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Zwitterionic conformers of pyrrolysine and their interactions with metal ions—a theoretical study

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Abstract A total of 16 pyrrolysine conformers in their zwitterionic forms are studied in gas and simulated aqueous phase using a polarizable continuum model (PCM). These conformers are selected on the basis of our study on the intrinsic conformational properties of non-ionic pyrrolysine molecule in gas phase [Das and Mandal (2013) J Mol Model 19:1695−1704]. In aqueous phase, the stable zwitterionic pyrrolysine conformers are characterized by full geometry optimization and vibrational frequency calculations using B3LYP/6-311++G(d,p) level of theory. Single point calculations are also carried out at MP2/6-311++G(d,p) level. Characteristic intramolecular hydrogen bonds present in each conformer, their relative energies, theoretically predicted vibrational spectra, rotational constants and dipole moments are systematically reported. The calculated relative energy range of the conformers at B3LYP/6-311++G(d,p) level is 5.19 kcal mol^{-1} whereas the same obtained by single point calculations at MP2/6-311++G(d,p) level is 4.58 kcal mol^{-1}. A thorough analysis reveals that four types of intramolecular H-bonds are present in the conformers; all of which play key roles in determining the energetics and in imparting the observed conformations to the conformers. The vibrational frequencies are found to shift invariably toward the lower side of frequency scale corresponding to the presence of the H-bonds. This study also points out that conformers with diverse structural motifs may differ in their thermodynamical stability by a narrow range of relative energy. The effects of metal coordination on the relative stability order and structural features of the conformers are examined by complexing five zwitterionic conformers of pyrrolysine with Cu^{+2} through their carboxylate groups. The interaction enthalpies and Gibbs energies, rotational constants, vibrational frequencies and dipole moments of the metal complexes calculated at B3LYP level are also reported. The zwitterionic conformers of pyrrolysine are not stable in gas phase; after geometry optimization they are converted to the non-ionic forms.

Introduction

Nature has expanded the genetic code by including pyrrolysine (Pyl) as the 22nd member in the family of genetically encoded amino acids. In certain methanogenic archaea and bacteria, pyrrolysine is co-translationally incorporated into protein in response to an in-frame UAG codon which in other organisms terminates the translation process of protein biosynthesis. Thus, pyrrolysine, a lysine homologue with chemical identity N^6-[(4R,5R)]-4-methyl-1-*pyrro*line-5-carbonyl]-L-*lysine*, has been recognized as the 22nd genetically encoded natural amino acid [2–8]. Known for its catalytic activities, pyrrolysine is an important constituent in the active site of methylamine methyltransferases involved in methylamine metabolism in methanogenic archaea [4, 9]. Since its discovery in 2002, there have been prolific studies to understand the biosynthetic pathway which is still unclear [10], on metal-binding affinity/selectivity of pyrrolysine [11], on the scope of synthesizing pyrrolysine analogues [12, 13] and on the intrinsic conformational properties of non-ionic pyrrolysine molecule in gas phase [1]. However, neither experimental nor theoretical investigations are carried out to date

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concerning the stability and structural features of zwitterionic conformers of pyrrolysine as well as their metal complexes, which to a large extent determine the dynamic properties and functional specificity of proteins and polypeptides containing pyrrolysine residues.

It is difficult to implement theoretical or computational approaches directly for large systems such as proteins. Therefore, over the last few decades, solitary amino acids and their analogues have served as important model systems for the computational studies concerning the structure of protein and energetics of protein folding. The low-energy structures and their related properties derived from such computations have a meaningful relationship with their presence and functional activities performed in the macromolecular context of real life systems. Besides structural knowledge, conformational flexibility is also important to understand the reactivity and biological roles of bioactive molecules [14–16]. Amino acids are highly flexible molecules since they possess many rotatable single bonds. Internal rotations around these single bonds require a very small amount of energy and therefore even at room temperature a particular amino acid molecule may change form one conformation to another [17]. Often it has been found that the conformers of an amino acid differ from one another by small energy differences though their conformations are very different [1, 18, 19]. The knowledge of different conformers of an amino acid is important since they may have different functional aspects in bio-chemical processes. Amino acids are known to exist in non-ionic forms in gas phase while as zwitterions in solvent and solid phases [19, 20]. The predominance of the zwitterionic forms of the amino acids in solid and solution phases has been attributed to electrostatic, polarization and H-bonding interactions with the solvent [21–25]. Amino acid molecules in their zwitterionic forms are of special importance since amino acids normally occur in zwitterionic forms in biological media [26–28]. Thus, it is of fundamental importance for the theoretical studies concerning conformational details of a biological molecule to include solvent effects of aqueous phase since the vast majority of biochemical processes occur in an aqueous environment. Solvation effects may be estimated either by considering the solvent molecules explicitly or alternatively by modeling the solvent as a dielectric continuum following the Onsager reaction field approach as developed by Kirkwood [29, 30]. However, on many occasions it has been found that several explicit water molecules are required to stabilize a particular zwitterionic conformer of an amino acid and consequently such quantum chemical studies become computationally expensive [31]. Moreover, the complexity of the different possible arrangements of such solvent molecules also makes such investigations complicated. As a result, the use a continuum model to simulate solvent effects has turned into an efficient

alternative approach. Computational studies on zwitterionic conformers of amino acids [31–34] have been performed with a view toward understanding various chemical and biochemical processes taking place in the macromolecular context of real life systems. It has now been realized that computational techniques are indispensable in elucidating atomic level structural information about biologically active molecules owing to certain limitations of experimental techniques as pointed out in the literature [35–37].

