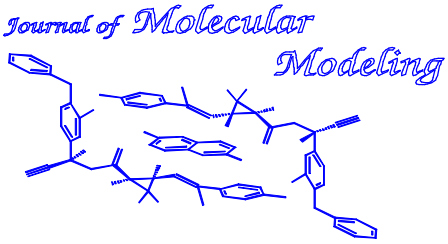
J. Mol. Model. **1996**, *2*, 51 - 61



Conformational Behavior and Flexibility of Terminally Blocked Cysteine and Cystine

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Received: 18 October 1995 / Accepted:11 March 1996 / Published:22 April 1996

Abstract

Conformational potential energy hypersurfaces, *PES*, for the terminally blocked L-Cysteine, L,L-Cystine and D,L-Cystine have been analyzed by means of molecular mechanics in combination with the programs ROSE, CICADA, PANIC and COMBINE. Low energy conformations and conformational transitions, conformational channels, have been located. Global and fragmental flexibility and conformational softness have been calculated for each conformer as well as for the entire molecule. The *PES* analyses were used for simulation of conformational movement based on Boltzmann probability of the points obtained on the *PES*. Boltzmann travelling revealed interesting correlated conformational movement where three or even more dihedral angles changed simultaneously. It could be shown that conformational behavior and flexibility were strongly influenced by the absolute configurations of the amino acids in the peptides.

Keywords: Conformational search, Molecular mechanics, Potential Energy (hyper)surface, D,L-form of amino acid, Conformational flexibility.

Introduction

Amino acids are interesting and important targets for the computational chemist because they contain a variety of intramolecular interactions, are conformationally labile, and are of a tractable size for calculations. Detailed knowledge of the conformational properties of disulphide bridges is essential for studies of all molecules in this group, e.g. for the understanding of numerous extracellular properties of biologically active oligopeptides [1]. Recently, we have reported a study [2] of the conformational behavior of the 20 common amino acids. We have shown that the ability of single residue to form a specified secondary peptide structure is not only dependent on low energy conformers, but also on the flexibilities of the conformers. In that study, it was shown that the conformational behavior of L-Cysteine exhibited the largest deviations between calculated and X-ray data of real proteins. This may be ascribed to the disulfide bridges present in real proteins. The above problem prompted us to elaborate on the study of a small molecules containing disulfide bridges, i. e., terminally blocked Cystine. We have chosen two forms of Cystine for our study, L,L– and D,L-Cystine. The reason is that peptides with a combination of L-forms

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and D-forms of amino acids play an important role in transport processes within membranes. D-forms of amino acids play a key role in the transport activity (e.g., Gramicidin A). Another motivation for studying the D-form of Cystine is that D-Cysteine is included in cyclic analogues of enkephalins, which have specific activity to receptors in the brain. The comparison of the conformational behavior of a single residue and Cystine as a part of cyclic peptide will follow this work.

The present study has three objectives.

1. To continue the studies on relationships between conformational behavior and flexibility of amino-acids as separate entities and as part of peptides and proteins.
2. To test the ability of the programs ROSE, CICADA, PANIC and COMBINE to perform conformational potential energy hypersurface PES analyses.
3. To test performance of the software PMMX [3] in describing the conformational space of single amino acid residues and short peptides. Conformational properties of amino acid residues L-Cysteine and Cystine obtained by the molecular mechanics method have been reported in the literature[4, 5, 6]. These published data there were used as a starting point for our calculations.

Method

All computations were performed with the ROSE [7] (version 2.9) and CICADA [8] (version 3.0) software interfaced with molecular mechanics program PMMX which is the MMX program [9] specially parametrized for peptides using parameters obtained from the literature [10]. Final analysis of data and exibility computations were performed with PANIC [11] (version 3.0). Conformational movements have been simulated by the program COMBINE [12]. All the molecular mechanics calculations were performed for a dielectric constant $\varepsilon = 78.5$ in order to mimic an aqueous environment. The approach is expressed by the following steps.

1. We have extracted from the literature [4, 5, 6] structural data for the rotatable backbone bonds of the low energy conformers for each terminally blocked amino acid. For the side chain we used the ±gauche and trans geometry for the torsion χ_1 , χ_2 and ±90 for the disulphide bridge of Cystine (for more details see Figure 5). These values have been used as input data for the program ROSE which generates low energy conformers by a combinatorial algorithm. The Cartesian coordinates of conformers have been generated by ROSE for L-Cysteine, L,L- and D,L-Cystine, respectively.
2. The structures generated by ROSE were next fully relaxed and minimized. The conformations with relative energies less than 1.5 kcal·mol^{-1} were then selected and used as starting set for the CICADA calculation. CICADA performs a systematic search for low energy conformational interconversions, i.e., conformational channels on the PES. The following parameters were used during the CICADA calculations for all molecules. The limiting difference for dihedral angle (Dmin) above which two conformers are considered as different was set to 30 degrees. This means that two conformers are considered identical if all their dihedral angles differ by less than Dmin. The smallest energy differences (Emin) taken into account was fixed at 0.1 kcal·mol^{-1}. An energy cutoff of 30 kcal·mol^{-1} has been applied. This means that no interconversion with a transition energy higher than 30 kcal·mol^{-1} has been taken into account. For more details about CICADA parameters see [8].
3. CICADA results were then analyzed by the program PANIC. The theory used by the program is based on a graph theoretical model of the *PES* [13, 14]. The program produces a list of minima and transition states along the *PES*. Interconversion pathways and their transition energies are generated by the program. Moreover, PANIC calculates absolute flexibility ϕ, and relative flexibility *f*, and con-formational softness f^0 .
4. All the results were used for the program COMBINE [12] which simulates travelling on the *PES* based on the Boltzmann distribution of conformers. The geometrical parameters for structures along the Boltzmann trajectories were extracted by the program ANALYSE [15]. The same software was used to elucidate correlated conformational movement. The drawings were made by the "in house" software TULIP and FROG ([16]).

