Conformational Analysis by Molecular Mechanics Energy Minimizations of the Tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe, a Recurring Sequence of Elastin

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The amino acid sequences Gly-X-Gly-Gly (X = Ala, Val, Leu, Ile) frequently recur in elastin and might play a role in the molecular mechanism of protein elasticity. In this paper we report on a theoretical conformational analysis by molecular mechanics energy minimizations of the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe, using a strategy based on the build-up method for searching exhaustively the conformational space. The set of 3785 conformers has been determined and a statistical analysis has been carried out. The conformers' energy, end-to-end distance, torsions and >C=O • • • HN< interaction distance distributions are discussed. The stability of type II β-turn Gly-1-C=O • • • HNGly-4, within the conformer family (experimentally observed), and C_7 HNLeu-2, C_7 HNMe and C_{10} HNMe hydrogen bonds (hypothesized according to experimental results), have been theoretically verified. The possible role of librational motions and sliding β-turns for the entropic elasticity mechanism of elastin is discussed.

The amino acid sequences Gly-X-Gly-Gly (X = Ala, Val, Leu, Ile) frequently recur in the elastic protein (elastin) of the tissues of many vertebrates (*e.g.* in human, avian and bovine tropoelastin 14, 33 and 18 times, respectively) and it is thought that they play an important role in the molecular mechanism of elasticity.[1] Some synthetic polypeptides comprising these sequences have been the subject of detailed and extensive circular dichroism (CD) and NMR conformational studies.[2,3] In particular, for synthetic pentapeptides Boc-Gly-X-Gly-Gly-Y–OMe (X, Y = Val, Leu) the main conformational features in solution are the type II β-turn Gly-1-C=O • • • HNGly-4† and the involvement of HNX-2 and HNY-5 in intramolecular hydrogen bonds. For the HNX-2 hydrogen bond, the simplest interpretation is a seven-membered cycle BocC=O • • • HNX-2. For the HNY-5 hydrogen-bond, three different possibilities should be taken into account. The first considers only a simple C_7 ring Gly-3-C=O • • • HNY-5; the second is an equilibrium, which would involve sliding type II β-turns, between Gly-1-C=O • • • HNGly-4 and Leu-2-C=O • • • HNY-5; the third is a consecutive double type II–IV β-turn[4] involving both Gly-1-C=O • • • HNGly-4 type II and X-2-C=O • • • HNY-5 type IV in the same conformer. Furthermore, the experimental data reveal multiple conformational equilibria with substantial molecular flexibility. Finally, theoretical conformational studies have been carried out for the dipeptide Boc-Gly-Leu–NMe and the tripeptide Boc-Gly-Leu-Gly–NMe by molecular mechanics[5] and the pentapeptide Boc-Gly-Val-Gly-Gly-Leu–OMe by molecular dynamics simulations.[6] In the last case there has been evidence that the β-turn Gly-1-C=O • • • HNGly-4 is not stable in the presence of Val-2-C=O • • • HNLeu-5. Moreover, Gly-3-C=O • • • HNLeu-5 and Val-2-C=O • • • HNLeu-5 oscillate with 'reverse phase' which indicates fast equilibrium between the C_7 ring and the C_{10} turn. In all cases the peptides are characterized by a combination of flexibility and conformational preferences.

In this paper we describe a conformational analysis by molecular mechanics calculations, using a strategy based on the build-up method[7] for searching exhaustively the conformational space in order to determine the set of conformers[8] (*i.e.* energy local minima) for the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe. A statistical analysis of the conformers' data set has been carried out. The conformational energy distribution and end-to-end distance, torsional and hydrogen bond interaction distances *vs.* energy scatter-plots have been calculated. The conformers' Ramachandran scatter-plots and λ-plots[9] are reported and discussed within the framework of the entropic librational mechanism of elasticity proposed for elastin by Urry.[10] Lastly, the conformers' families whose secondary structures are in agreement with the experimental evidence have been selected in order to verify the structural hypotheses previously proposed.

† The symbolism *e.g.* Gly-1-C=O • • • HNGly-4 refers to a hydrogen bond formed between the carbonyl and NH groups of the amino acids at the first and fourth position, respectively, of the peptide chain.

Theoretical Computations

The Model Force Field.—The AMBER program[11–14] was used for molecular mechanics energy minimizations. The force field equation is of the form:

$$E_{tot} = \Sigma_{bonds}\, k_r(r - r_{eq})^2 + \Sigma_{angles}\, k_\tau(\tau - \tau_{eq})^2 + \Sigma_{dihedrals}\, U_n/2\,[1 + \cos(n\varphi - \delta)] + \Sigma_{i<j} w(A_{ij}/R_{ij}^{12} - B_{ij}/R_{ij}^{6} + 332.2\, q_i q_j/\varepsilon R_{ij}) + \Sigma_{H\text{-}bonds}(C_{ij}/R_{ij}^{12} - D_{ij}/R_{ij}^{10})$$

The calculations were carried out on a VAX 8530 computer under the VMS 5.4 operating system. As in our previous paper[5] the all-atoms force field representation was used, the force field parameters are those of ref. 12, the 1–4 non-bonded and electrostatic interactions are weighted by a scale factor $w = 0.5$ and no cut-off for energy interactions was used. Moreover, in the Coulomb term the value of the relative permittivity $\varepsilon = 1$ has been taken. Net atomic charges q_k were those of refs. 6 and 12 calculated by fitting to a point charge model the quantum mechanically derived electrostatic potential.[15] All energies are expressed as $\Delta E = E - E_0$, where E_0 is the energy corresponding to the most stable conformer.

Multiple-minima Search.—The molecular structure with the notation of φ_i, ψ_i, χ_i^j, torsional angles for the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe is presented in Fig. 1. The quoted values of torsion angles are reported according to the current IUPAC–IUB convention.[16] The multiple-minima search for the tetrapeptide was performed using a modified build-up procedure.[5] The search started from 5 minima for *N*-acetylglycyl-

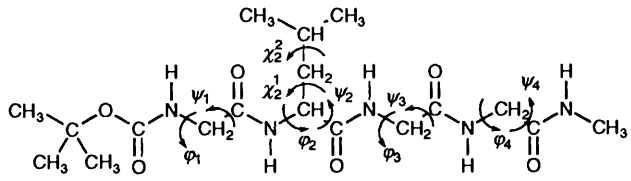

Fig. 1 Molecular structure of the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe with the notation of φ_i, ψ_i and χ_i^j

N'-methylamide and 1015 conformers for the tripeptide Boc-Gly-Leu-Gly–NMe as determined in our laboratory (ref. 5). By combination of the torsional sets φ, ψ and χ, assuming a *trans*-conformation for the peptide bonds and for the Boc-urethane group, and the staggered one for the methyl group, 1015 × 5 = 5075 starting conformations for the tetrapeptide were identified.

The calculation was made more manageable by using a strategy whereby firstly only partial minimizations and a sorting procedure to remove duplicate conformations were carried out. Then, the selected conformations were subjected to full minimization and to a final sorting procedure.

The conjugate gradient method in Cartesian coordinates, in which geometry optimization of all freedom atomic degrees is carried out, was used for all the energy minimizations. The energy-minimized structures were selected by the following sorting procedure: they were ordered by increasing energy, then duplicate structures were identified and only the ones with the lowest energy were selected. Two structures are defined as duplicate when all torsions are equal with a tolerance (*i.e.* absolute value of the angular difference) of 15°. A similar value is typically observed in molecular dynamics simulations for molecules fluctuating around stationary states.