Here, attempts are made to obtain full knowledge about the relative stabilities of 16 different zwitterionic conformers of pyrrolysine in gas phase as well as in simulated aqueous phase; and to provide theoretical results such as rotational constants, vibrational frequencies, dipole moments and the characteristic intramolecular hydrogen bonds present in each conformer that may be helpful for future experimentalists. The effects of metal coordination on the relative stability order and structural features of the conformers are examined by complexing five zwitterionic pyrrolysine conformers, viz. Pyl-1, Pyl-c, Pyl-g1, Pyl-b and Pyl-p5, with Cu^{+2} through their carboxylate groups. These five conformers are selected on the basis of their positions in the relative stability order of the 16 different zwitterionic conformers of pyrrolysine in aqueous phase (see the discussion on the relative energies of the zwitterionic conformers of pyrrolysine offered in a succeeding section of this paper). Interaction enthalpies (metal ion binding affinities) and Gibbs energies, rotational constants, vibrational frequencies and dipole moments of the metal complexes calculated at B3LYP level are also analyzed. Figure 1 schematically represents the zwitterionic form of pyrrolysine molecule. The atom numbering and the torsion angle definitions are given in accordance with the schemes used earlier in various literatures [13, 38]. To facilitate a clear representation of the intramolecular hydrogen bond interactions present in the zwitterionic pyrrolysine conformers some of the hydrogen atoms are named as H_a, H_b or H_c. This DFT study on the zwitterionic pyrrolysine conformers and their

$\chi_a = C_4 - C_5 - C_6 - N_7$ $\chi_b = C_5 - C_6 - N_7 - C_8$
$\chi_c = C_6 - N_7 - C_8 - C_9$ $\chi_d = N_7 - C_8 - C_9 - C_{10}$
$\chi_e = C_8 - C_9 - C_{10} - C_{11}$ $\chi_f = C_9 - C_{10} - C_{11} - C_{12}$
$\chi_g = C_{10} - C_{11} - C_{12} - C_{13}$ $\chi_h = C_{11} - C_{12} - C_{13} - O_{14}$
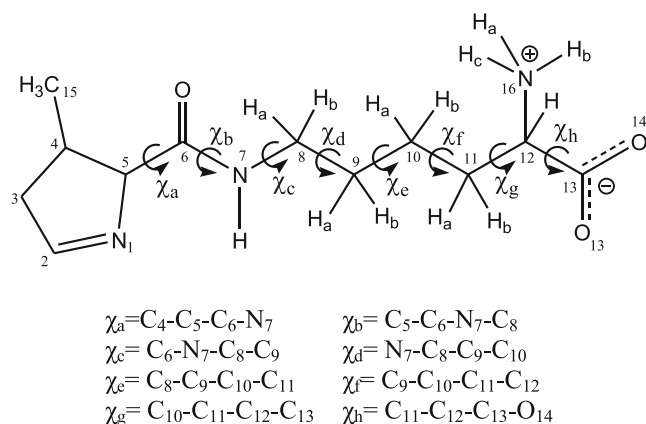
Fig. 1 Schematic representation of the eight rotatable internal backbone torsional angles in zwitterionic pyrrolysine

Table 1 Dihedral angles (in degrees) about the eight internal back-bone torsional angles of zwitterionic pyrrolysine conformers after full geometry optimization at B3LYP/6-311++G(d,p) level in aqueous phase

Conformers	χ_a	χ_b	χ_c	χ_d	χ_e	χ_f	χ_g	χ_h
Pyl-1	127.05	178.28	−97.37	179.82	178.15	−178.21	171.70	124.36
Pyl-b	−168.67	−3.59	−115.21	−177.60	179.77	−177.56	172.37	123.59
Pyl-c	127.61	−179.72	97.81	179.01	179.72	−178.81	172.18	124.40
Pyl-d1	129.83	179.11	−88.75	−63.98	−179.67	−178.12	172.71	123.70
Pyl-d2	128.86	179.76	−113.53	65.71	−179.86	−179.07	171.77	124.91
Pyl-e1	127.49	178.88	−102.62	−175.53	−69.75	−177.48	171.52	123.83
Pyl-e2	124.83	178.90	−93.08	176.43	68.73	179.84	172.80	124.67
Pyl-f1	125.89	178.52	−95.68	−179.91	−176.22	−67.42	171.93	124.40
Pyl-f2	127.54	178.39	−92.65	−179.83	172.89	67.63	160.32	122.77
Pyl-g1	124.94	179.14	−94.66	−178.40	−179.33	−179.25	−55.69	121.01
Pyl-g2	128.39	177.97	−92.61	−178.68	−179.15	178.99	70.82	102.80
Pyl-p1	129.21	177.82	−93.51	−178.56	−170.78	−68.15	−46.44	−62.43
Pyl-p2	126.53	178.45	−97.56	−178.34	175.35	67.60	69.54	−77.10
Pyl-p3	128.23	178.16	−96.56	179.43	−179.88	−92.85	64.31	−84.66
Pyl-p4	126.34	178.42	−97.74	−179.08	173.59	71.42	−65.15	−58.05
Pyl-p5	−162.68	2.12	96.88	177.44	179.40	−179.21	171.78	124.23

metal complexes is expected to provide the opportunity to know its conformational properties at an atomic level which in turn may help us to understand the dynamics and functional specificity of proteins, in discovering its biosynthetic pathway, to synthesize a new generation of pyrrolysine analogues, in understanding the nature of the genetic code or amino acid code which seems to be still evolving [39] and in enhancing this rapidly expanding area of research.

