-cc-pVDZ//RHF/3-21G level is in good agreement with NMR experimental values of 20.4 and 19.8 Kcal/mol [21]. In addition our results are in agreement with those previously reported using RHF/6-31G(d) level in water [13]. Observing the results shown in Figures 13 and 14 it is possible to appreciate that there is a good agreement between these experimental results with our theoretical results.

In summary, the overall results clearly indicate that *cis-trans* isomerization is possible with barriers height no higher than 24.6 kcal/mol at the aug-cc-pVDZ//RHF/3-21G level of theory. In solutions reactions which have a barrier less than 25 kcal/mol occur spontaneously. Also it is interesting to remark that the *cis-trans* isomerization involves not only the twist of ω but a co-ordinated mechanism involving the torsional angle ψ as well as the ring puckering ω.

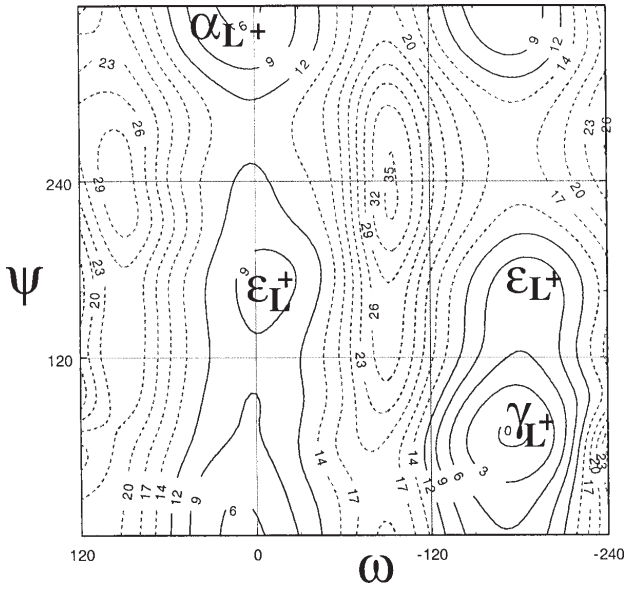


Figure 7. *Energy contour diagram E = F(ψ,ω) at the (HF/3-21G) level of theory of* ***For-L-Pro-NH$_2$*** *with syn puckering. Contour lines up to 12 Kcal/mol are solid lines, above 12 Kcal/mol broken lines.*

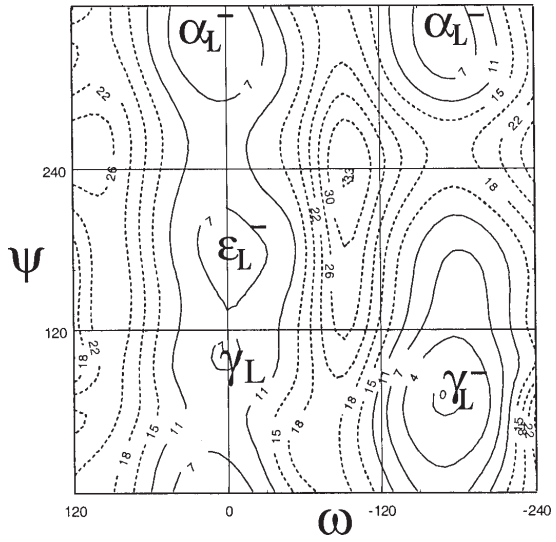


Figure 8. *Energy contour diagram E = F(ψ,ω) at the (HF/3-21G) level of theory of* ***For-L-Pro-NH$_2$*** *with anti puckering. Contour lines up to 12 Kcal/mol are solid lines, above 12 Kcal/mol broken lines.*