Subsequently, the tetrapeptide starting conformations were subjected twice to 50 iteration steps of partial energy minimization and sorting procedure. Accordingly, firstly 4475, then 4193 non-equivalent conformations were determined. Finally, the selected conformations were subjected to full energy minimization with a convergence criterion of $\Delta E = 10^{-6}$ kcal mol^{-1} * between two following iterations, obtaining values of the r.m.s. energy gradient lower than 10^{-2} kcal mol^{-1} Å^{-1} for the final structures. After the sorting procedure 3785 non-equivalent conformers were located.

Results and Discussion

As mentioned, the outcome of the search consisted of 3785 conformers, spread over an energy range of 30 kcal mol^{-1}. The conformers' energy distribution is reported in Fig. 2. The plot reveals a continuous and symmetric distribution of the energy states with a Gauss-like functional shape. No significant increase in degeneracy of energy levels is observed until more than 5 kcal mol^{-1} above the global minimum. The highest frequency occurs in the range 11–19 kcal mol^{-1} above the lowest-energy minimum. Nevertheless the occupation of higher-energy levels is such that they will not contribute, given the energy disadvantage, at a significant degree.

Gaussian energy distributions have been previously found both in our laboratory[5] (for oligopeptides where six or eight torsion angles were considered in a build-up procedure) or by Tosi *et al.*[17] (in a molecule where 13 torsions were considered in a Monte Carlo energy minimization method), by using completely different approaches, *i.e.* either systematic or stochastic.

* 1 cal = 4.1868 J.

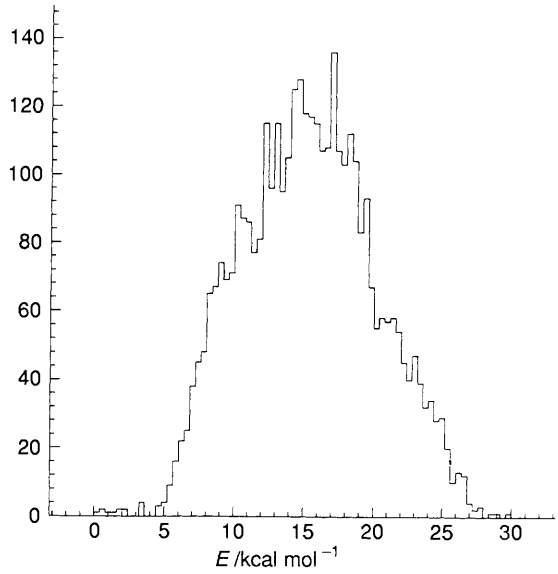

Fig. 2 Histogram of energy distribution for the 3785 conformers. Energy is expressed in kcal mol^{-1}.

A Gaussian energy distribution would indicate a small coupling among the torsional degrees of freedom of the molecule (*e.g.* between the φ, ψ pairs of the different residues), even if strong long-range hydrogen bond interactions, and especially C_7 and C_{10} rings, are present.

By comparing the conformers' energy distribution of the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe with those of the dipeptide Boc-Gly-Leu–NMe and of the tripeptide Boc-Gly-Leu-Gly–NMe of ref. 5, a trend is observed toward a progressive shift of the distribution to higher energy values, with a decrease of the low-energy population on increasing the length of the peptide chain.

This behaviour agrees with the experimental evidence of progressive increase of conformational disorder and disappearance of well-defined structures for similar sequences in solution, that is passing from the pentapeptide Boc-Gly-Val-Gly-Gly-Leu–OMe to the decapeptide Boc-(Gly-Val-Gly-Gly-Leu)$_2$–OMe and to the pentadecapeptide Boc-(Gly-Val-Gly-Gly-Leu)$_3$–OMe.[18]

In this context the isolated tetrapeptide could be considered as a model for similar amino acid sequences in anhydrous elastin. In fact, the presence of lowest energy states of the tetrapeptide, which are poorly populated with respect to those at higher energy, could agree with a possible role for the sequences Gly-X-Gly-Gly (X = Ala, Val, Leu, Ile) in the observed drastic change of the dynamic-mechanical properties of elastin on hydration. As a matter of fact, the protein is brittle, when dry or poorly hydrated, and becomes highly viscoelastic, when swelled in water.[19] Accordingly, it may be hypothesized that the hydration, by destabilizing the low-energy folded states and stabilizing the high-energy less folded ones, could shift the distribution to lower energy values, and therefore modify the Boltzmann distribution of the conformational ensemble. Then, for the anhydrous protein the sequence Gly-X-Gly-Gly would assume an enthalpically stable, but at low entropy, state while for the swelled form one could expect that the entropy should increase with consequent larger number of accessible states.

In Fig. 3 the distances between α-C of Gly-1 and α-C of Gly-4, $d_{\alpha\alpha}$ = α-C-1–α-C-4 and d_{OH} = BocC=O · · · HNMe are taken into account as different expressions of the end-to-end molecular distance. Their distributions and $d_{\alpha\alpha}$ and d_{OH} *vs.* conformers' energy scatter-plots are presented. The distance $d_{\alpha\alpha}$ corresponds to α-C-*i*–α-C-(*i* + 3) for the *i*-th amino acid residue in a polypeptide chain and has been considered as an index of the folding degree of protein chains;[20] the value of $d_{\alpha\alpha}$ = 7 Å is