Computational methodology

The molecular geometries of all the zwitterionic pyrrolysine conformers are subjected to full geometry optimization and vibrational frequency calculations using the B3LYP/6-311++G(d,p) level of theory [40, 41] of Gaussian 09 package [42]. The efficiency of B3LYP/6-311++G(d,p) in studying conformational behavior and various other properties of amino acids

Table 2 The relative energies (kcal mol^{-1}), theoretical rotational data (GHZ) and dipole moments (D) of the zwitterionic pyrrolysine conformers at B3LYP/6-311++G(d,p) level in aqueous phase

Conformers	Relative energies		Rotational constants			Dipole moments
	ΔE_1	ΔE_2	A	B	C	
Pyl-1	0	0	1.28243	0.10052	0.09675	13.427
Pyl-c	0.24	0.02	0.94667	0.10770	0.10601	13.478
Pyl-d1	0.53	−0.52	0.83943	0.12370	0.12158	16.378
Pyl-d2	0.57	−0.62	0.83189	0.13325	0.12751	15.639
Pyl-g1	0.99	−0.36	1.16511	0.11220	0.10641	16.816
Pyl-f1	1.03	0.39	1.01296	0.11253	0.11107	16.222
Pyl-e1	1.16	0.57	1.02908	0.11614	0.10943	17.340
Pyl-e2	1.17	0.57	1.16214	0.10752	0.10394	11.024
Pyl-g2	1.25	0.62	1.25755	0.10499	0.10163	10.575
Pyl-f2	2.17	1.49	1.08358	0.11562	0.11322	16.256
Pyl-p2	2.26	0.77	0.99372	0.12596	0.11945	15.982
Pyl-p4	3.03	1.04	0.84886	0.14821	0.14413	11.623
Pyl-p1	3.22	0.88	0.97839	0.13238	0.12611	11.755
Pyl-p3	3.98	2.04	0.97111	0.13150	0.12287	9.029
Pyl-b	5.18	4.58	0.93315	0.11972	0.11192	15.157
Pyl-p5	5.19	4.04	0.77596	0.12510	0.11889	10.890

Relative energies: ΔE_1 = at B3LYP/6-311++G(d,p) and ΔE_2 = single point at MP2/6-311++G(d,p)

Table 3 H-bond distances (in angstrom) of the intramolecular H-bond interactions detected in the zwitterionic pyrrolysine conformers in aqueous phase

H-bonds	Pyl-1	Pyl-b	Pyl-c	Pyl-d1	Pyl-d2	Pyl-e1	Pyl-e2	Pyl-f1	Pyl-f2	Pyl-g1	Pyl-g2	Pyl-p1	Pyl-p2	Pyl-p3	Pyl-p4	Pyl-p5
$N_1 \cdots H-N_7$	2.17	abs	2.16	2.17	2.16	2.16	2.17	2.17	2.17	2.16	2.17	2.17	2.17	2.17	2.17	abs
$O_6 \cdots H-N_7$	abs	2.40	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	2.41
$O_6 \cdots H_a-C_8$	abs	abs	2.50	abs	2.45	abs	2.52	abs	2.53	abs	2.53	2.52	2.50	2.51	2.50	abs
$N_1 \cdots H_a-C_8$	abs	2.63	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	2.64
$O_6 \cdots H_b-C_8$	2.49	abs	abs	2.56	abs	2.47	abs	2.50	abs	2.52	abs	abs	abs	abs	abs	abs
$N_7 \cdots H_a-C_9$	2.74	2.71	2.74	abs	abs	2.69	2.70	2.72	2.72	2.73	2.74	2.74	2.73	2.73	2.73	abs
$N_7 \cdots H_b-C_9$	2.73	2.73	2.72	abs	2.73	abs	2.75	2.74	2.73	2.75	2.73	2.71	2.72	2.73	2.73	2.72
$O_{13} \cdots H_a-C_{10}$	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	2.55	abs	2.54	abs	abs	abs
$O_{13} \cdots H_b-C_{10}$	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs
$N_{16} \cdots H_a-C_{11}$	abs	abs	abs	abs	abs	abs	abs	abs	2.53	abs	2.63	2.72	2.64	2.64	abs	abs
$O_{13} \cdots H_a-C_{11}$	2.61	2.62	2.62	2.62	2.61	2.62	2.60	2.61	2.68	abs	abs	abs	abs	abs	abs	2.61
$N_{16} \cdots H_b-C_{11}$	2.73	2.74	2.73	2.73	2.73	2.72	2.74	2.71	abs	abs	2.75	abs	2.73	2.67	abs	2.73
$O_{13} \cdots H_b-C_{11}$	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs
$O_{14} \cdots H_b-N_{16}$	1.75	1.76	1.76	1.76	1.76	1.76	1.76	1.76	1.74	1.78	1.81	1.79	1.82	1.87	1.78	1.75
$O_{13} \cdots H_b-N_{16}$	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs	abs

Only the (B⋯H) distances are listed where B is H-bond acceptor; *abs* = absent

has been explained in literature [43]. The computations are conducted in gas phase as well as in solvent phase using a polarizable continuum model (PCM) [44]. The accuracy of self-consistent reaction field (SCRF) model in predicting the structure and energetics of conformers of alanine dipeptide has already been justified in literature [45]. The B3LYP hybrid functional in combination with 6-311++G(d,p) (for H, C, N and O atoms) and LANL2DZ (for Cu atoms) [46–48] basis sets is used to optimize the molecular geometries of the metal complexes. Absence of imaginary frequency values in the vibrational frequency calculations proves that all the optimized geometries are precise minima. Zero point energy (ZPE) corrections are applied to the total energies of all the conformers using a correction factor 0.9877 [49]. The vibrational frequencies below 1800 cm^{-1} are scaled with 1.01 and for those above 1800 cm^{-1} a correction factor 0.9679 is used [49]. Use of diffuse functions is important to take into account the relative diffuseness of lone pair of electrons when a molecule under investigation contains lone pair of electrons [50] while polarization functions are useful in studying the conformational aspects where stereoelectronic effects play an important role [51].

