# Solvent effect on population distribution of conformers of N-acetyl L-prolyl N′-methylamide

L-Proline (L-Pro) is one of the naturally occurring twenty amino acid constituents of proteins. It is the only amino acid which has a side chain cyclising on the backbone nitrogen building a pyrrolidine moiety. This structural feature restricts the conformational space of the amino acid backbone with Φ, –90° to –30° and Ψ, –60° to 180° approximately[1] from Φ, Ψ angle distributions for 4415 Pro residues with backbone *B*-factor <30 from the 500-structures of high-resolution database. The unique structure has attracted the attention of researchers[2–6] for conformational studies of L-Pro. We have studied the population distribution of N-acetyl L-prolyl N′-methylamide (AceProNMe) as a model system. QM/MM methods are applied to study the conformational preferences of AceProNMe in vacuum and in different solvents. Population distribution of the conformers is observed to be correlated with the polarity function of the solvent. The results suggest that surface area and volume of the conformers may be important factors in determining the solvent effect.

The model for AceProNMe in beta-strand conformation is built using database module of HyperChem Pro8.0 and optimized by molecular mechanics with Amber99 force field using Polak-Ribiere conjugate gradient method in vacuum terminating at 0.01 kcal mole$^{-1}$ Å$^{-1}$ RMS gradient. Conformational search module of HyperChem is applied with pyrrolidine ring flexibility (0° to ±60°) and variation of Ψ torsional angle of Pro between 0° and ±180°. The conformers are chosen by random walk using Metropolitan criterion, using 300 K switched to 2000 K after 15 repeated or 30 rejected conformers. An optimization termination criterion is set for RMS gradient 0.01 kcal mole$^{-1}$ Å$^{-1}$ and maximum 1000 cycles. Random seeding is adapted from the computer clock. Conformational search is terminated after 1000 optimizations or 100,000 iterations. The variation of Φ torsional angle is included in the ring flexibility. The conformers with local energy minima in the Ramachandran landscape are further subjected to quantum mechanical optimization for more accurate energy and geometry using ORCA software[7]. DFT method is used with B3LYP functional and 6-311G++ basis set in vacuum or conductor-like screening model (COSMO) of solvents with various dielectric constants, i.e. polarity function (Table 1). The molecular properties namely, surface area and volume are calculated using the QSAR module of HyperChem. Population distributions of conformers are computed by the Boltzman distribution law

$$P_i = [g_i\, e^{-(E_i/RT)}]/[\sum g_j\, e^{-(E_j/RT)}],$$

where $g_i$ is the degeneracy of $i$th conformer, $E_i$ the energy of $i$th conformer, $R$ the gas constant, $T$ the temperature in Kelvin and $P_i$ is the fraction of population of $i$th conformer.

Polarity function of a solvent is defined as

$$f(\varepsilon, n) = (\varepsilon - 1/2\varepsilon + 1) - (n^2 - 1/2n^2 + 1),$$

where $\varepsilon$ and $n$ are the dielectric constant and refractive index of the solvent respectively[8].

AceProNMe with Φ(C–N–Cα–C′), Ψ(N–Cα–C′–N′), $\chi_1$(N–Cα–Cβ–Cγ) values (–74.95°, 29.45°, 32.00°); (–78.29°, 45.99°, 34.33°); (–71.72°, 1.44°, 28.55°); (–55.97°, 1.40°, –26.89°) and (–70.41°, 152.89°, 28.91°) are obtained as local minima in the Ramachandran landscape[9]. These conformers are subjected to optimization by quantum mechanical method. There is only one minimum in vacuum with Φ, Ψ, $\chi_1$ values (–81.47°, 69.28°, 31.45°). It is designated as gamma conformer. However, in solvent media, three minima for conformers are obtained: one in right handed α-helical region (Φ ~ –80° and Ψ ~ –15°), one in gamma (Φ ~ –82° and Ψ ~ 70° to 75°), and the other in polyproline II (PPII) region (Φ ~ –75° and Ψ ~ 150°) of Ramachandran landscape (Table 1).

The effect of solvents on molecular spectra is discussed in terms of solvent polarity functions since 1950s (refs 8, 10–12). Different conformations of an *N*-terminal Pro–Pro segment in polypeptides are reported to be dependent on the nature of the solvents[13]. Zhao and Xia[14] have reported that energy barrier of isomers of rhodamine dyes correlate linearly with the solvent polarity. In the present study we establish a quantitative relationship between the solvent polarity function and population distribution of AceProNMe (Figure 1).

The amino acid residues in a protein experience different environment with different dielectric constants. For example, dielectric constant in a protein cavity is ~5.0 similar to the solvent chloroform whereas the dielectric constant at the surface of a protein is ~80.0 similar to that of water. The membrane spanning residues experience the dielectric constant similar to that of *n*-hexane.