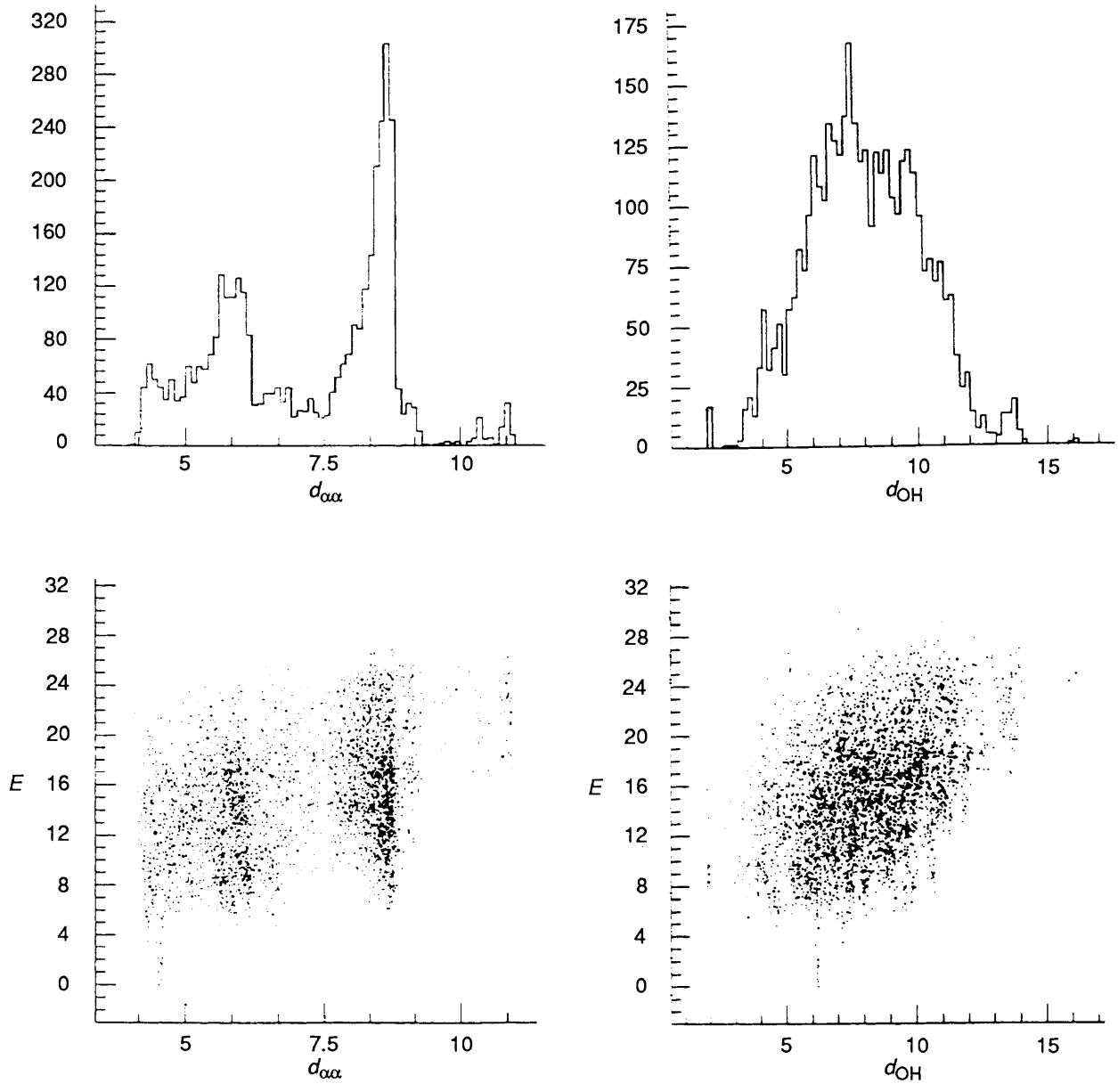

Fig. 3 Distances $d_{\alpha\alpha}$ = α-C-1–α-C-4 and d_{OH} = BocC=O · · · HNMe distributions (upper figures) and scatter-plots *vs.* conformers energy (lower figures). Distance is expressed in Å and energy in kcal mol^{-1}.

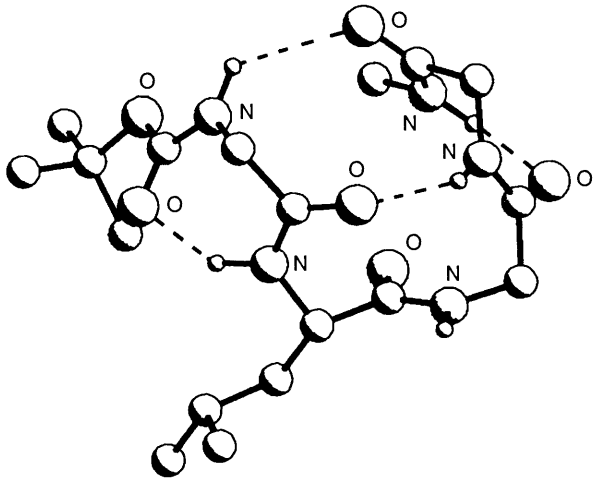

Fig. 4 The lowest-energy conformer of the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe. Hydrogen bonds are shown as dashed lines. Labels are given for non-hydrogen and -carbon atoms.

taken as a borderline between folded chains ($d_{\alpha\alpha}$ < 7 Å) and extended chains ($d_{\alpha\alpha}$ > 7 Å). Nevertheless, as $d_{\alpha\alpha}$ is not a direct function of φ_1 and ψ_4, the distance d_{OH} in evaluating the end-to-end distance has also been taken into account. The $d_{\alpha\alpha}$ distribution shows two different peaks of high frequency corresponding to folded and extended chains; the more intense and well-resolved the second one, the more ill-defined the first one. In contrast, the d_{OH} distribution exhibits a bell-like functional shape that demonstrates the continuity of the values from folded to extended chains. The different $d_{\alpha\alpha}$ and d_{OH} distribution shapes show a complementarity, with respect to the molecular folding degree of φ_1 and ψ_4: the ends tend to depart in folded conformations and to approach in extended ones. The scatter-plots E *vs.* $d_{\alpha\alpha}$ or d_{OH} evidence a weak, but real correlation between conformers' energy and end-to-end distance. In both cases folded and extended conformations correspond to lower and higher energy values, respectively. Although in the plots the points are very scattered, the clouds appear to be approximately centred around a line not orthogonal to the plot axes.

The computed structure for the lowest-energy conformer is

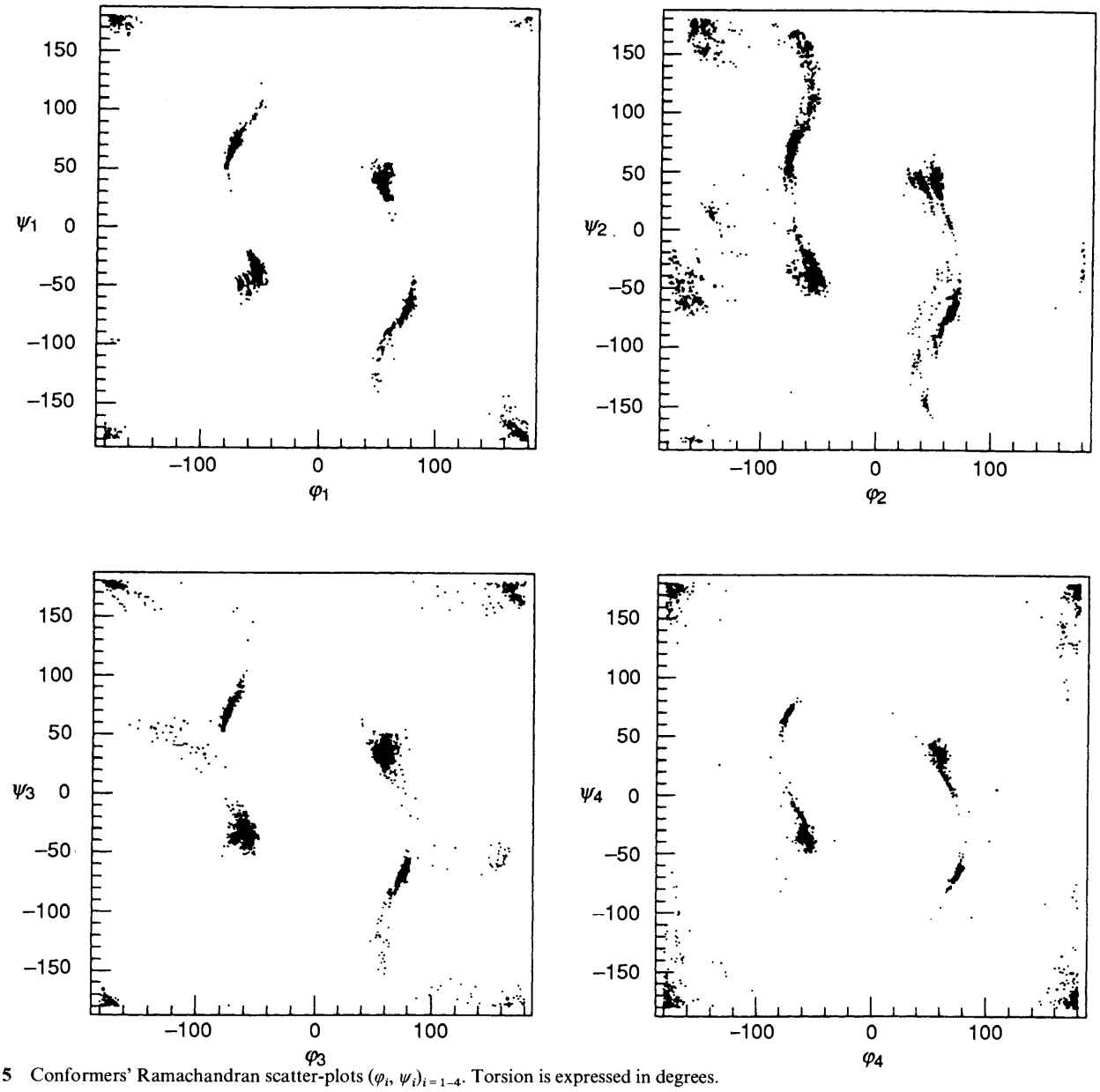

Fig. 5 Conformers' Ramachandran scatter-plots $(\varphi_i, \psi_i)_{i=1-4}$. Torsion is expressed in degrees.

reported in Fig. 4. The folded conformation is stabilized by the seven-membered hydrogen-bonded rings (C_7) BocC=O···HNLeu-2 and Gly-3-C=O···HNNMe, and by the hairpin structure formed by the C_{10} type II β-turn Gly-1-C=O···HNGly-4 and by the C_{14} Gly-4-C=O···HNGly-1. The seven- and ten-membered rings are in agreement with the experimentally found secondary structure features.[2] In contrast, the C_{14} ring was not evidenced and probably exists only in strongly apolar solvents (*i.e.* hydrocarbons).