Results and discussion

With a large number of possible rotational degrees of freedom, the pyrrolysine molecule is a highly flexible molecule and it has been found that a total of 1296 different conformers may result for the non-ionic pyrrolysine molecule if all the possible combinations of rotations about the nine internal back-bone torsional angles are included [1]. The zwitterionic conformers of pyrrolysine considered in this study are selected on the basis of our previous study on the intrinsic conformational properties of non-ionic pyrrolysine molecule in gas phase [1]. The zwitterionic conformers of pyrrolysine are not stable in gas phase; after geometry optimization they are converted to the non-ionic forms. It is needless to mention that there are a number of theoretical studies which have indicated that amino acids like leucine [52], lysine [53], cysteine [33] *etc.* do not exist as zwitterions in gas phase. Table 1 lists the aqueous phase values of the internal back-bone torsional angles of all 16 stable zwitterionic conformers of the pyrrolysine molecule. Table 2 presents the aqueous phase data on their relative energies, rotational constants and dipole moments calculated at B3LYP/6-311++G(d,p) level of theory (the total energies of the conformers are given in the Supplementary information). Table 3 lists some important intramolecular H-bonds that play crucial roles in determining the energetics and in conferring the observed conformations to the zwitterionic conformers of pyrrolysine in aqueous phase. Table 4 lists some of the characteristic frequency and intensity values (given in brackets) of the 16 conformers calculated at the B3LYP/6-311++G(d,p)

Table 4 Frequencies (cm^{-1}) and IR intensities (km mol^{-1}) of various vibrational modes obtained from the theoretical vibrational spectra of zwitterionic pyrrolysine conformers in aqueous phase. Intensity values are given in brackets

Vibrational modes	$\nu(N_7{-}H)$	$\nu(C_6{=}O)$	$\nu(C_2{=}N_1)$	$\nu(C_6{-}N_7)$	$Sis(N_{16}{-}H)$	$\nu(C_8{-}H_a)$	$\nu(C_8{-}H_b)$	$\nu(C_9{-}H_a)$	$\nu(C_9{-}H_b)$	$\nu(C_{11}{-}H_a)$	$\nu(C_{11}{-}H_b)$	$\nu(C_5{-}C_4)$	$\gamma(N_{16}{-}H)$
Pyl-1	3455 (140)	1684 (371)	1705 (112)	1572 (498)	1646 (87)	2947 (45)	3003 (35)	2959 (86)	2922 (14)	2988 (12)	2916 (11)	812 (8)	256 (12)
Pyl-b	3452 (37)	1688 (905)	1696 (69)	1515 (68)	1646 (68)	2930 (73)	3035 (17)	2965 (85)	2922 (73)	2988 (34)	2918 (8)	813 (10)	275 (3)
Pyl-c	3456 (141)	1685 (749)	1704 (124)	1574 (487)	1648 (108)	3004 (34)	2947 (65)	2959 (80)	2921 (67)	2989 (11)	2918 (7)	812 (7)	290 (19)
Pyl-d1	3463 (144)	1687 (620)	1704 (119)	1567 (505)	1647 (109)	2952 (8)	3003 (26)	2966 (121)	2923 (62)	2989 (14)	2919 (15)	810 (7)	270 (12)
Pyl-d2	3446 (137)	1686 (595)	1705 (115)	1575 (471)	1644 (90)	3007 (20)	2944 (57)	2958 (96)	2922 (127)	2987 (17)	2916 (11)	810 (3)	293 (2)
Pyl-e1	3450 (142)	1685 (654)	1704 (117)	1575 (480)	1647 (74)	2941 (52)	3016 (22)	2960 (67)	2923 (22)	2987 (14)	2931 (17)	815 (0.7)	265 (5)
Pyl-e2	3428 (110)	1684 (590)	1704 (118)	1572 (486)	1648 (100)	3006 (28)	2957 (38)	2964 (53)	2923 (17)	2932 (40)	2919 (80)	812 (4)	293 (6)
Pyl-f1	3456 (140)	1685 (684)	1705 (115)	1571 (490)	1643 (73)	2950 (73)	3003 (30)	2962 (72)	2924 (47)	2989 (14)	2927 (27)	813 (9)	279 (10)
Pyl-f2	3457 (143)	1686 (499)	1705 (118)	1574 (496)	1648 (105)	3004 (36)	2953 (44)	2963 (104)	2917 (21)	2989 (8)	2917 (21)	813 (12)	305 (2)
Pyl-g1	3458 (141)	1686 (568)	1705 (120)	1573 (493)	1651 (124)	2950 (90)	3011 (40)	2962 (65)	2922 (38)	2931 (7)	2987 (37)	812 (11)	297 (19)
Pyl-g2	3458 (142)	1685 (573)	1704 (118)	1571 (495)	1647 (63)	3001 (45)	2954 (55)	2949 (30)	2923 (88)	2910 (24)	2946 (27)	811 (9)	258 (7)
Pyl-p1	3455 (143)	1686 (567)	1705 (116)	1571 (502)	1644 (113)	3005 (33)	2953 (40)	2966 (79)	2916 (10)	2928 (21)	2985 (24)	812 (11)	280 (4)
Pyl-p2	3455 (140)	1685 (571)	1704 (121)	1572 (491)	1638 (134)	2946 (36)	3005 (33)	2959 (71)	2919 (43)	2912 (33)	2950 (19)	812 (10)	261 (11)
Pyl-p3	3455 (140)	1684 (518)	1705 (110)	1572 (489)	1636 (170)	3004 (45)	2948 (67)	2928 (32)	2928 (32)	2956 (36)	2919 (33)	811 (12)	262 (9)
Pyl-p4	3455 (139)	1685 (924)	1704 (117)	1573 (494)	1649 (101)	2946 (64)	3005 (38)	2981 (30)	2933 (28)	3000 (17)	2985 (25)	810 (19)	291 (12)
Pyl-p5	3465 (43)	1691 (896)	1694 (69)	1444 (93)	1652 (122)	3015 (23)	2951 (45)	2960 (88)	2924 (60)	2989 (19)	2924 (60)	814 (12)	291 (9)