The approach is schematically demonstrated in Figure 1. The calculations have been performed on i386 based PC running

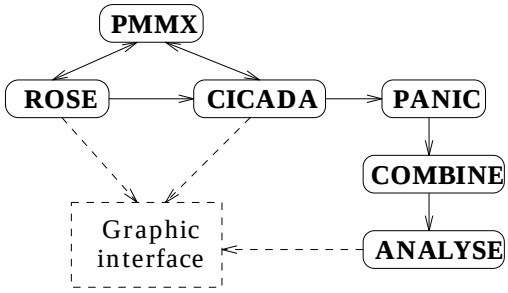


Figure 1. *Scheme of the communication between single programs. Energies of the structures are computed by PMMX which is an MMX program specially parametrized for peptides. The program ROSE is used for generation of starting conformations by a combinatorial algorithm. CICADA performs travelling along the PES. Results from the program CICADA are used to PES and flexibility analysis which is performed by the program PANIC. Results from the programs CICADA and PANIC serve for the simulation of the conformational analysis which is performed by COMBINE. ANALYSE is used to extract geometry parameters of structures obtained by a COMBINE run.*

under the operating system UNIX (ROSE), on SGI Indy running operating system IRIX 5.1 and SGI POWER Challenge L running operating system IRIX 6.0 (all other calculations).

Flexibility

Detailed knowledge of the low energy regions of the potential energy surface is essential in order to quantify conformational flexibility. For that purpose, the following values have previously been defined: f and ϕ for relative and absolute flexibility [17], respectively, and f^0 for the conformational softness [18]. Relative and absolute flexibilities can be evaluated for single conformers, for molecular fragments as well as for an entire molecule. As a consequence, the relative flexibility ${}_i f$ of conformer i is evaluated as a product of three terms, thermodynamic p_i, kinetic k_i, and geometrical g_i:

$$ {}_i f = p_i \cdot k_i \cdot g_i \tag{1}$$

Each term can be defined as follows:
• the thermodynamic contribution p_i is the probability of each conformation at absolute temperature (T), calculated using the Boltzmann distribution.

$$p_i = exp\left(-E_i \big/ KT\right) \Big/ \sum_{j=1}^{n} exp\left(-E_j \big/ KT\right) \tag{2}$$

where K is the Boltzman constant and E_i the relative energy of conformer i.
• the kinetic term k_i is expressed by the Arrhenius rate constant term as follows:

$$k_i = \sqrt[n_i]{\prod_{j=1}^{n_i} exp\left(-E_j / RT\right)} \tag{3}$$

where R is the gas constant.
• the geometrical term g_i is computed by:

$$g_i = \left[\sum_{j=1}^{n_i}\sum_{k=1}^{m}\left|t_k^j - t_k^i\right|\right] \Big/ 2\pi m n_i \tag{4}$$

where n_i is the number of transition states (torsion angle t^j) surrounding the conformation being considered (torsion angle t^i) and m is the number of dihedral angles followed. This term is therefore the sum of geometrical movements from the conformer i via the various transition states measured in the dihedral angle space, and normalised by the number of transition states and the number of rotatable bonds. By analogy, the absolute flexibility; ${}_i\phi$, of the conformer i is expressed by:

$${}_i\phi = p_i \sum_{j=1}^{n_i} g_{ij} \cdot exp\left(-E_j / KT\right) \tag{5}$$

where g_{ij} is analogous to g_i but normalised only by the number of rotatable bonds.

From the conformer flexibility values, it is then possible to calculate the relative f and absolute ϕ flexibility of the molecule as a summation over all conformers. While the absolute flexibility value reflects the best (lowest energy) conformational interconversions, the relative flexibility expresses "an average conformational interconversion". Both flexibility values increase when the energy barriers between the conformers decrease and both are temperature depend-

Table 1. *Global descriptors of the CICADA analysis of residues [a]*

	Number of torsions		PES description		
Residue	**Global**	**Active**	**points**	**conform. [b]**	**pathways**
L-Cysteine	6	4	260	49(40)	448
L,L-Cystine	13	9	4999	1550(37)	6776
D,L-Cystine	13	9	5000	1473(293)	7188

[a] Active torsions: torsions driven during the calculation; point on PES is any nuclear configuration of importance and thus saved by the program (i.e. energy minimum, transition state). For details of CICADA and its parameters see Ref. [7].

[b] Numbers in parentheses: number of conformers (energy minima) with relative energies less than 1.5 kcal/mol.

ent. For detailed descriptions see references [17, 19]. Another value of interest is the conformational softness f^0 which reflects the total number of interconversions and also their energy barriers. It can be evaluated for the entire molecule using:

$$f^0 = \left[n/ln(n-0.67523)\right] \cdot \left[\sum_{i=1}^{n}\sum_{j=1}^{k} p_i \, exp\left(-E_j/RT\right)\Big/ln(k+1224.84)\right] \quad (6)$$

where n represents the total number of conformers, i. e., (energy minima) and k the total number of interconversions [17].

Results and discussion

Global descriptors of the CICADA analyses are summarized in Table 1. It is revealed that the number of low energy conformers (local energy minima on the *PES*), as well as the number of interconversion pathways (conformational channels), are very strongly dependent on the number of rotatable bonds. It is seen by comparison of both forms of Cystine that the total number of the points on the *PES* as well as the number of the conformations that have been found by program CICADA are comparable. This means that both CICADA runs (for D,L and L,L forms) are comparable. In contrast the total number of conformations with relative energy less than 1.5 kcal·mol^{-1} is much higher for the D,L-form. This fact is also reflected in computed flexibilities that are summarized in Table 2 for successive torsions as well as for the entire molecules.