In Fig. 5 the conformers' Ramachandran scatter-plots $(\varphi_i, \psi_i)_{i=1-4}$ are reported. The plots reveal the presence of five distinct and symmetric point clusters and of large forbidden angular regions. A strong correlation between the torsional pairs in each residue is observed: in fact the number of clusters is lower than that expected in the case of independent variables.

For the glycyl residues the clusters are centred near the regions of right- and left-handed α-helices (φ, ψ = 60, 40° and −60, −40°, respectively), β-turns (φ, ψ = −70, 70° and 70, −70°) and fully-extended chains (φ, ψ = 180, 180°). The finding of the expected symmetry for Gly residues can be considered a positive test of the exhaustiveness of the multiple-minima exploration.[21] For the leucyl residue the clusters are broader and ill-delimited, evidencing some disordering of the backbone conformation, evidently induced by the branched side-chain.

In Fig. 6 the conformers scatter-plots of energy *vs.* φ_i and ψ_i (i = 1–4) and related torsional histograms are presented. The molecule shows well-defined conformational preferences, in fact the torsional values are distributed in a pentamodal way with dihedral ranges sharp and well-delimited around the t and split $g^{\pm}$ positions (only the torsion ψ_2 shows broad and ill-delimited dihedral ranges).

In all cases the *gauche* states are energetically favoured with respect to the *trans* states. The *gauche* substates are always degenerate except than for Gly-4. Lastly, we observe that for each conformational state the density of distribution as a function of the energy is continuous and bell-like shaped in the whole populated range.

The conformers scatter-plots of energy *vs.* the interaction distances >C=O···HN< and distance histograms which present a significant population in correspondence with the hydrogen bond distance (about 2 Å) are reported in Fig. 7. All possible C_7 and C_{10} secondary structures are populated according to literature data on oligopeptides.[22] Nevertheless, in the low-energy region (ΔE < 5 kcal mol^{-1}), only the hydrogen bonds BocC=O···HNLeu-2 (C_7), Gly-1-C=O···HNGly-4

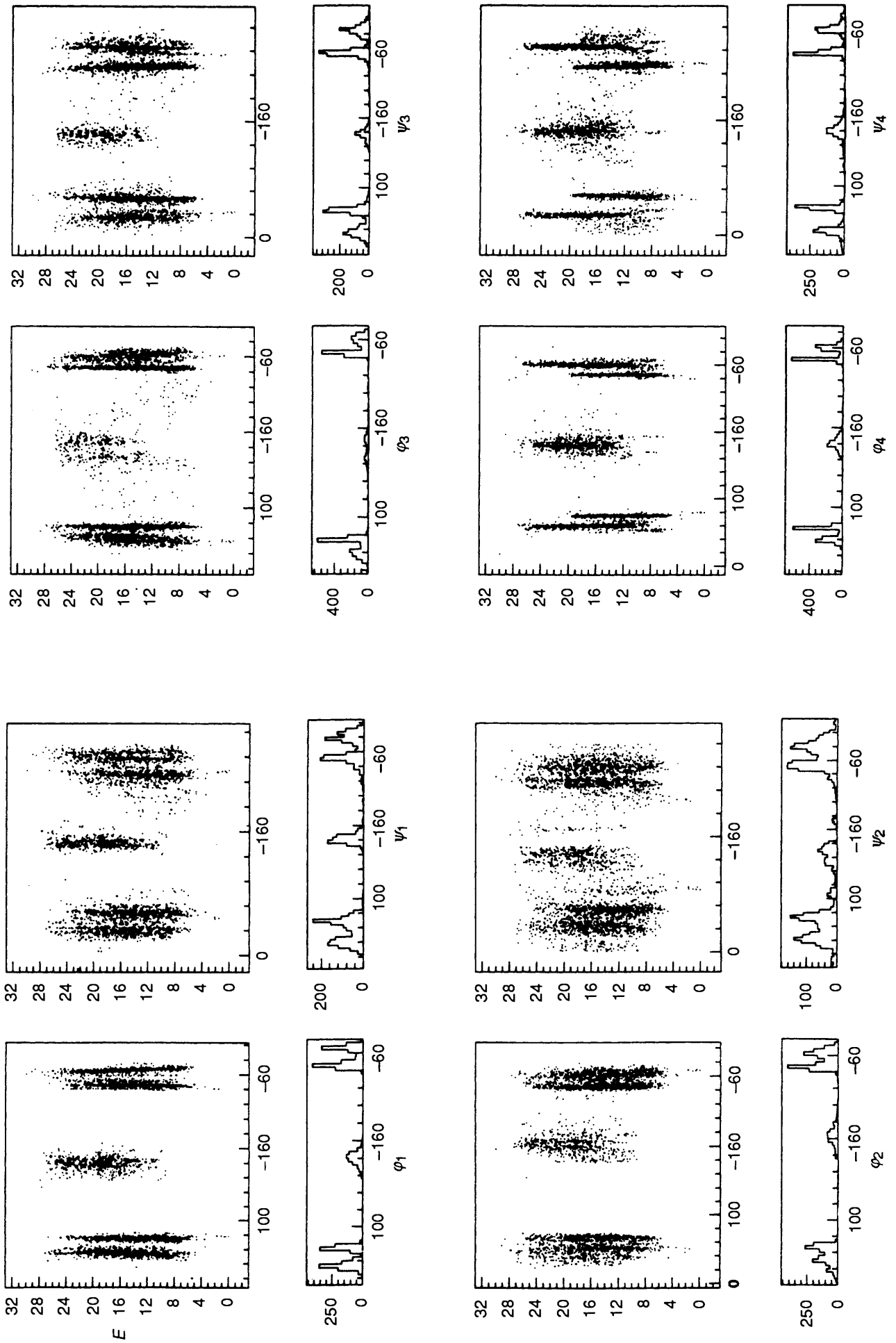

Fig. 6 Conformers' scatter-plots of energy *vs.* φ_i and ψ_i (i = 1–4) (upper figures) and torsional histograms (lower figures). Energy is expressed in kcal mol^{-1} and torsion in degrees.