The frequencies below 1800 cm^{-1} are scaled with 1.01 and for those above 1800 cm^{-1} a correction factor 0.9679 is used

Vibrational modes: ν = stretching; γ = rocking; Sis = scissoring; s = symmetric; as = asymmetric

Table 5 Calculated relative energies (kcal mol^{-1}), interaction enthalpies ΔH (kcal mol^{-1}) and Gibbs energies ΔG (kcal mol^{-1}), theoretical rotational data (GHZ) and dipole moments (D) of the metal complexes of the conformers in aqueous phase

Complexes	Relative energies	ΔH	ΔG	Rotational constants			Dipole moments
				A	B	C	
Pyl-1-Cu	0	−9.05	−4.05	0.70590	0.06392	0.06082	18.399
Pyl-c-Cu	0.20	−9.09	−3.73	0.51678	0.07126	0.06643	18.385
Pyl-g1-Cu	0.68	−9.36	−4.61	0.53029	0.08310	0.07376	21.981
Pyl-p5-Cu	5.20	−9.04	−3.42	0.46549	0.08104	0.07368	15.409
Pyl-b-Cu	5.40	−8.83	−2.29	0.79205	0.06648	0.06334	19.917

level. Table 5 presents the relative stability order, interaction enthalpies and Gibbs energies, rotational constants and dipole moments of the metal complexes of the zwitterionic conformers of pyrrolysine calculated at B3LYP level while Tables 6 and 7 list their eight back-bone dihedral angles and data on the vibrational spectra (intensity values are given in brackets) respectively. The optimized structures of the conformers are depicted in Figs. 2 and 3 while their theoretical IR spectra are reported in the Supplementary information (scaled with a correction factor 0.9679). Figure 4 schematically represents the chemical structure of Pyl-1-Cu complex in aqueous phase.

Conformations and relative energies

Intramolecular H-bonds are the strongest non-covalent interactions that play important roles in stabilizing the different conformations of an amino acid molecule. The number of intramolecular H-bonds and the strength of these interactions are the two important factors responsible for bringing differences in relative energies among the various conformers of an amino acid. The strength of these H-bonds depends on two factors, (a) the shorter the distance A–H…B is than the sum of their van der waals radii and (b) closer the angle A–H…B to 180° [18], where A–H is H-bond donor and B is H-bond acceptor. In the case of zwitterionic pyrrolysine molecule the COO$^-$, NH$_3^+$, N-atom of the imine group, C$_6$=O and N$_7$—H bonds of the amide linkage and all the CH$_2$ groups participate actively in intramolecular H-bond formation stabilizing the various conformers. The H-bond combinations in zwitterionic

pyrrolysine conformers are complex and various types of intramolecular H-bonds may coexist in one conformer. A thorough analysis reveals that four types of intramolecular H-bonds, namely O…H—N, N…H—N, O…H—C and N…H—C, are present in the conformers (Table 3). All these H-bonds play key roles in determining the energetics of the conformers. The relative energies of the 16 zwitterionic conformers shown in Table 2 are determined relative to the energy of Pyl-1 which is predicted as the most stable conformer at B3LYP/6-311++G(d,p) level of theory. Pyl-p5 is the least stable in the relative stability order with an energy difference of 5.19 kcal mol^{-1} compared to Pyl-1. The single point calculations at MP2/6-311++G(d,p) level reveal that the stability order of the conformers depends upon the level of theory used. However, the range of relative energies calculated at the two level of theories are very similar; 5.19 kcal mol^{-1} at B3LYP/6-311++G(d,p) while 4.58 kcal mol^{-1} when MP2/6-311++G(d,p) is used (listed in Table 2). It has been pointed out that full geometry optimization of gaseous tryptophan conformers at B3LYP/6-311G(d,p) and MP2/6-311++G(d,p) levels do not produce any noticeable structural changes, only the conformer energies change by small amounts [54]. Therefore, it is expected that the conformations of the 16 conformers predicted at B3LYP/6-311++G(d,p) level of theory will not change even if higher level of quantum mechanical theories are used; only the conformer energies may change a little. The data on the energetics of the conformers presented in Table 2 suggest that many of the conformers differ from one another by small energy differences though their conformations are very different (see the discussion on the vibrational spectra of the

Table 6 Dihedral angles (in degrees) about the eight internal back-bone torsional angles of the metal complexes of the zwitterionic pyrrolysine conformers after full geometry optimization in aqueous phase

Complexes	χ_a	χ_b	χ_c	χ_d	χ_e	χ_f	χ_g	χ_h
Pyl-1-Cu	127.61	177.48	−93.85	179.70	179.03	−179.48	171.61	123.97
Pyl-c-Cu	128.85	179.99	97.10	178.62	178.62	179.66	173.13	123.74
Pyl-g1-Cu	125.53	178.88	−100.32	−179.52	178.56	179.60	−56.66	127.25
Pyl-p5-Cu	−163.58	1.51	97.84	175.93	177.97	178.71	170.53	124.02
Pyl-b-Cu	−168.53	0.60	−132.24	−178.89	177.88	179.80	172.38	121.35

Table 7 Frequencies (cm^{-1}) and IR intensities (km mol^{-1}) of various vibrational modes obtained from the theoretical vibrational spectra of the metal complexes of the conformers in aqueous phase. Intensity values are given in brackets