It is seen from this table that the flexibilities of the investigated molecules are dependent on their sizes. The computed relative flexibility of L-Cystine is much more higher than that of L,L-Cystine and also higher than that of D,L-Cystine. The differences between the computed absolute flexibilities are smaller because the absolute flexibility reflects only the lowest energy interconversion pathways. It is also seen that a significant part of the flexibility has been localized to the torsions ϕ and ψ around the backbone. On the other hand, the lowest computed flexibility has been concentrated on the disulphide bridges of the Cystine. The largest differences between flexibility of torsions are seen for L,L-Cystine.

Table 2. *Relative and absolute fragmental flexibilities of single residues. (For denotation see Figure 2 and 5).*

	L–Cysteine	L,L–Cystine	D,L–Cystine
		Flexibility	
Torsion	Relative (×10^5) Absolute (×10^3)	Relative (×10^5) Absolute (×10^3)	Relative (×10^5) Absolute (×10^3)
ϕ	12.0100	0.1114	0.4406
	3.6640	0.2601	0.8030
ψ	13.5100	0.1933	0.9440
	9.0410	1.2510	2.3950
χ_1	9.6100	0.0676	0.2200
	0.6630	0.0446	0.2841
χ_2	6.2400	0.0859	0.2975
	3.1900	0.0841	0.3284
χ_3	–	0.0279	0.1160
	–	0.0339	0.1858
χ_{21}	–	0.0811	0.3147
	–	0.0812	0.3494
χ_{22}	–	0.0648	0.2138
	–	0.0493	0.2670
ϕ_2	–	0.1283	0.5632
	–	0.2427	0.8149
ψ_2	–	0.2204	0.8232
	–	1.2550	2.1900
Total flexibility	41.5800 16.6400	0.9995 3.3410	4.0230 7.7280

L-Cysteine

The terminally blocked form of the L-Cysteine, N-acetyl-N'-methylamide of L-Cysteine, has been investigated. The usual notation ϕ, ψ, χ_1, χ_2 is used to describe the rotatable bonds. The description is illustrated in Figure 2.

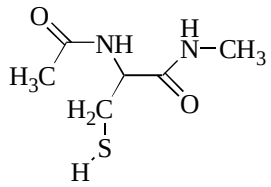
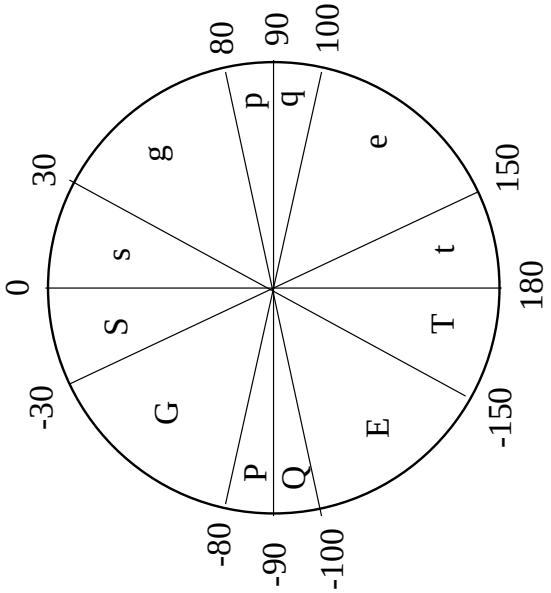


Figure 2. *Schematic representation of the Cysteine studied here along with the labelling of torsional angles of interest. (ϕ) -C-N-C^{α}–C'- , (ψ) -N-C^{α}-C'-N- , (χ_1) -N-C^{α}-C^{β}-S^{γ}- , (χ_2) -C^{α}-C^{β}-S^{γ}-H-*

The results from the conformational behavior analyses of the molecule are summarized in Tables 3 and 4. Table 3 shows geometries and energies of the best 5 conformations, from the relative energy point of view, which were found by the program CICADA. For the synopsis, the geometry parameters are combined with the letter code according to Zimmerman et al. [5].

It is seen from this table that the global conformational minimum is in the α-helix region with dihedral angle values ϕ = –69° and ψ = –45°. Calculated flexibility data are summarized in Table 4.

The table shows that the global minimum (conformation number 29) exhibits the highest relative flexibility. The highest absolute flexibility has been calculated for two conformations (1 and 2) with relative energies 0.0671 and 0.1546 kcal·mol^{-1}, respectively. The first one adopts a geometry similar to the global minimum, only with torsion χ_2 in the trans geometry. The second one belongs to the β-pleated sheet region. The ϕ–ψ plot for the conformations and transition states obtained is shown in Figure 3.



Scheme 1. *Assignment of letter codes for the side chain dihedral angles.*

Table 3. *Selected conformations found for L-Cysteine (for denotation see Figure 2).*

[a]	[b]	Torsion ϕ	Torsion ψ	Torsion χ_1	Torsion χ_2	Energy (kcal/mol)
1	AGt	-69	-45	-64	179	0.0671
2	CGT	-70	127	-64	-180	0.1546
29	AGG	-69	-45	-62	-60	0.0000
34	AGg	-69	-46	-65	63	0.0002
61	FGg	-71	132	-66	63	0.0889

[a] Numbering of structures (conformations) as generated by the software.

[b] Letter code is composed of Zimmerman notation [5] (first character) and letter abbreviation for the side chain. For denotation see Scheme 1. Starred letters in the original Zimmerman code are substituted by small letters.