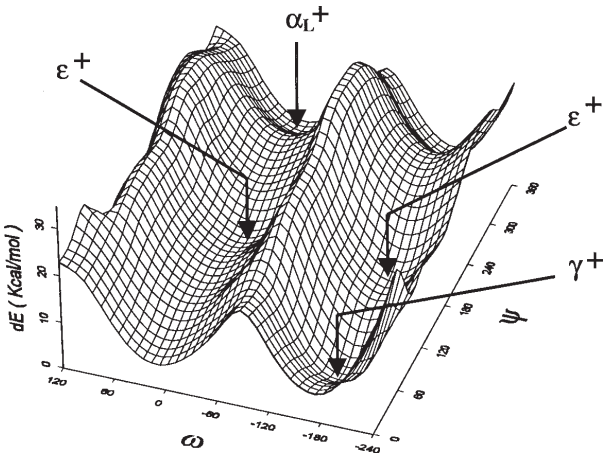


Figure 9. *Potential energy, E = F(ψ,ω), landscape for syn puckering of* ***For-L-Pro-NH$_2$***

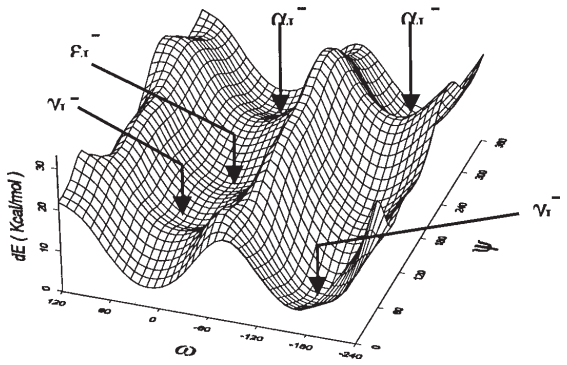


Figure 10. *Potential energy, E = F(ψ,ω), landscape for anti puckering of* ***For-L-Pro-NH$_2$***.

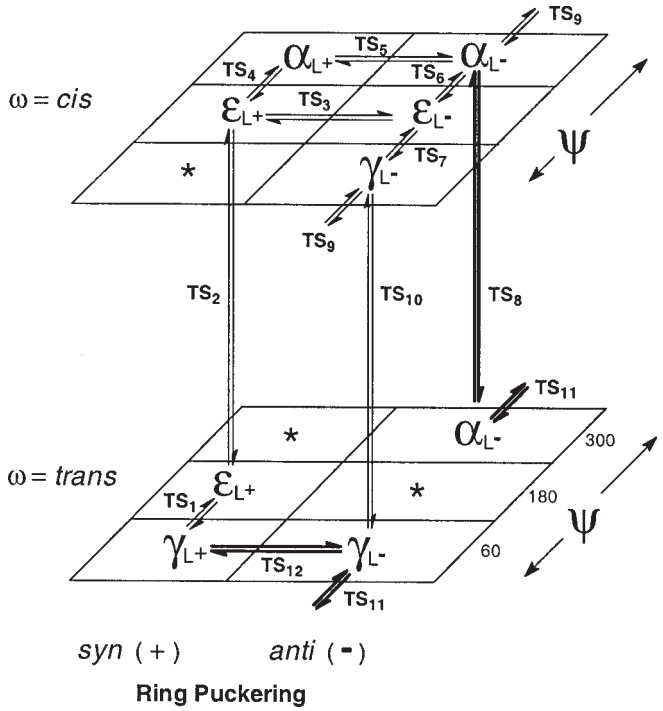


Figure 11. *Topology of Potential energy hypersurface including ring puckering as well as rotation about ψ and ω for* ***For-L-Pro-NH***$_2$. *The low-energy pathway for cis-trans topoisomerization is denoted with arrows in bold.*