Vibrational Modes	$\nu(N_7$—H)	$\nu(C_6$=O)	$\nu(C_2$=N$_1$)	$\nu(C_6$—N$_7$)	Sis(N$_{16}$—H)	$\nu(C_8$—H$_a$)	$\nu(C_8$—H$_b$)	$\nu(C_9$—H$_a$)	$\nu(C_9$—H$_b$)	$\nu(C_{11}$—H$_a$)	$\nu(C_{11}$—H$_b$)	$\nu(C_5$—C$_4$)	$\gamma(N_{16}$—H)
Pyl-1-Cu	3456 (135)	1685 (591)	1705 (117)	1570 (507)	1669 (394)	2950 (42)	3002 (42)	2924 (57)	2961 (72)	2992 (20)	2922 (7)	812 (4)	237 (13)
Pyl-c-Cu	3455 (143)	1685 (585)	1705 (119)	1571 (490)	1647 (387)	3003 (34)	2949 (68)	2961 (75)	2924 (49)	2992 (13)	2922 (2)	812 (6)	277 (3)
Pyl-g1-Cu	3454 (140)	1685 (573)	1704 (119)	1574 (496)	1645 (285)	2943 (13)	3006 (30)	2949 (77)	2963 (73)	2935 (15)	2993 (26)	812 (12)	230 (1)
Pyl-p5-Cu	3464 (41)	1691 (862)	1695 (70)	1509 (41)	1645 (331)	3017 (22)	2950 (48)	2961 (88)	2922 (15)	2993 (15)	2924 (54)	813 (12)	242 (17)
Pyl-b-Cu	3453 (38)	1690 (880)	1696 (71)	1512 (56)	1647 (324)	2917 (35)	3036 (11)	2921 (61)	2919 (95)	2993 (17)	2924 (73)	882 (24)	266 (1)

The frequencies below 1800 cm^{-1} are scaled with 1.01 and for those above 1800 cm^{-1} a correction factor 0.9679 is used

Vibrational modes: ν = stretching; γ = rocking; Sis = scissoring; s = symmetric; as = asymmetric

conformers given in a succeeding section of this paper); and therefore they may have different functional aspects in biochemical processes. It is interesting to note that in Pyl-b and Pyl-p5 the N$_7$–H and C$_6$=O bonds of the amide linkage orient themselves *cis* to each other (the χ_b values being −3.59° and 2.12° respectively) whereas in the other 14 conformer the N$_7$—H and C$_6$=O bonds are found to possess *trans* orientations (χ_b values range form 177.82 to −179.72°). As a result of this *cis* orientation of N$_7$—H and C$_6$=O bonds the values of χ_a change to −168.67° and −162.68° for Pyl-b and Pyl-p5 respectively from the usual range of 124.83° to 129.83° observed in the other zwitterionic conformers of pyrrolysine. This indicates that orientation of the five membered methylpyrroline ring of pyrrolysine may be influenced by rotation about C$_6$—N$_7$ bond. Similar situations are also observed in three more cases- (a) change in χ_d value to 65.71° in Pyl-d2 from the usual range of 176.43 to −175.53° changes the χ_c value to −113.53°; (b) changes in χ_g, −55.69° in Pyl-g1 and 70.82° in Pyl-g2, change the χ_h values to 121.01° and 102.8° respectively; and (c) in Pyl-f2 when χ_f is changed to 67.63° the χ_g value changes to 160.32°. The other internal torsional rotations are more or less independent of each other, *i.e.*, rotation around one dihedral angle does not alter the conformation of the other part of the molecule.

Vibrational spectra

Study of aqueous phase vibrational spectra is important to understand the existence and nature of various types of intramolecular H-bonds in the zwitterionic conformers of pyrrolysine molecule. Each zwitterionic conformer of pyrrolysine has a total of 111 normal modes of vibration. It is evident from Table 4 that some vibrational modes, viz. Sis(N$_{16}$—H), $\nu(C_5$—C$_4$) stretch *etc.*, basically remain unaltered along with the change of the conformation while some are very sensitive to even small changes in the configurations of the conformers and consequently leave noticeable signatures in the IR spectra. The frequency change reflects the internal information about each conformer and the relative strength of different intramolecular H-bond interactions. For example, in Pyl-b and Pyl-p5, because of the *cis* orientation of the N$_7$—H and C$_6$=O bonds of the amide linkage, there exist strong and unique O$_6$…H-N$_7$ interactions with O$_6$…H distances equal to 2.40 Å and 2.41 Å respectively. Consequently these two conformers possess many distinct structural features and some of the normal modes of vibration, for example the intensity of $\nu(N_7$—H), $\nu(C_2$=N$_1$) and $\nu(C_6$—N$_7$), band position of $\nu(C_2$=N$_1$) and $\nu(C_6$—N$_7$) *etc.*, differ appreciably from the other conformers. The results of this DFT study also point to the fact that vibrational frequencies are lowered corresponding to the presence of the intramolecular H-bond interactions. For instance, the lowering of $\nu(C_{11}$—H$_b$) stretching values in all
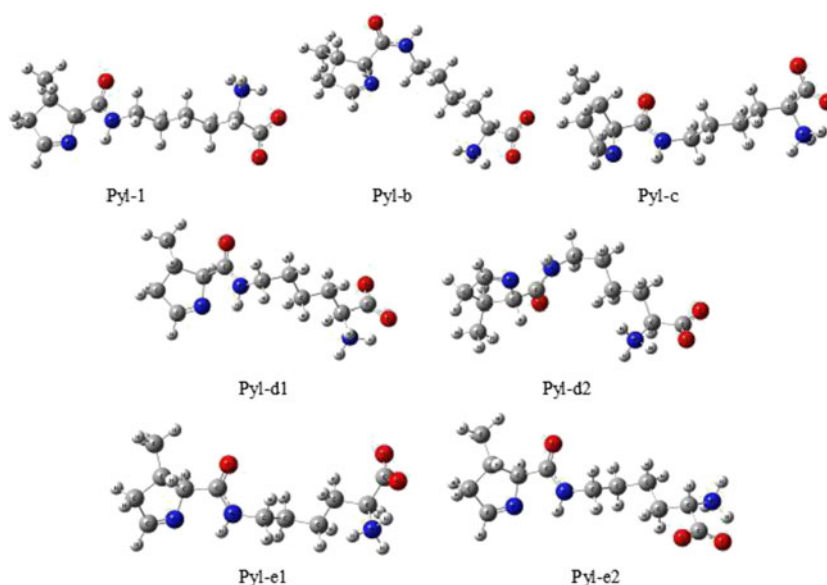
the conformers, except for three cases Pyl-g1, Pyl-p1 and Pyl-p4, can be attributed to the fact that the C_{11}—H_b bonds in the conformers participates in H-bond formation with the N_{16} and O_{13} atoms. Similarly, increase in $\nu(C_{11}$—$H_a)$ stretching value up to 3000 cm^{-1} in the case of Pyl-p4 is because the N_{16} and O_{13} atoms do not form any H-bonds with the C_{11}—H_a bond of the conformer. Thus, it is expected that the data listed in Table 4 would greatly aid future experimentalists in detecting the zwitterionic pyrrolysine conformers even though they differ by small energy differences from one another, for example, conformer Pyl-b and Pyl-p5 can be distinguished from others by simply referring to their $\nu(C_6$—$N_7)$ band positions occurring at 1515 cm^{-1} and 1444 cm^{-1} respectively while for the other conformers the same occur above 1567 cm^{-1}; similarly Pyl-e2 by its $\nu(N_7$—$H)$ stretching occurring at 3428 cm^{-1} (for others the same appear above 3446 cm^{-1}).