Table 4. *Relative and absolute flexibilities of selected conformations of L-Cysteine [a]*

Conf.	p_i	k_i	g_i	f_T	ϕ_T
1	0.5519	0.1091	0.5152	0.3103	0.2335
2	0.4762	0.1260	0.5088	0.3052	0.2180
29	0.6181	0.1054	0.5023	0.3273	0.1635
34	0.6179	0.0965	0.5062	0.3020	0.1599
61	0.5320	0.0844	0.5194	0.2331	0.1494

(p_i ×10, k_i ×10, g_i ×10, f_T ×10^4, ϕ_T ×10^2).
[a] For geometry and energy see Table 3

The areas designated by α and β show the combinations of ϕ, ψ torsions to form secondary structure α–helix and β–pleated sheet. This figure illustrates the location of conformations and transition states on the *PES*. The inter-conversion pathways between the conformations obtained could be estimated from the positions of the transition states. Figure 4 shows the relative energy and the values of torsions χ_1 and χ_2 for obtained conformations and transition states.

Energy barriers for rotation of the χ_1, χ_2 torsions are clearly seen from Figure 4. The transition states with torsion ±gauche and trans geometry correspond to transition states on the

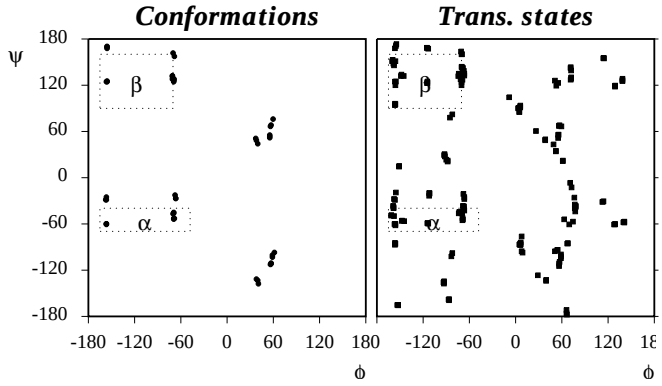


Figure 3. *Plots of the conformers (left part) and transition states (right part) found by CICADA as a function of the ϕ and ψ torsion angles of L-Cysteine. Areas α and β indicate the possible combinations of torsions ϕ and ψ in peptides to form the secondary structure of α-helix and β-pleated sheet, respectively.*

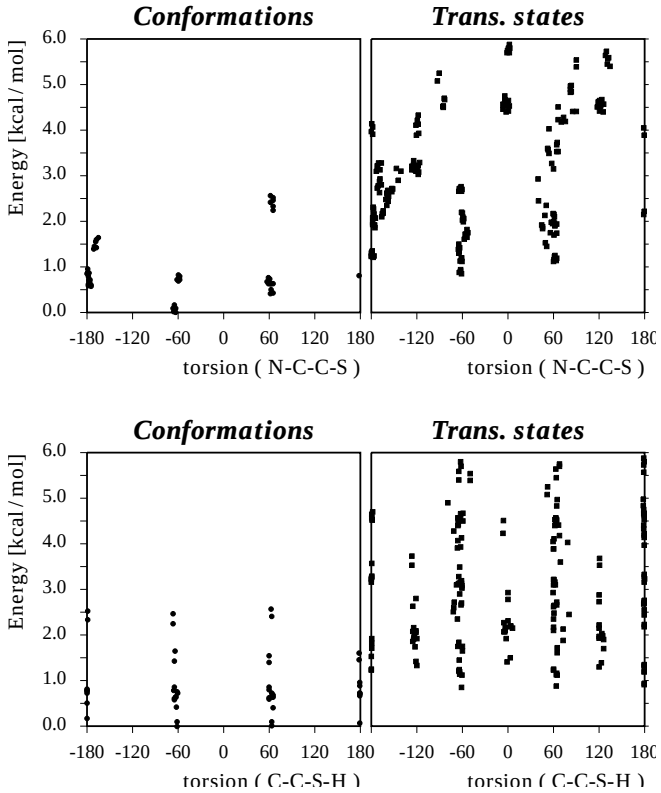


Figure 4. *Energy vs geometry plot for dihedral angles χ_1 (upper figure) and χ_2 (bottom figure) of L-Cysteine. Data are shown for all the conformers (left part) and the transition states (right part) found by CICADA in an energy window of 6 kcal/mol.*

interconversion pathways within other parts of the *PES*. It is seen from Figure 4 that the rotation of torsion χ_1 is energetically unfavorable compared with a rotation of the terminal -SH group. These observations are reflected in the computed absolute flexibilities of the torsions investigated. Our results are compared with a conformational analysis of L–Cysteine, that was performed by the program Sybyl using the AMBER molecular mechanics force field and starting with X-ray data obtained from Cambridge Structural Database (CSD) [20]. We have found a preference for the –gauche geometry for the χ_1 torsion, whereas the +gauche geometry is preferred by X–ray analysis. Conformations with +gauche geometry of the χ_1 torsion are found at 0.4 kcal·mol^{-1} higher energy in our results relative to the conformations in –gauche χ_1 geometry. The population of torsion χ_2 is also in a good agreement with the X–ray analysis.

Cystine

Two structures of the Cystine have been selected: D – Cys – S – S – L – Cys and L – Cys – S – S – L – Cys in the terminally blocked form (for denotation see Figure 5).

Geometry parameters of the best 5 conformations for L,L-Cystine are summarized in Table 5. It is seen that the global minimum (conformation number 590) adopts an α–helix geometry in both parts of the molecule. The table shows that the preferred value for the disulphide bridge is about 90°. The distribution of torsions χ_1, χ_2, χ_{21} and χ_{22} is similar to that of corresponding torsions in the L–Cysteine. Our results are in a good agreement with results obtained by X–ray structural analysis of peptides containing Cystine and molecular mechanics and statistic analysis studies[20]. The results of relative and absolute flexibility are summarized in Table 6.