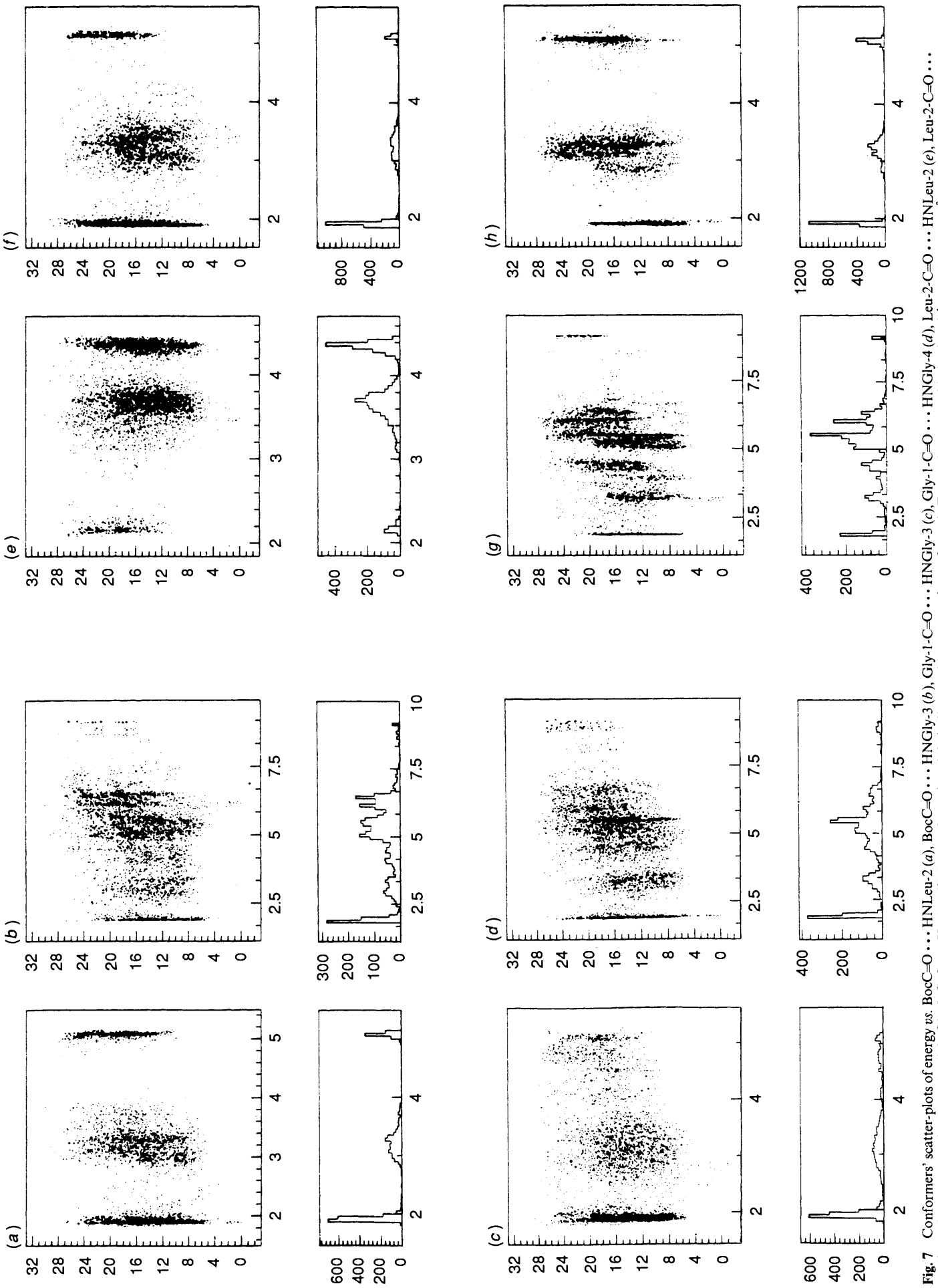

Fig. 7 Conformers' scatter-plots of energy *vs.* BocC=O · · · HNLeu-2 (*a*), BocC=O · · · HNGly-3 (*b*), Gly-1-C=O · · · HNGly-3 (*c*), Gly-1-C=O · · · HNGly-4 (*d*), Leu-2-C=O · · · HNLeu-2 (*e*), Leu-2-C=O · · · HNGly-4 (*f*), Leu-2-C=O · · · HNMe (*g*), Gly-3-C=O · · · HNMe (*h*) (upper figures) and their distance histograms (lower figures). Energy is expressed in kcal mol^{-1} and distance in Å.

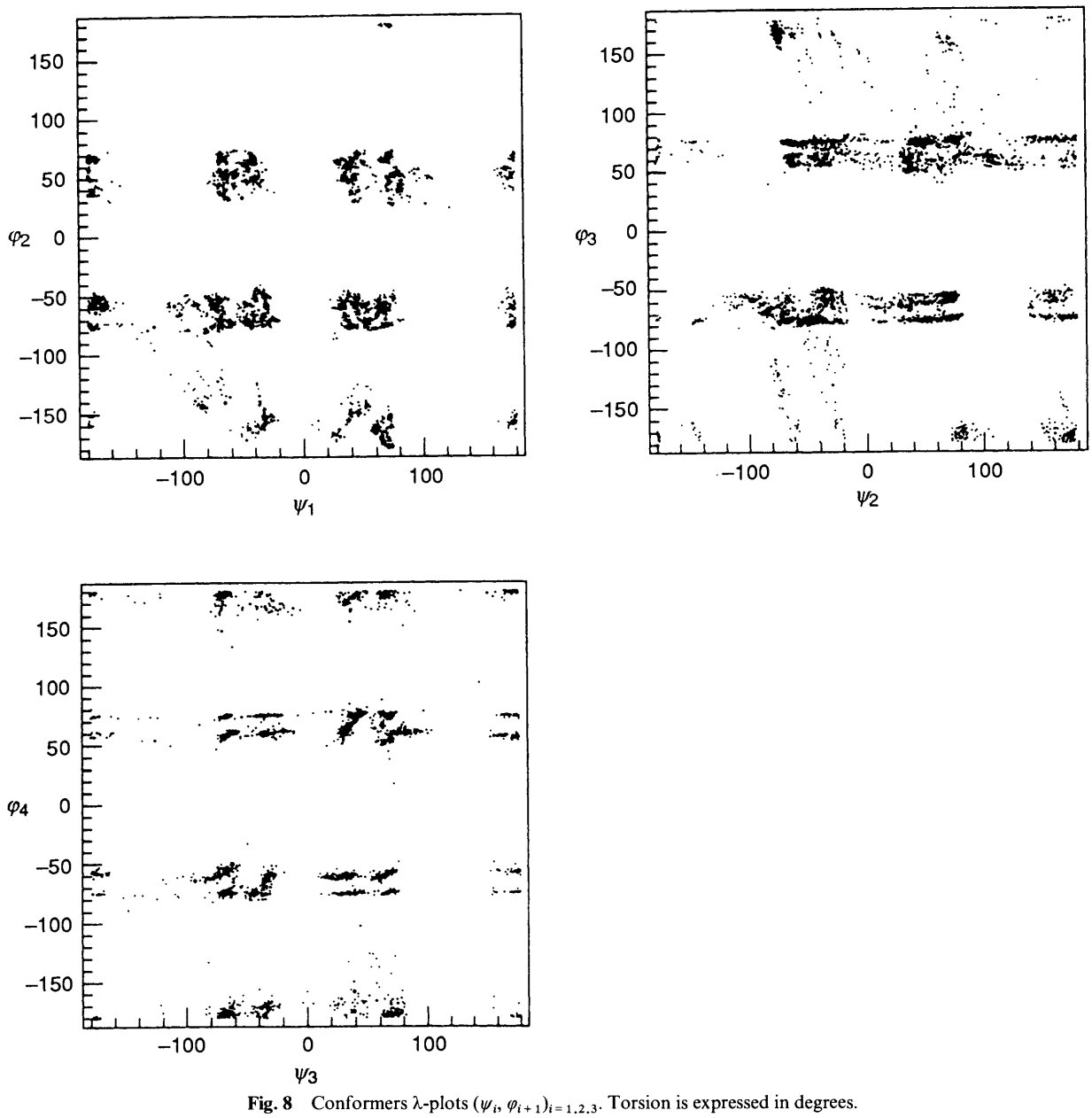

Fig. 8 Conformers λ-plots $(\psi_i, \varphi_{i+1})_{i=1,2,3}$. Torsion is expressed in degrees.

