

# Conformational analysis of the 13/4 to 4/1 helical transition in poly( $\alpha$ -isobutyl $\beta$ -L-aspartate)

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## Abstract

The mechanism for the conversion of the 13/4 helix into the 4/1 helix of poly( $\alpha$ -isobutyl  $\beta$ -L-aspartate) was simulated at the atomic level. A computational procedure based on a potential of mean force capable of creating multiple conformation states compatible with a given helical symmetry was used. Intermediate helical arrangements differing in 0.1 residues/turn were modeled and energetically evaluated to delineate the reaction coordinate. The conversion was found to proceed through poorly defined hydrogen bonded structures having an energy surplus of about 6 kcal/mol-residue. Results were consistent with experimental data and helpful for understanding fundamental aspects of the transition as it is observed in the solid state. © 1999 Elsevier Science Ltd. All rights reserved.

*Keywords:* Poly( $\beta$ -peptide)s; Potential of mean force; Poly( $\beta$  aspartate)s

## 1. Introduction

Poly( $\alpha$ -alkyl  $\beta$ -L-aspartate)s are a class of poly( $\beta$ -peptide)s, i.e., polymers derived from  $\beta$ -amino acids, that have attracted great interest because of their capability to take up helical conformations similar to the  $\alpha$ -helix of poly( $\alpha$ -amino acid)s [1,2]. Recently  $\beta$ -peptides containing as few as six residues have been shown to form stable helices with conformational features resembling those described earlier for poly( $\beta$ -L-aspartate)s [3,4].

As poly( $\beta$ -peptide)s contain an additional freely rotating methylene in the main chain, they are expected to display a greater conformational flexibility. Among the variety of conformations described for poly( $\alpha$ -alkyl  $\beta$ -L-aspartate)s, the right-handed 13/4 and 4/1 helices with 3.25 and 4.0 residues per turn respectively, are the structures most frequently found [1]. Whereas the former is observed for side groups of any size and shape, the existence of the later appears to be restricted to polymers bearing acyclic side groups with less than six carbon atoms. As illustrated in Fig. 1, the 13/4 helical conformation is defined by interwoven 14-membered ring hydrogen bonds which run from a backbone NH to the backbone carbonyl of the third amide group in the C-terminal direction. Conversely, the 4/1 helix has the hydrogen-bonds set between the  $i$  and  $i + 4$  amide groups in the C direction as well, so that 18-membered ring hydrogen bonds are formed in this case. The 13/4 helix

crystallizes usually in a pseudohexagonal lattice with chains oriented up-and-down while the 4/1 helix invariably adopts a tetragonal structure in which all chains seem to point to the same direction [5].

The hexagonal crystal form converts into tetragonal upon heating through a mechanism that implies a rearrangement in both the helical conformation and the position of the chains within the lattice. Although the transition is known to occur for almost all poly( $\beta$ -L-aspartate)s displaying hexagonal-tetragonal dimorphism [6–9], it is best observed for poly( $\alpha$ -isobutyl  $\beta$ -L-aspartate), abbreviated PAIBLA. The phase change is detected in the DSC heating traces of the hexagonal form as an exothermic peak in the proximity of 130°C with an enthalpy of around  $-3$  kcal/mol [10]. Structural variations involved in the transition have been characterized by both X-ray [11] and electron diffraction [12]. The phenomenon is of interest owing to the scarcity of transitions involving 4/1 helices reported so far, the closest example being the  $\alpha$ -to- $\omega$ -helix transition undergone by the polypeptide poly( $\beta$ -benzyl  $\alpha$ -L-aspartate) [13,14].

In the last few years a considerable number of studies have been devoted to investigate conformational transitions in poly( $\alpha$ -amino acid)s that take place between two well-defined end structures differing in their hydrogen bond schemes [15–27]. In such studies the relative energy of the intermediate states is estimated by means of a potential of mean force that induces hydrogen bonds to change according to the scheme required by each of the two end

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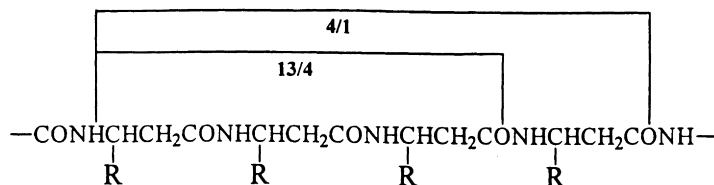


Fig. 1. Hydrogen bonding schemes for the 13/4 and 4/1 helices of poly( $\beta$ -L-aspartate)s.

conformations. Although the hexagonal–tetragonal transition occurring in poly( $\beta$ -L-aspartate)s is experimentally well supported, it has not been modeled at the atomic level so far. In the present work we present a computational study of the conversion of the 13/4 helix into the 4/1-helix of PAIBLA. This is the first stage for a better understanding of the transition observed in poly( $\beta$ -L-aspartate)s. The results obtained in this study provide a picture of the change between the two helical conformations at the atomic level that is of help to rationalize the process.