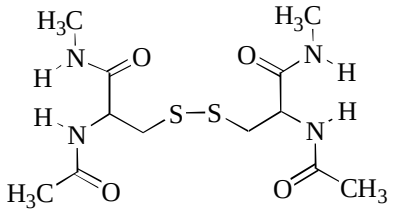


Figure 5. *Schematic representation of the Cystine studied here along with the labelling of torsional angles of interest. (ϕ) -C-N-C^{α}-C'- , (ψ) -N-C^{α}-C'-N- , (χ_1) -N-C^{α}-C^{β}-S^{γ}- , (χ_2) -C^{α}-C^{β}-S^{γ}-C'^{2}- , (χ_3) -C^{β}-S^{γ}-$S^{\gamma 2}$-$C^{\beta 2}$- , (ϕ_2) -C^{2}-N^{2}-$C^{\alpha 2}$-C'^{2}- , (ψ_2) -N^{2}-$C^{\alpha 2}$-C'^{2}-N^{2}- ,(χ_{21}) -N^{2}-$C^{\alpha 2}$-$C^{\beta 2}$-$S^{\gamma 2}$- , (χ_{22}) -$C^{\alpha 2}$-$C^{\beta 2}$-$S^{\gamma 2}$-S^{γ}- .*

The same parameters as for L,L–Cystine are summarized for the D,L–form in Tables 7 and 8. The results for D,L-Cys-

Table 5. *Selected conformations found for L,L-Cystine (for denotation see Figure 5).*

Conf. no. [a]	Zimmerman notation [b]	Torsion angles									Energy (kcal=mol)
		ϕ	ψ	χ_1	χ_2	χ_3	χ_{22}	χ_{21}	ϕ_2	ψ_2	
553	AGGpgGA	-76	-44	-55	-67	88	65	-73	-77	-54	0.0015
566	AGGpgGC	-74	-43	-55	-67	89	65	-74	-76	124	0.5939
569	FGGpgGA	-76	133	-56	-67	88	65	-73	-76	-54	0.0900
590	AGgpGGA	-77	-55	-72	65	88	-67	-55	-77	-44	0.0000
596	AGgpGGF	-77	-55	-72	65	88	-67	-55	-77	133	0.0888

[a] Numbering of structures as generated by the software.
[b] Letter code is composed of Zimmerman notation [5] (first and last character) and letter abbreviation for the side chain. For denotation see Scheme 1. Stared letters in the original Zimmerman code are substituted by small letters.

Table 6. *Relative and absolute exibilities of selected conformations of L,L-Cystine [a]*

Conf.	p_i	k_i	g_i	f_T	ϕ_T
553	0.4423	0.0216	0.3122	0.2982	0.5883
566	0.1628	0.0132	0.3273	0.0702	0.1114
569	0.3810	0.0156	0.3217	0.1910	0.4747
590	0.4435	0.0238	0.3188	0.3368	0.5846
596	0.3817	0.0273	0.3082	0.3215	0.4859

($p_i \times 10$, $k_i \times 10$, $g_i \times 10$, $f_T \times 10^4$, $\phi_T \times 10^2$).
[a] For geometry and energy see Table 5.

tine are similar to those obtained for L,L–Cystine, except for dihedral angle values for torsions ϕ and ψ, which have opposite signs. It is shown that the global minimum (conformation 1473) exhibits lower relative and absolute flexibility than conformations with relative energy 0.4 kcal·mol^{-1} (e.g. conformations 120 and 176). This means that the global minimum is located in a relatively narrow valley on the *PES* and conformations 120 and 176 are located on energetically higher but shallower parts of the *PES*.

The dependency of the relative energies of conformations and transition states on the values for torsion χ_3 is shown for L,L– and D,L–Cystine in Figure 6.

This figure can be used to rationalize the different behaviors of the two forms of Cystine. It is seen that for the L,L-form values of χ_3 about +90° are preferred, whereas for the D,L-form, both values (+90° and –90°) are populated. The barriers of rotation are lower for the D,L-form than for the L,L-form. This is also reflected by the flexibilities obtained, which are about 6 times higher for the D,L-form than for the L,L-form.

Table 7. *Selected conformations found for D,L-Cystine. (For denotation see Figure 5).*

Conf. no. [a]	Zimmerman notation [b]	Torsion angles									Energy (kcal=mol)
		ϕ	ψ	χ_1	χ_2	χ_3	χ_{22}	χ_{21}	ϕ_2	ψ_2	
120	cgTggGA	69	-129	64	-178	77	66	-74	-78	-51	0.4395
152	agTggGA	68	46	64	-178	78	66	-74	-78	-50	0.3182
176	agTggGC	68	46	65	-179	78	65	-75	-79	117	0.4224
236	ggTqGGF	136	59	69	-152	94	-57	-58	-72	134	0.2642
514	cggqGGF	73	-124	61	63	90	-67	-56	-76	133	0.2463
568	aggQGGC	75	44	56	66	-91	-65	-62	-72	127	0.1895
708	agGGtGA	77	50	74	-66	-78	178	-65	-68	-46	0.3175
1473	agGQgtC	76	54	72	-67	-90	69	177	-74	102	0.0000
1489	agGQgtD	76	53	72	-67	-90	69	175	-155	99	0.1687
1847	cggqGGA	72	-127	61	64	90	-66	-56	-76	-44	0.1878

[a] and [b] see Table 5.