Rotational constants and dipole moments

Table 2 presents the rotational constants and dipole moments of the zwitterionic pyrrolysine conformers calculated at the B3LYP/6-311++G(d,p) level. The accuracy of DFT method in predicting the rotational constants of conformers of some aliphatic amino acids has been discussed in the literature [55, 56]. The results of this DFT study suggest that the geometries of all 16 zwitterionic pyrrolysine conformers exhibit large values of total dipole moments ranging from 9.029 to 17.340 D. These high values of dipole moments indicate that the conformers have greater polar character and consequently possess greater affinity to polar solvents. In the absence of any experimental data on rotational constants and dipole moments, the theoretical data presented in Table 2 may be useful for experimentalists.

Interactions with metal ions

In recent years, interactions of metal ions with amino acids have been extensively studied due to their immense importance in the life supporting processes [57–59]. The optimized geometries of the five metal complexes of the zwitterionic conformers of pyrrolysine at B3LYP level of theory reveal that the metal cations are bicoordinated to the carboxylate groups of the zwitterionic conformers in unsymmetrical fashion, where the $Cu^{+2} \ldots O_{13}$ bond distances are about 2.04 Å while the $Cu^{+2} \ldots O_{14}$ are about 3.25 Å (see Fig. 4). This type of unsymmetrical binding pattern of Cu^{+2} to the zwitterionic forms of phenylalanine, tyrosine and tryptophan has been well discussed in literature [60]. Table 5 lists the aqueous phase data on relative energies, interaction enthalpies (ΔH) and Gibbs energies (ΔG), rotational constants and dipole moments of the five metal complexes. As evident from Table 5, the range of relative energies of the five metal complexes, 5.40 kcal mol^{-1}, is very similar to that of their corresponding free zwitterionic pyrrolysine conformers. Similarly, the stability order of the metal complexes also remains relatively unaltered after metal coordination, except for Pyl-p5 which attains more thermodynamic stability than Pyl-b as a result of metal coordination. The negative values of interaction enthalpies and Gibbs energies for all five metal complexes indicate that the interaction of metal ions with the free zwitterionic pyrrolysine conformers lowers the total electronic energies of the metal complexes, and that the metal complexes are thermodynamically stable. On the other hand, all five metal complexes, like their corresponding free zwitterionic pyrrolysine conformers, exhibit larger values of total dipole moments ranging from 15.409 to 21.981 D.

It is evident from Table 6, which lists aqueous phase data on the eight back-bone dihedral angles of the metal

Fig. 3 The optimized structure of conformers Pyl-f1, Pyl-f2, Pyl-g1, Pyl-g2, Pyl-p1, Pyl-p2, Pyl-p3, Pyl-p4 and Pyl-p5