**Table 1.** Parameters of geometrically optimized conformers of AceProNMe in vacuum and solvents

| Medium | Polarity function | Conformers | Energy in au | Φ | Ψ | $\chi_1$ |
|---|---|---|---|---|---|---|
| Vacuum | 0.000 | α-helical | – | – | – | – |
| | | Gamma | –573.257633 | –81.25 | 69.28 | 31.45 |
| | | PPII | – | – | – | – |
| Chloroform | 0.148 | α-helical | –573.258039 | –86.03 | –9.73 | 30.43 |
| | | Gamma | –573.261240 | –82.05 | 64.68 | 31.35 |
| | | PPII | –573.259981 | –74.33 | 146.40 | 29.97 |
| Ethanol | 0.288 | α-helical | –573.265538 | –83.79 | –15.17 | 29.49 |
| | | Gamma | –573.266867 | –81.94 | 69.63 | 31.29 |
| | | PPII | –573.266770 | –74.62 | 149.38 | 29.30 |
| DMSO | 0.309 | α-helical | –573.260422 | –83.87 | –14.95 | 29.44 |
| | | Gamma | –573.263191 | –82.11 | 63.53 | 31.35 |
| | | PPII | –573.261751 | –73.51 | 149.09 | 29.39 |
| Water | 0.320 | α-helical | –573.258226 | –83.05 | –16.84 | 29.51 |
| | | Gamma | –573.258553 | –82.28 | 64.77 | 31.34 |
| | | PPII | –573.260096 | –72.75 | 150.93 | 28.65 |

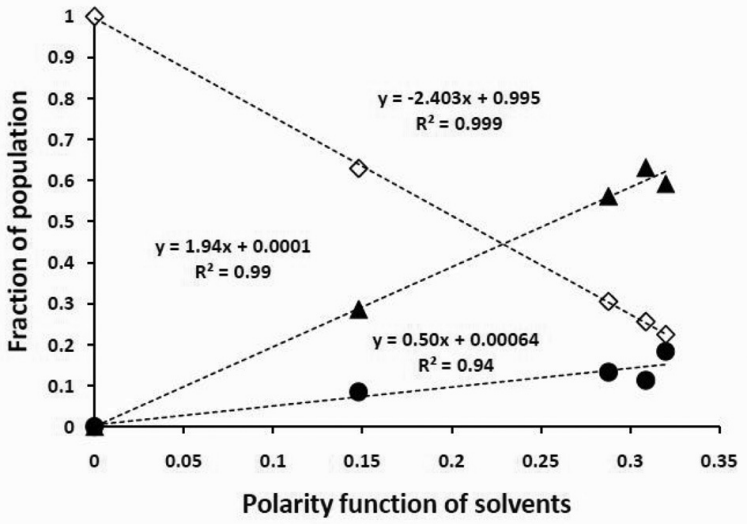


**Figure 1.** Dielectric constant of solvents exhibit correlation to the population distribution of three major conformers (◊, gamma; ▲, PPII; ●, $\alpha$-helical) of AceProNMe. Correlation equations and $R^2$ values are embedded in the figure.

The present results showed a new direction to research in the area conformational studies of amino acids and peptides. The study will be extended to conformers of all the 20 naturally occurring amino acids in the proteins and inference can be drawn on the solvent effect on the conformers of the single amino acid residues. The results may provide information on the contribution of individual amino acids to the overall conformation of peptides or proteins under the environment of different dielectric constants.

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# ICP-OES analysis of *Naja naja karachiensis* venom: inorganic ions for turning on and off enzymatic actions

Snake bite envenomation is resulting in high rates of mortality and morbidity all over the world. In developing countries like Pakistan, the burden of snake bite is hard to estimate due to the insufficient epidemiological data, as victims receive traditional health care instead of hospitalization. Four types of snakes cause most of envenomation. They are *Bungarus sindanus*, *Daboia russelii*, *Echis carinatus sochureki* and *Naja naja*[1]. *Naja naja karachiensis* is a subspecies of Pakistani *Naja naja*, which is responsible for large number of deaths in southern Punjab province of Pakistan. Among various complications caused by *Naja naja karachiensis* venom are bleeding wounds, haematuria, haemolysis, inflammation, bleeding gums, necrosis, coagulopathies, damage to liver, heart and kidneys[2]. Many toxic proteins in the venom have been considered to cause the detrimental effects of *Naja naja karachiensis* bites. Among them phospholipase $A_2$ ($PLA_2$), protease, alkaline phosphatase (ALPase), L-amino acid oxidase (LAAO), 5′-nucleotidase (5′-ND) and three finger toxins (3FTXs) are deadly venoms[1,3]. Snake venom additionally comprises inorganic constituents, which play a pivotal role in its toxicity. According to the literature survey, metallic ions are of prime importance in