(C_{10}) and Gly-3-C=O · · · HNMe (C_7) appear to be stabilized in agreement with the experimental results obtained *via* CD and NMR.[2] Lastly, the distance histograms show frequency maxima characteristic of each type of >C=O · · · HN< interaction and independent of the involved amino acid sequence.

Urry proposed an entropic librational mechanism to explain the molecular nature of the elasticity of elastin.[9] In particular, molecular mechanics and dynamics calculations on the repeating sequence of elastin, poly(Val-Pro-Gly-Val-Gly), show that the torsions φ, ψ of the sequence Pro-Gly specify a type II β-turn, while the torsion pairs ψ_i, φ_{i+1} in Val-Gly-Val segments are highly anticorrelated with large amplitude rocking motions of peptide units, *i.e.* peptide librations. Then, the elastomeric restoring force would originate from reduction of librational entropy in those Val-Gly-Val segments linking successive β-turns as elastin is stretched. Moreover, Wasserman and Salemme,[23] by molecular dynamics (MD) simulations on hydrated poly(Val-Pro-Gly-Val-Gly), described state-switching crankshaft motions of ψ_i, φ_{i+1} torsions of the portions bridging β-turns with the peptide units oscillating around one local librational state and then hopping to another one.

In Fig. 8 the λ-plots $(\psi_i, \varphi_{i+1})_{i=1-3}$ are reported. In all cases at least nine substructured point clusters, corresponding approximately to values t, g^+, g^- (180, 60, −60°) of the torsions, are identified. A high degree of symmetry in the high-frequency regions is present for $i = 2$ and 3. In particular, $F(\psi_i, \varphi_{i+1}) = F(-\psi_i, -\varphi_{i+1})$, where F is the torsional distribution density. Moreover, it is generally observed for each cluster a larger distribution of ψ_i values, that is continuous or bimodal, than for φ_{i+1}, generally bimodal. Although these λ-plots evidence equivalent angular regions, they do not show any correlation between the variables.

The Gly-1-C=O · · · HNGly-4- β-turn in tetrapeptide conformers is characterized by the values of the torsion pairs (φ, ψ) in the residues Leu-2 and Gly-3. Accordingly, the conformers scatter-plots of the interaction distance Gly-1-C=O · · · HNGly-4 *vs.* φ_i, ψ_i (i = 2, 3) are presented in Fig. 9. The plots show a high symmetry degree and the β-turn containing conformers correspond to hydrogen bond (*i.e.* $d_{OH} \leqslant 2.3$ Å[24]) point clusters. As expected,[25] in all plots the hydrogen bond clusters fall in the angular regions characteristic of β-turn types I, II, III and their inverse I′, II′, III′. Then, we identify the clusters containing the β-turn II, the group I + III and their inverse. In the plots concerning φ_2, φ_3 and ψ_3 there are only two hydrogen bond

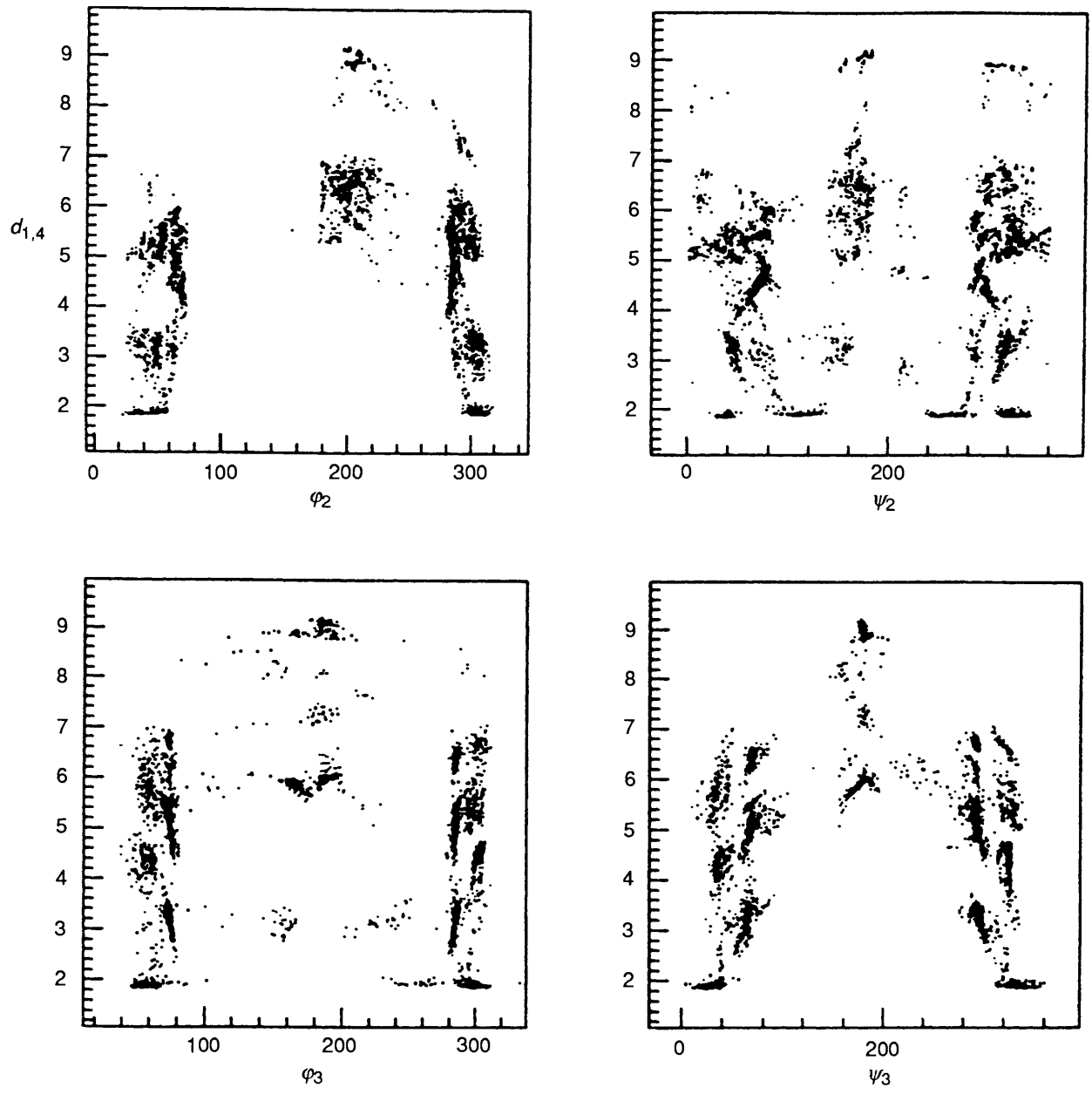

Fig. 9 Conformers' scatter-plots of the interaction distance $d_{1,4}$ = Gly-1-C=O · · · HNGly-4 *vs.* φ_i, ψ_i (i = 2, 3). Distance is expressed in Å, torsion in degrees.