Table 8. *Relative and absolute exibilities of selected conformations of D,L-Cystine [a]*

Conf.	p_i	k_i	g_i	f_T	ϕ_T
120	0.0540	0.0685	0.3206	0.1185	0.1566
152	0.0662	0.0885	0.3075	0.1803	0.2394
176	0.0556	0.0609	0.3412	0.1154	0.1750
236	0.0726	0.1214	0.2975	0.2622	0.1329
514	0.0748	0.0178	0.4557	0.0606	0.4224
568	0.0823	0.0534	0.3133	0.1376	0.6084
708	0.0663	0.0676	0.3149	0.1413	0.1699
1473	0.1133	0.0195	0.2970	0.0656	0.1072
1489	0.0852	0.0141	0.2925	0.0352	0.0609
1847	0.0826	0.0360	0.3055	0.0907	0.4372

($p_i \times 10$, $k_i \times 10$, $g_i \times 10$, $f_T \times 10^4$, $\phi_T \times 10^2$).
[a] For geometry and energy see Table 7.

Simulation of the conformational movement

The results of the conformational analysis obtained were next used for simulation of travelling along the *PES*. Both simulations were performed using the program COMBINE [12] with the same parameters. The global minimum was used as a starting point. The number of steps was set to 1 000 000 and the temperature to 300 K. After each 1000 steps a new starting conformer has been selected. We have focused our attention on the side chain torsions χ_1, χ_2, χ_3, χ_{22} and χ_{21}. The changes of torsions χ_1, χ_2 and χ_3 within a selected 300 steps window are shown in Figures 7 and 8.

The differences in flexibilities of the L,L– and D,L–forms are clearly seen from these pictures. It is also seen that the more flexible torsions result in a larger number of changes relative to the more rigid torsions. It is evident (at least for this particular case) that the more rigid torsion can evoke changes of flexible torsions. This is seen from the correlation between torsion changes. The data have been calculated for the representative torsions and are summarized in Table 9 for L,L-Cystine and in Table 10 for D,L-Cystine, respectively. It can be seen that a smaller number of torsional changes occure in the L,L–form than in the D,L–form. No significant

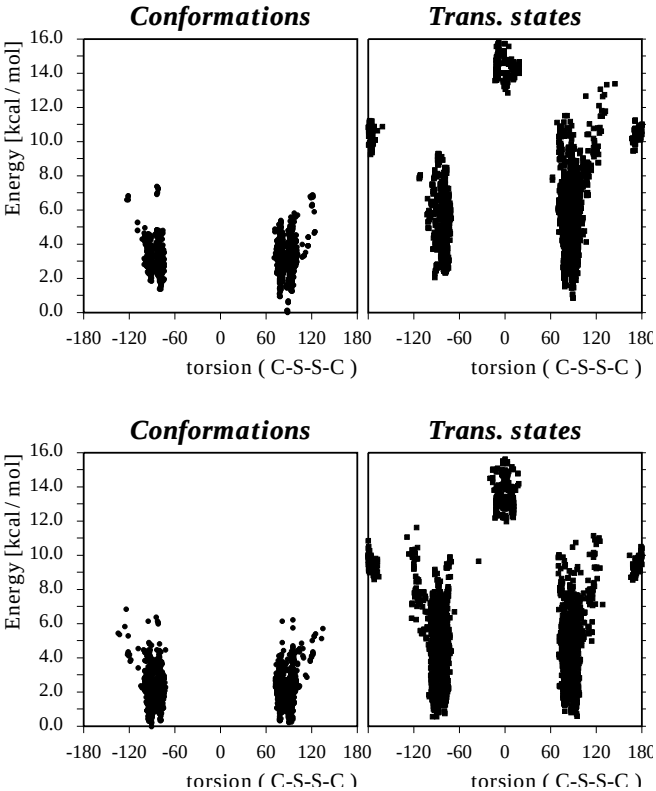


Figure 6. Energy vs geometry plot for dihedral angle χ_3 of L,L-Cystine (upper picture) and D,L-Cystine (bottom picture). Data are shown for all the conformers (left part) and the transition states (righ part) found by CICADA in an energy window of 16 kcal/mol.

Table 9. *Matrix of correlated movement for L,L-Cystine [a] for 1000000 steps simulation at 300 K.*

Tors.	No. of changes [b]	Torsions ϕ_1	ψ_1	χ_1	χ_2	χ_3	χ_{22}	χ_{21}	ϕ_2	ψ_2
ϕ_1	48967	100.0	5.7	0.3	1.4	0.0	1.2	0.0	0.0	0.0
ψ_1	134122	2.1	100.0	0.2	0.7	0.0	0.4	0.0	0.0	0.2
χ_1	1522	8.9	15.8	100.0	2.3	0.0	8.4	0.0	0.1	2.0
χ_2	6899	9.9	13.6	0.5	100.0	0.0	0.9	1.4	4.8	9.3
χ_3	3	0.0	0.0	0.0	0.0	100.0	100.0	100.0	66.7	100.0
χ_{22}	4876	12.0	11.0	2.6	1.3	0.1	100.0	1.6	3.4	12.3
χ_{21}	1556	0.1	0.7	0.0	6.4	0.2	4.9	100.0	8.3	23.3
ϕ_2	42860	0.0	0.0	0.0	0.8	0.0	0.4	0.3	100.0	6.6
ψ_2	141604	0.0	0.2	0.0	0.5	0.0	0.4	0.3	2.0	100.0

[a] and [b] see Table 10.