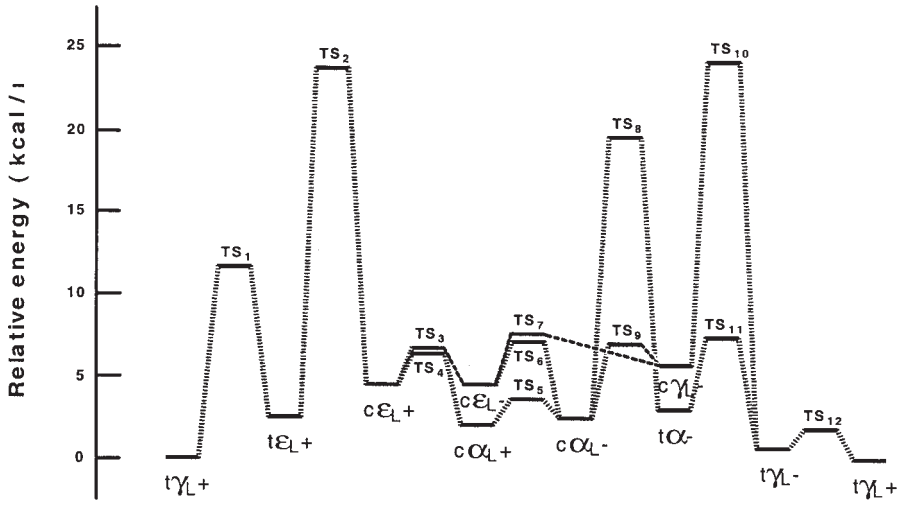


Figure 12. *Topomerization energy profile obtained at aug-cc-pVDZ//RHF/3-21G level for* ***For-L-Pro-NH***$_2$, *including trans-cis isomerization.*

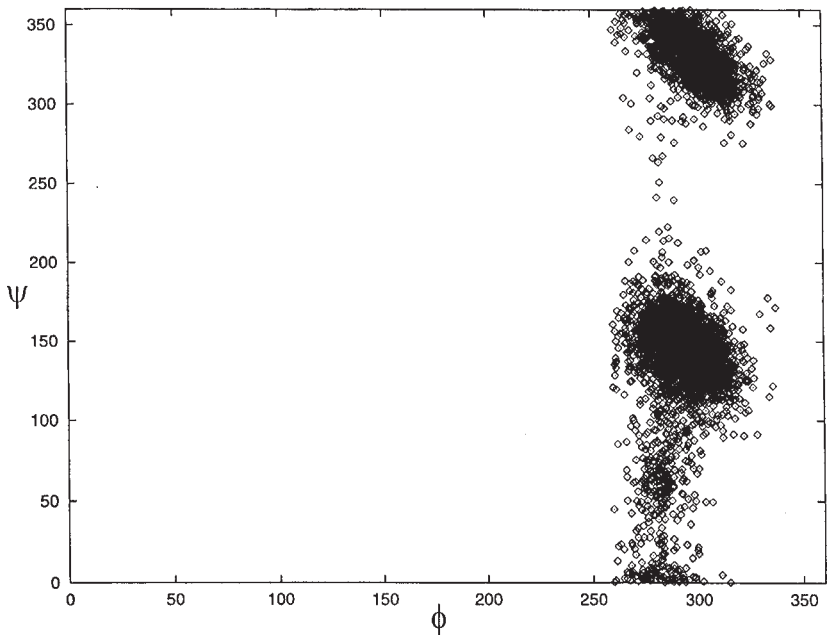


Figure 13. *PDB occurrance of trans-proline residue in proteins.*

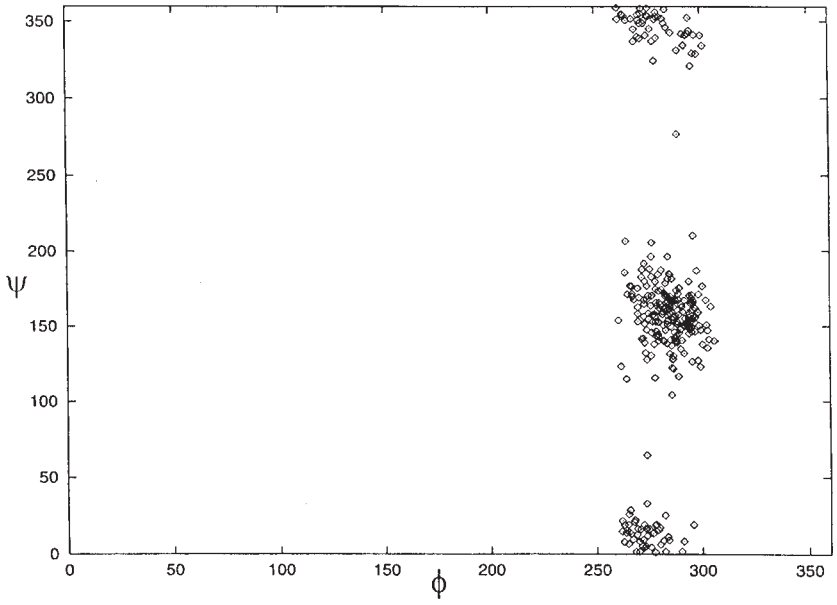


Figure 14. *PDB occurrance of cis-proline residue in proteins.*

Theoretical calculations reported here are in agreement with the statistical distribution of proteins. This is a clear experimental indication of the fact that the *cis-trans* isomerization of proline is part of the physical reality.