clusters originated by overlapping of different β-turn types which does not allow us to discriminate between them. In contrast, in ψ_2 plots the four hydrogen bond clusters centred at 120, −120, −40 and 40° correspond to β-turns type II, II′, I + III and I′ + III′, respectively. We have selected the subset comprising the type II β-turn. Gly-1-C=O · · · HNGly-4, in agreement with the previous experimental results,[2] on the basis of d(Gly-1-C=O · · · HNGly-4) ≤ 2.3 Å[24] and 80° ≤ ψ_2 ≤ 160°.[25] 142 Conformers resulted and we have also verified that the torsions φ_2, φ_3 and ψ_3 are compatible with the type II β-turn. The same result is obtained if the conformers with all torsions $(\varphi_i, \psi_i)_{i=2,3}$ of type II β-turn are selected, in agreement with Nemethy and Scheraga.[25] However, the conformers with at least 3 torsions $(\varphi_i, \psi_i)_{i=2,3}$ of type II β-turn are 366, and are not satisfying as a large number of structures devoid of the Gly-1-C=O · · · HNGly-4 hydrogen bonds are selected.[24]

In Fig. 10 the λ-plots $(\psi_i, \varphi_{i+1})_{i=1-3}$ and energy distribution for such conformers are reported. The ψ_2, φ_3 torsion pair are anticorrelated: the points in the plot (ψ_2, φ_3) fall around a line with an approximately −0.5 slope, *i.e.* for a change of φ_3 a double oppositely signed change in ψ_2 corresponds. The presence of the Gly-1-C=O · · · HNGly-4 hydrogen bond induces a different variability of the torsions ψ_2 and φ_3. In contrast, the ψ_i, φ_{i+1} (i = 1–3) torsion pairs appear completely uncorrelated. In the models proposed in the literature[9] the torsion pairs ψ_i, φ_{i+1} outside the β-turn rings are correlated. Nevertheless, although it is clear that the β-turn is not a rigid structure but exhibits a number of accessible conformational states, the correlations of φ, ψ pairs inside the turn are not discussed. Furthermore, in these models the β-turns correspond to the corner sequences Pro-Gly where a drastic reduction of the torsional freedom degrees because of Pro takes place. In our case, the obtained results evidence that the ψ_2, φ_3 torsions inside the β-turn II for corner sequences Leu-Gly may be anticorrelated and contribute with their mobility to the librational entropy of the peptide. The energy distribution is roughly bell-like shaped with the highest frequency region at about the same value found for the conformers distribution of Fig. 2.

The conformer subset characterized, according to the experi-

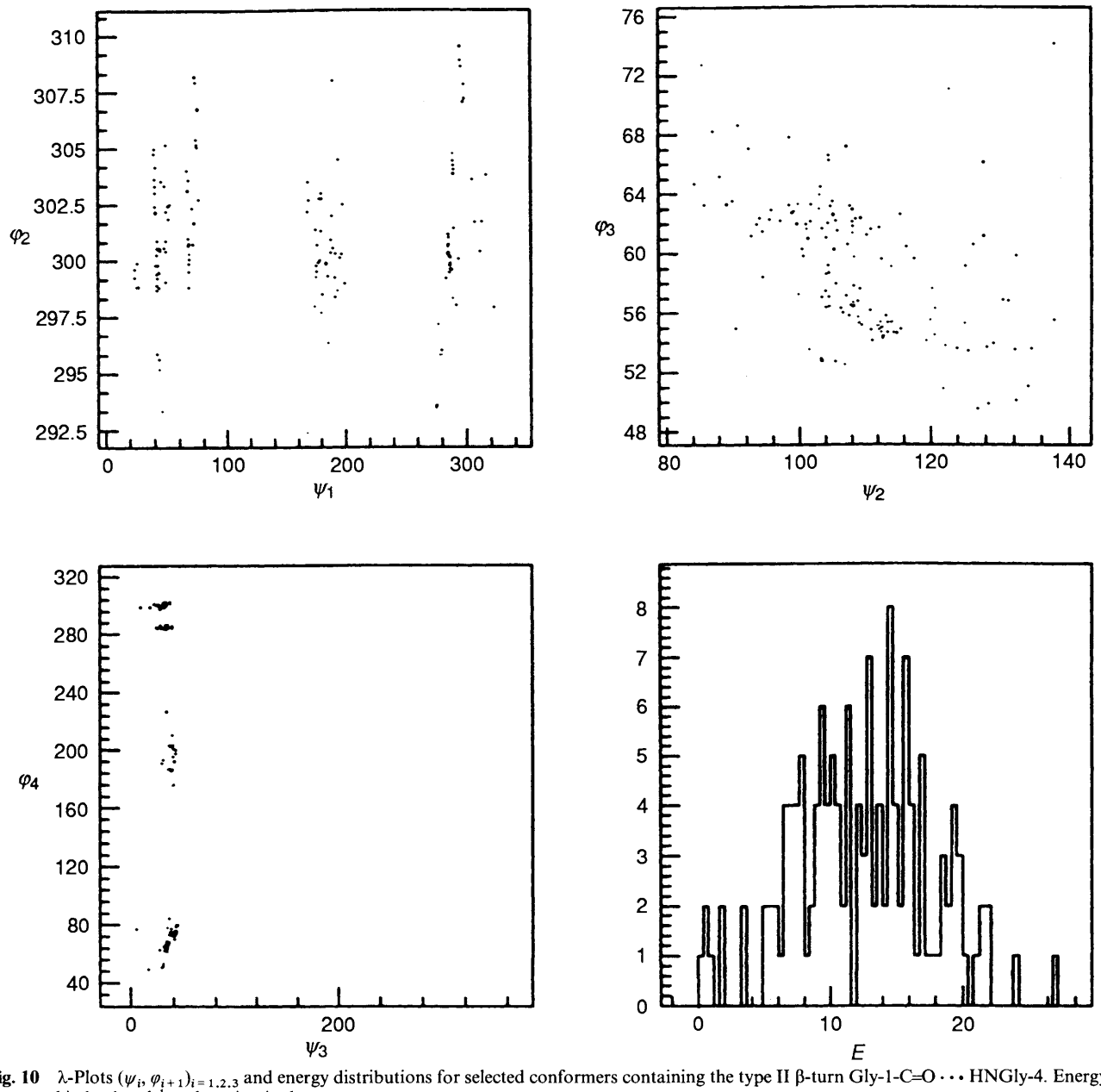

Fig. 10 λ-Plots $(\psi_i, \varphi_{i+1})_{i=1,2,3}$ and energy distributions for selected conformers containing the type II β-turn Gly-1-C=O · · · HNGly-4. Energy is expressed in kcal mol^{-1} and torsion in degrees.

mental evidence, by the co-presence of the seven-membered ring BocC=O · · · HNLeu-2 and β-turn II Gly-1-C=O · · · HNGly-4 and by the absence of hydrogen bonded rings with more than ten members ($C_{n>10}$), as C_{14} Gly-4-C=O · · · HNGly-1, has been selected. 50 Conformers have been obtained spread on an energy range from 3.5 to 19 kcal mol^{-1}. The analysis of the interaction distances >C=O · · · HN< revealed that about 40% of conformer population show the C_7 Gly-3-C=O · · · HNMe and 28% the C_{10} Leu-2-C=O · · · HNMe. The first structure is present in the whole energy range; the second one is present in the energy range from 6.2 to 10 kcal mol^{-1}, always a type III' β-turn and never coexisting with Gly-3-C=O · · · HNMe. The selected Gly-3-C=O · · · HNMe conformer family fully agrees with the known experimental data and confirms the hypothesis of the C_7 HNMe hydrogen bond.[2] The lowest energy conformer at ΔE = 3.5 kcal mol^{-1} is reported in Fig. 11(*a*). The presence of the Leu-2-C=O · · · HNMe type III' β-turn family is in contrast with previous CD results.[2]

To analyse the chance of β-turn sliding between Gly-1-C=O · · · HNGly-4 and Leu-2-C=O · · · HNMe, as previously reported,[2] we have selected the conformer family characterized by the co-presence of BocC=O · · · HNLeu-2 and type II β-turn Leu-2-C=O · · · HNMe. 15 Conformers have been obtained in an energy range from 9.7 to 17 kcal mol^{-1}, and the lowest energy conformer is reported in Fig. 11(*b*). The hydrogen bond C_7 Gly-1-C=O · · · HNGly-3 is always present and the secondary structures Leu-2-C=O · · · HNMe type II β-turn and Gly-1-C=O · · · HNGly-4 hydrogen bond never coexist.