Table 10. *Matrix of correlated movement for D,L-Cystine [a] for 1,000,000 steps simulation at 300 K.*

Tors.	No. of changes [b]	Torsions ϕ_1	ψ_1	χ_1	χ_2	χ_3	χ_{22}	χ_{21}	ϕ_2	ψ_2
ϕ_1	67784	100.0	32.3	3.6	3.3	2.6	2.3	3.2	0.7	8.2
ψ_1	166303	13.2	100.0	3.5	3.5	1.6	2.9	3.2	2.0	4.0
χ_1	8711	28.1	67.7	100.0	78.7	34.1	59.4	74.4	24.2	68.8
χ_2	11094	20.2	52.1	61.8	100.0	25.7	47.4	57.3	18.0	55.6
χ_3	3020	58.6	86.8	98.4	94.4	100.0	55.0	99.9	3.6	90.0
χ_{22}	13878	11.3	34.8	37.3	37.9	12.0	100.0	37.3	12.9	39.8
χ_{21}	7973	27.3	66.9	81.3	79.7	37.8	64.8	100.0	27.6	79.7
ϕ_2	75842	0.6	4.5	2.8	2.6	0.1	2.4	2.9	100.0	21.0
ψ_2	152067	3.7	4.4	3.9	4.1	1.8	3.6	4.2	10.5	100.0

[a] Correlation is in percentage. The matrix is not symmetrical as sometimes rotation around one bond induces rotation around another one but not "vice versa".

[b] Energy change larger than 30 degrees in one step is taken into account.

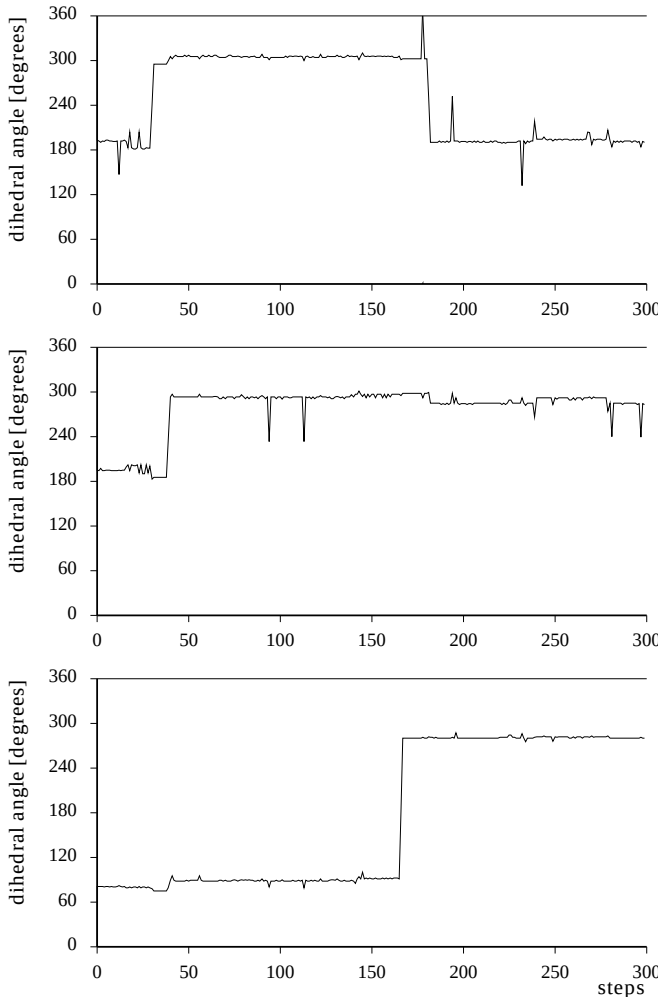


Figure 7. *History of selected dihedral angles during the simulation of conformational movement on L,L-Cystine within the step window 0–300 at the thermodynamic temperature 300 K (χ_1 (upper part), χ_2 (middle part) and χ_3 (bottom part)).*

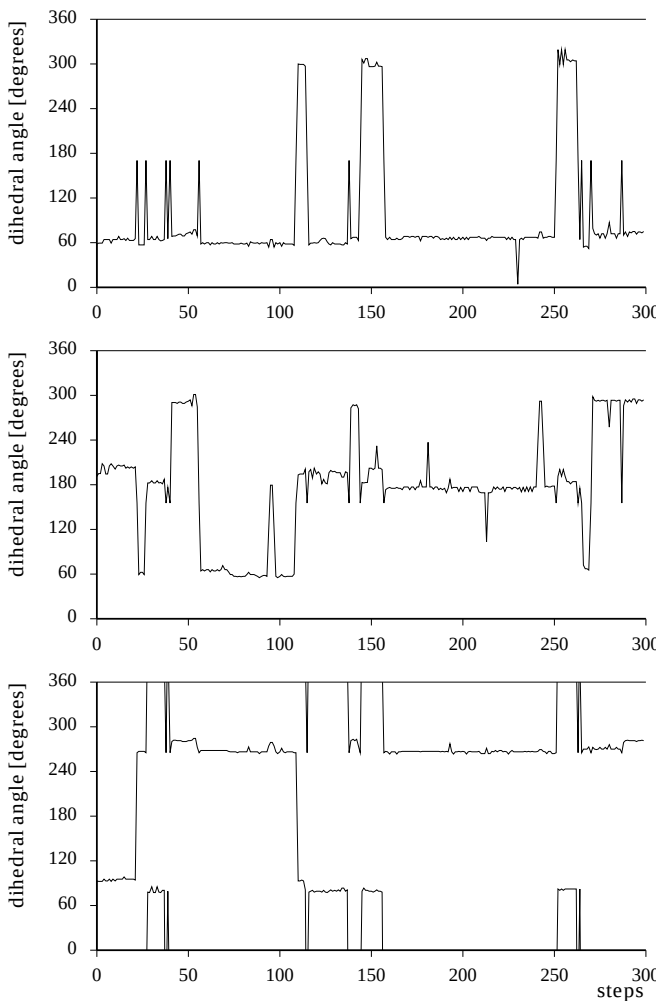


Figure 8. *History of selected dihedral angles during the simulation of conformational motion on D,L-Cystine within the step window 0–300 at the thermodynamic temperature 300 K (χ_1 (upper part), χ_2 (middle part) and χ_3 (bottom part)).*

correlation between the torsional changes is observed for L,L-Cystine (cf. Table 9). A correlation not only between neighboring torsions, but also between relative distant torsions of molecule, e.g., between χ_3 and ψ, has been observed for D,L-Cystine.