Table 1. *Torsional angles, total energy and energy gap of critical points of N-formylprolinamide computed at various levels of theories and several geometry for the optimized backbone in syn and anti ring puckered conformations.*

Conf.	Energy [a]	ω_0 [b]	φ [b]	ψ [b]	χ [b]	ω_1 [b]	A [b]	P [b]	ΔE [c]
HF/3-21G									
$t\alpha_L+$				Not found					
$t\varepsilon_L+$	-488.943851	175.81	-70.98	150.62	32.22	178.25	39.79	-113.00	6.59
$t\gamma_L+$	-488.954348	-172.98	-83.28	67.96	33.59	-178.84	39.84	-112.46	0.00
$t\alpha_L-$	-488.941003	-173.05	-74.49	-23.44	-24.82	-179.21	39.13	83.32	8.37
$t\varepsilon_L-$				Not found					
$t\gamma_L-$	-488.951951	-171.71	-82.79	68.77	-12.75	178.87	38.13	109.52	1.50
$c\alpha_L+$	-488.945374	10.24	-96.36	2.02	33.12	179.97	39.93	-115.40	5.63
$c\varepsilon_L+$	-488.941638	-7.77	-62.41	172.36	31.87	178.53	40.14	-109.52	7.98
$c\gamma_L+$				Not found					
$c\alpha_L-$	-488.943170	9.37	-67.45	-26.13	-28.74	-176.84	38.83	75.08	7.01
$c\varepsilon_L-$	-488.940219	-3.72	-51.16	178.52	-27.31	179.30	38.51	80.76	8.87
$c\gamma_L-$	-488.937928	1.98	-59.67	101.35	-27.04	-178.03	38.43	83.37	10.30
HF/6-31G(d)									
$t\alpha_L+$				Not found					
$t\varepsilon_L+$				Not found					
$t\gamma_L+$	-491.689586	-172.16	-84.90	73.81	28.03	-173.09	37.24	-111.61	0.00
$t\alpha_L-$	-491.683753	-164.28	-79.23	-12.57	-24.95	172.91	38.44	89.45	3.66
$t\varepsilon_L-$				Not found					
$t\gamma_L-$	-491.687963	-170.46	-83.94	78.83	-13.52	-171.19	38.24	103.38	1.02
$c\alpha_L+$	-491.684991	10.92	-91.73	-2.47	32.93	179.39	37.49	83.37	2.88
$c\varepsilon_L+$	-491.681233	-5.24	-67.37	164.21	30.64	177.62	36.56	-116.43	5.24
$c\gamma_L+$				Not found					
$c\alpha_L-$	-491.683803	10.51	-72.00	-19.88	-23.87	-176.72	37.49	83.37	3.63
$c\varepsilon_L-$	-491.680538	-3.71	-51.07	167.36	-22.35	-179.69	37.28	83.16	5.68
$c\gamma_L-$				Not found					
B3LYP/6-31G(d)									
$t\alpha_L+$				Not found					
$t\varepsilon_L+$				Not found					
$t\gamma_L+$	-494.638904	177.10	-81.01	70.36	29.64	-173.55	37.35	-109.60	0.00
$t\alpha_L-$				Not found					
$t\varepsilon_L-$				Not found					
$t\gamma_L-$	-494.638557	-170.01	-81.76	73.56	-14.21	-171.95	37.82	104.73	0.22
$c\alpha_L+$	-494.631996	11.91	-96.73	5.51	28.97	178.39	37.48	-116.63	4.33
$c\varepsilon_L+$	-494.628113	-2.80	-71.84	-148.14	29.02	-178.47	36.05	-116.55	6.77
$c\gamma_L+$				Not found					
$c\alpha_L-$	-494.630785	11.52	-74.47	-19.23	-21.81	179.06	37.45	86.79	5.09
$c\varepsilon_L-$	-494.627543	1.53	-56.71	118.97	-21.74	-177.44	37.36	79.32	7.13
$c\gamma_L-$				Not found					