In conclusion, the selected conformers' families confirm the stability of type II β-turn Gly-1-C=O · · · HNGly-4 and the chance of C_7 HNLeu-2, C_7 HNMe and C_{10} HNMe hydrogen bonds. The possibility of a conformational equilibrium implying a sliding between Gly-1-C=O · · · HNGly-4 and Leu-2-C=O · · · HNMe hydrogen bonds, which may contribute to the peptide entropy, appears to be verified.

Conclusions

The set of the tetrapeptide Boc-Gly-Leu-Gly-Gly–NMe conformers has been determined by molecular mechanics energy minimizations using a strategy based on the build-up method. The outcome of the search was constituted by 3785

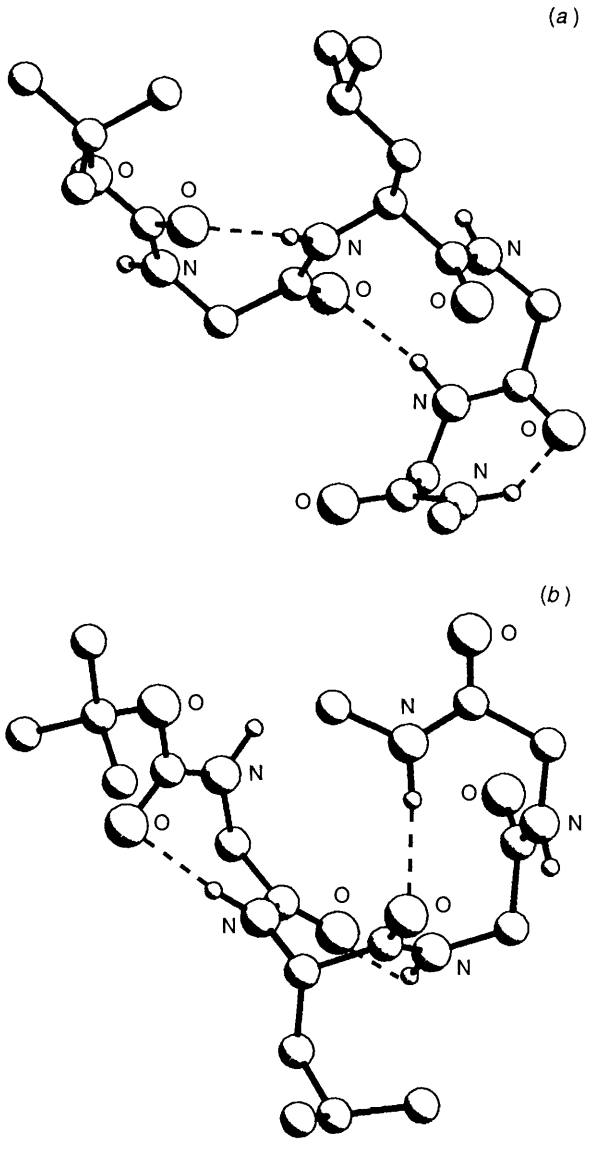

Fig. 11 The lowest-energy conformer containing the type II β-turn Gly-1-C=O ··· HNGly-4 (*a*) and the corresponding containing the type II β-turn Leu-2-C=O ··· HNMe (*b*). Hydrogen bonds are shown as dashed lines. Labels are reported for non-hydrogen and -carbon atoms.

conformers spread on an energy range of 30 kcal mol^{-1} with a Gaussian distribution. This agrees with the experimental results pointing to high conformational flexibility and indicates a small coupling among the inter-residue torsional degrees of freedom of the molecule. The low-energy surface (ΔE < 5 kcal mol^{-1}) is poorly populated according to the shift of the energy distribution to higher energy values on increasing the length of the chain of similar sequences. Moreover, a correlation is observed between the conformer's energy and the end-to-end distance: the folding degree of the chain decreases passing from lower to higher energy values. Accordingly, it may be speculated about a possible role played by the sequence Gly-X-Gly-Gly-Y in inducing the drastic change of the dynamic-mechanical properties of elastin, *i.e.* from brittle to viscoelastic passing from the anhydrous to the water-swelled state.

The φ, ψ torsions show well-defined conformational preferences, in fact their distributions are essentially penta-modal with the frequency maxima near the *t* and the split $g^{\pm}$ positions. The φ, ψ energy distributions are Gaussian-like and in all cases the *gauche* states are energetically favoured with respect to the *trans* states. The conformers' Ramachandran scatter-plots reveal a strong correlation between the φ, ψ intra-residue pairs with five main clusters disposed in a highly symmetric way [*i.e.* $F(\varphi, \psi) = f(\varphi) \times f(\psi)$ and $F(\varphi, \psi) = F(-\varphi, -\psi)$, with *f* and *F* torsional distribution densities].

From the hydrogen bond distributions it is clear that all the C_7 and C_{10} secondary structures are populated. Nevertheless, according to the experimental results, in the low-energy region only the hydrogen bond BocC=O ··· HNLeu-2 (C_7), Gly-1-C=O ··· HNGly-4 (C_{10}) and Gly-3-C=O ··· HNMe (C_7) appear to be stable.

The calculated global minimum is highly folded with a C_{14} hairpin structure that is not confirmed by available experimental data in polar solvents, but it could be likely in apolar solvents as expected from the energy minimizations of the isolated molecule (*i.e. in vacuo* or gas-phase) which, in order to maximize the hydrogen bond interactions, generates globular-like structures not easily observable in polar solvents. Nevertheless, the conformation which is in full agreement with the experimental evidences is only at ΔE = 3.5 kcal mol^{-1}, confirming the need for searching in conformational analysis all low-energy conformers rather than the global minimum alone.

In our opinion, Urry's entropic librational mechanism of elastin elasticity is not applicable to Gly-X-Gly-Gly-Y amino acid sequences. As a matter of fact, in the conformer family containing the type II β-turn Gly-1-C=O ··· HNGly-4, experimentally evidenced, the ψ_2, φ_3 torsions pair inside the β-turn are anticorrelated and a change of φ_3 corresponds to a double change in ψ_2. These crankshaft rotations could contribute to the librational entropy of the peptide. In contrast, in Urry's model, only the ψ_i, φ_{i+1} pairs outside β-turns are responsible for the librational entropy. We propose that different librational entropic mechanisms occur for polypeptide sequences of the kind Gly-X-Gly-Gly-Y or Gly-X-Pro-Gly-Y, because of different cooperative rotations ψ_i, φ_{i+1} inside or outside the type II β-turn XC=O ··· HNY, respectively. Also, the type II β-turn Leu-2-C=O ··· HNMe conformer family has been selected confirming the possibilities of C_7 and C_{10} HNMe hydrogen bonds and of conformational β-turn II sliding equilibrium between Gly-1-C=O ··· HNGly-4 and Leu-2-C=O ··· HNMe hydrogen bonds. This could contribute to the peptide entropy.

Molecular dynamics simulations in progress should be useful to verify the librational and sliding type II β-turns motions.

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