This together with the observation of relatively stable conformations with higher relative energy within the simulations, leads to the presumption of the existence of hydrogen bonds in these conformations. We have followed the atom distances during the entire simulation period and found conformations stabilized by intramolecular hydrogen bonds. They are given in Figure 9.

No low energy conformations of L,L-Cystine have been found with intramolecular hydrogen bond.

Conclusions

Conformational potential energy hypersurface, PES, for terminally blocked L–Cysteine, L,L–Cystine and D,L-Cystine have been analyzed by means of molecular mechanics in combination with the programs ROSE, CICADA, PANIC and COMBINE.

- It has been observed that flexibility decreases as the size of the molecule increases. The reason is that the molecule becomes more organized. This feature is not generally observed for all molecules. For example, hydrocarbons exhibit a quite opposite behavior [21]. For molecules of the same size: D,L-Cystine is much more flexible than the structurally better organized L,L–form.
- The results obtained are in good agreement with experimental (X-ray) data and show that PMMX program is well parametrized for peptides.
- The interesting feature of strongly correlated conformational movement has been observed for D,L-Cystine. This phenomenon could be general for the D,L combination and may play a role, i.e. in processes within membranes.
- The above results confirm the ability of the software ROSE, CICADA, PANIC and COMBINE to describe the conformational behavior of flexible molecules.

Acknowledgment: The research has partially been supported by the Grant Agency of the Czech Republic, grant No: 203/94/0522 and by Chem–Consult, Hundhamaren, Norway. This financial support is gratefully acknowledged. The authors thank the Czech Academic Supercomputer Centre in Brno for providing them access to computer facilities. We would also like to thank BIOSYM Technologies, Inc., for providing the Academic licence for the INSIGHT II software.

Supplemetary material MMX input files of all the structures introduced in Tables as well as complete tables of low energy conformers are available in electronic form from the servers of the Springer-Verlag.

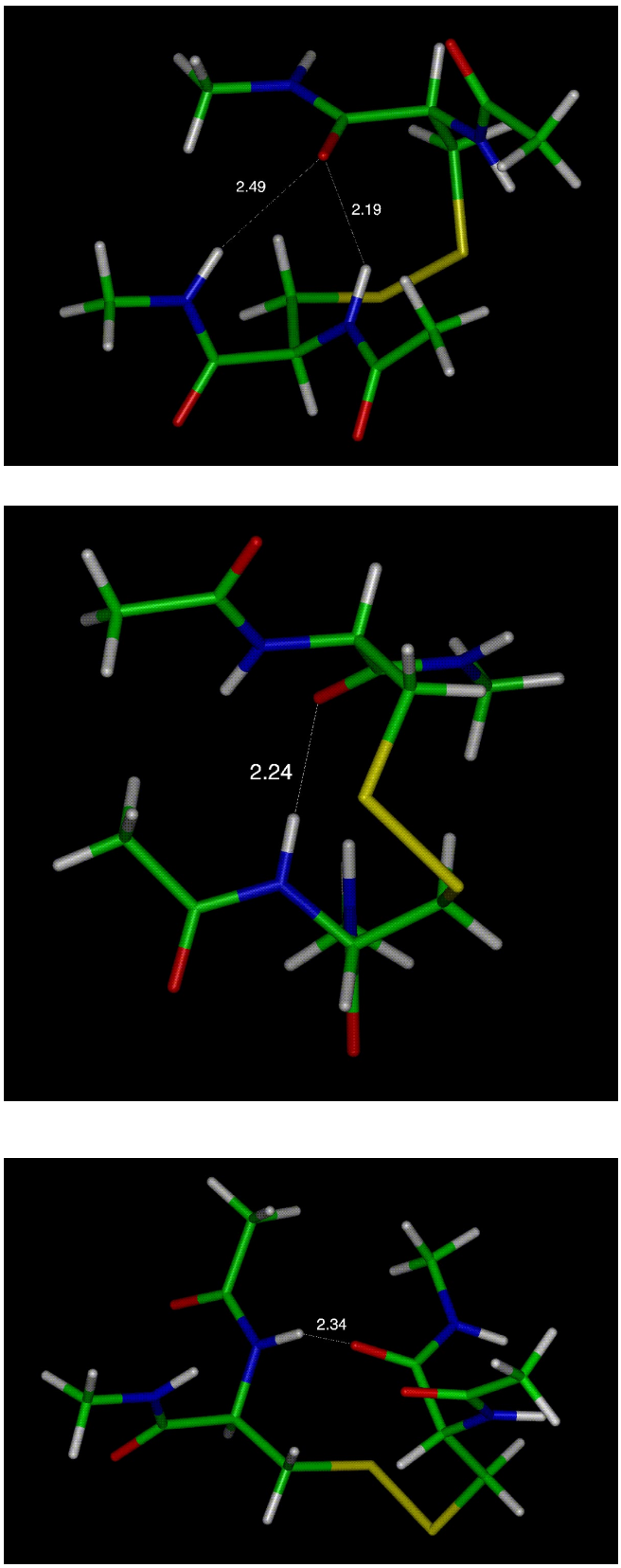


Figure 9. *Conformers with hydrogen bonding found for D,L-Cystine. The numbers indicate lengths of the hydrogen bond (Å).*

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