Pyl-f1

Pyl-f2

Pyl-g1
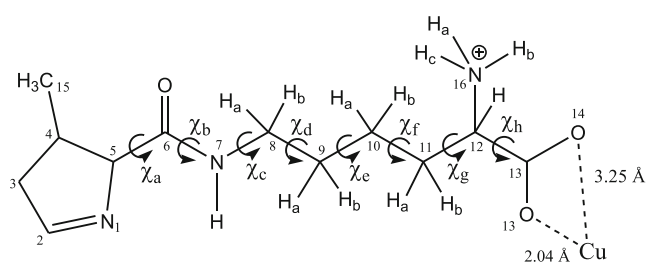
Pyl-g2

Pyl-p1

Pyl-p2
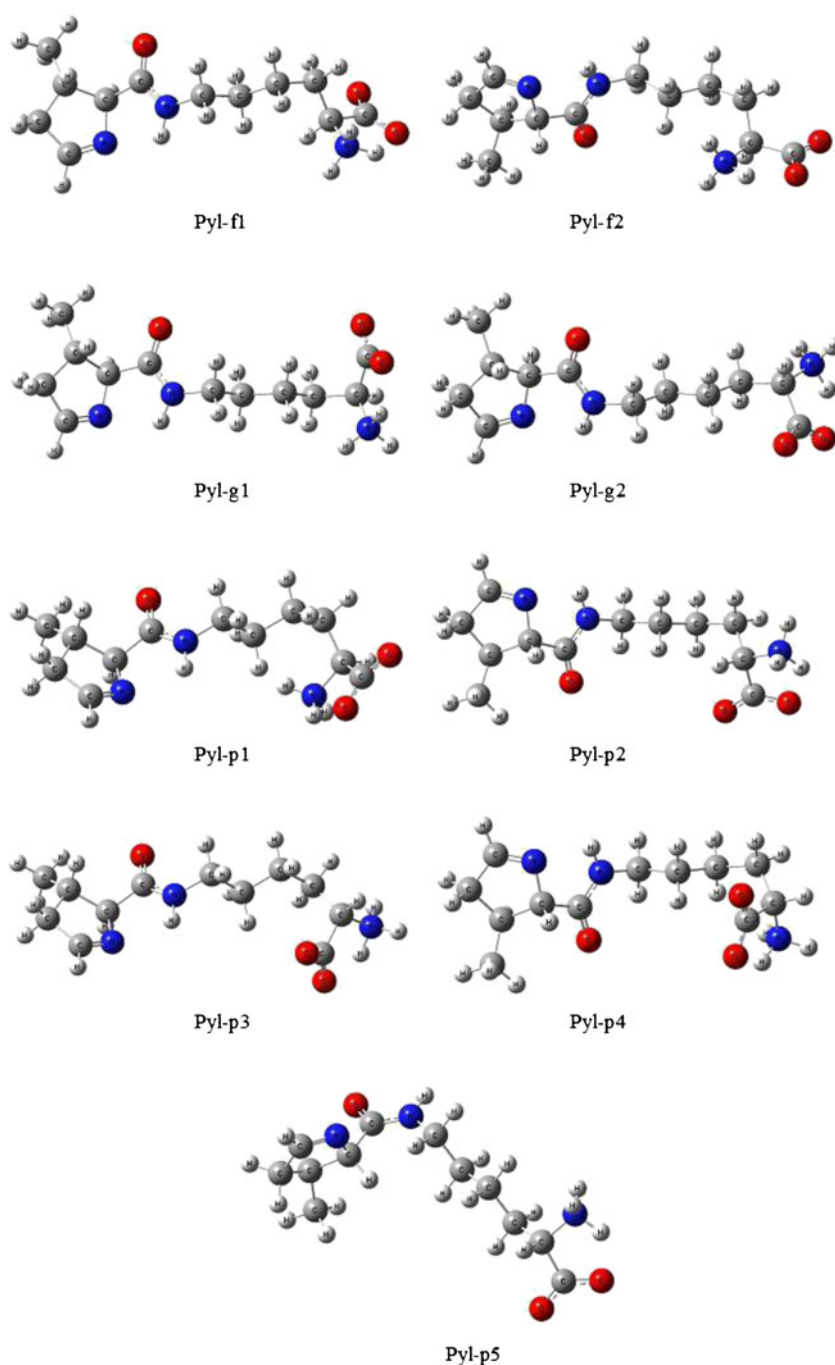
Pyl-p3

Pyl-p4

Pyl-p5

Fig. 4 Schematic representation of the Pyl-1-Cu complex in aqueous phase

complexes, that the internal structural features of the zwitterionic pyrrolysine conformers remain relatively unchanged due to metal coordination through their carboxylate groups. Most of the dihedral angle values of the metal complexes deviate within a range of 0.01 to 6.24° from their corresponding free zwitterionic conformers, except for χ_c value of Pyl-b which deviates up to a magnitude of 17.03°. Similarly, the data on the vibrational spectra of the metal complexes, presented in Table 7, also suggests limited changes in the structural features of the conformers as a result of metal coordination.

Conclusions

This DFT study at B3LYP/6-311++G(d,p) level of theory investigates the relative stabilities, theoretically predicted vibrational spectra, rotational constants, dipole moments and characteristic intramolecular hydrogen bonds present in 16 zwitterionic conformers of pyrrolysine. It has been observed that the COO^-, NH_3^+, N-atom of the imine group, $C_6{=}O$ and $N_7{-}H$ bonds of the amide linkage and all the CH_2 groups participate actively in intramolecular H-bond formation. A thorough analysis reveals that four types of intramolecular H-bonds, namely O…H—N, N…H—N, O…H—C and N…H—C, are present in the conformers; all of which play key roles in determining the energetics and in imparting the observed conformations to the zwitterionic pyrrolysine conformers. The calculated relative energy range of the conformers at B3LYP/6-311++G(d,p) level is 5.19 kcal mol^{-1} whereas the same obtained by single point calculations at MP2/6-311++G(d,p) level is 4.58 kcal mol^{-1}. Though the pyrrolysine conformers differ from one another by only small energy differences, their conformations are very different. The vibrational frequencies are found to shift invariably toward the lower side of the frequency scale corresponding to the presence of intramolecular H-bond ineractions in the conformers. The zwitterionic conformers of pyrrolysine are not stable in gas phase; after geometry optimization they are converted to the non-ionic forms. The effects of Cu^{+2} coordination on the relative stability order, structural features and vibrational frequencies of the zwitterionic conformers of pyrrolysine are examined. The interaction enthalpies and Gibbs energies, rotational constants and dipole moments of the metal complexes calculated at B3LYP level are also reported.

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