[a] *in Hartree,* [b] *in degree,* [c] *in kcal/mol.*

Table 2. *Total energy values and energy gap obtained for the critical points of N-formylprolinamide computed at aug-cc-pVDZ//RHF/3-21G level of theory.*

Conf. Minimun	**Energy** [a]	**ΔE** [b]
$t\alpha_L+$	Not found	
$t\varepsilon_L+$	-491.753306	2.48
$t\gamma_L+$	-491.757262	0.00
$t\alpha_L-$	-491.752586	2.93
$t\varepsilon_L-$	Not found	
$t\gamma_L-$	-491.756282	0.62
$c\alpha_L+$	-491.754106	1.98
$c\varepsilon_L+$	-491.750223	4.42
$c\gamma_L+$	Not found	
$c\alpha_L-$	-491.753456	2.39
$c\varepsilon_L-$	-491.750112	4.48
$c\gamma_L-$	-491.748254	5.65
Transition states		
TS_1	-491.738695	11.65
TS_2	-491.719523	23.68
TS_3	-491.746900	6.50
TS_4	-491.747020	6.42
TS_5	-491.745372	7.46
TS_6	-491.746030	7.04
TS_7	-491.751590	3.55
TS_8	-491.726045	19.58
TS_9	-491.746272	6.89
TS_{10}	-491.718920	24.06
TS_{11}	-491.745543	7.35
TS_{12}	-491.754403	1.79

[a] *in Hartree,* [b] *in kcal/mol.*

Table 3. *Torsional angles and total energy values of N-formylprolinamide computed at various levels of theories and several geometry optimized backbone (BB) in anti ring puckered conformations. The calculated relative energies (ΔE_{rel}) and stabilization energies (ΔE_{stabil}) are also shown.*

BB	Peptide Bond	ω_0 [a]	ϕ [a]	ψ [a]	ω_1 [a]	E [b]	ΔE [c]	ΔE_{stabil} [c]
	HF/3-21G							
α_L	*Cis*	9.37	-67.45	-26.13	-176.84	-488.943170	7.01	+1.90
α_L	*Trans*	-173.05	-74.49	-23.44	-179.21	-488.941002	8.37	+3.26
ε_L	*Cis*	-3.72	-51.16	+178.52	+179.30	-488.940219	8.86	+3.76
ε_L	*Trans*			Not				found
		—	—					
γ_L	*Cis*			Not				found
		—	—					
γ_L	*Trans*	-171.71	-82.79	+68.77	-178.87	-488.951950	1.50	-3.61
	HF/6-31G(d)							
α_L	*Cis*	+10.51	-72.00	-19.88	-176.72	-491.683803	3.63	+2.16
α_L	*Trans*			Not				found
		—	—					
ε_L	*Cis*	-3.71	-51.07	+167.36	-179.69	-491.680535	5.68	+4.21
ε_L	*Trans*			Not				found
		—	—					
γ_L	*Cis*			Not				found
		—	—					
γ_L	*Trans*	-170.46	-83.94	+78.83	-171.19	-491.687963	1.02	-0.45
	B3LYP/6-31G(d)							
α_L	Cis	+11.52	-74.47	-19.23	+179.06	-494.630785	5.66	+5.03
α_L	*Trans*			Not				found
		—	—					
ε_L	*Cis*	+1.53	-56.71	+118.97	-177.44	-494.627543	7.69	+7.07
ε_L	*Trans*			Not				found
		—	—					
γ_L	*Cis*			Not				found
		—	—					
γ_L	*Trans*	-170.01	-81.76	+73.56	-171.95	-494.638557	0.78	+0.16

[a] *degree,* [b] *hartree,* [c] *Kcal mol* $^{-1}$

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