## 2. Methods

### 2.1. Helix geometries

The conformations assumed for the right-handed 13/4 and 4/1 helices of PAIBLA were those previously derived from X-ray diffraction analysis [28]. The backbone and side-chain dihedral angles were taken thereof where such parameters were refined against X-ray diffraction data. In these structures hydrogen bonds are almost linear with N $\cdots$ O distances within the range 0.280–0.290 nm and N $\cdots$ H $\cdots$ O angles spanning between 172° and 178°. The dihedral angle notation used for PAIBLA is indicated in Fig. 2. Conformational and crystal parameters for the hexagonal and tetragonal forms of this polymer are compared in Table 1 and the

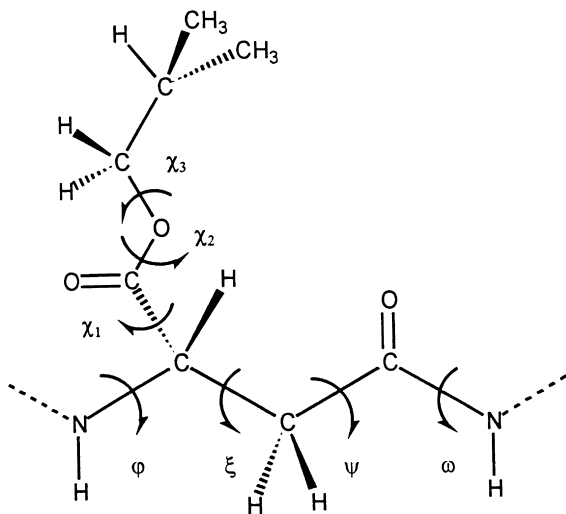


Fig. 2. The asymmetric unit of PAIBLA with indication of the notations used for the torsion angles.

axial projections of the corresponding 13/4 and 4/1 helices are depicted in Fig. 3.

### 2.2. Energy calculations

Computational procedures currently used to investigate conformational transitions in poly( $\alpha$ -amino acid)s are not feasible for poly( $\beta$ -aspartate)s as a result of the greater conformational flexibility and lower stability of the helical arrangements adopted by these polymers. In fact, preliminary molecular dynamics simulations carried out on PAIBLA oligomers have shown that this system tends to evolve towards a mixed structure when intramolecular distances are taken to define the reaction coordinate between the two end helical states.

In the present study we have taken advantage of GEMOX, a computational procedure recently developed in our laboratory for searching and generating all the conformations compatible with a given helical geometry. The program is based on systematic algorithm capable of creating multiple conformational states and, therefore, permits the design of a potential of mean force. A detailed description of the computational strategy used by GEMOX has been recently reported [29].

For the conformational analysis of the 13/4 to 4/1 transition, a polymer chain with 3.25 residues per turn was built with bond angles and distances fixed at their standard values. Such chain was generated taking advantage of the contour conditions applied by the GEMOX program which permits to generate an infinite molecular chain with all the residues in the same conformation. This helix was driven to the final symmetry of 4.0 residues/turn by a stepping increase of 0.05 residues/turn, the torsion angles being the only internal parameters subjected to modification. It is worth noting that in poly( $\beta$ -L-aspartate)s contain three flexible dihedral angles which considerably increase the difficulties involved in simulation studies. Due to computational limitations we have applied the approach that Tirado-Rives and Jorgensen [19] used for polypeptides derived from  $\alpha$ -amino acids. Thus, one of the flexible dihedral angles was fixed during the simulation. As the  $\phi$  backbone dihedral has very similar values for the initial and final states, only the  $\xi$  and  $\psi$  angles were allowed to change throughout the reaction coordinate. In all cases bond angles and distances were hold within standard values. The side chain torsions were fixed in the GEMOX calculations at the values previously derived from the X-ray diffraction data.

Table 1  
Conformational and crystal parameters of the two helical forms of PAIBLA

Helix	Torsion angles <sup>a</sup> (deg)			H-bonds			Crystal form	$\Delta E$ (kcal/mol-residue) <sup>b</sup>			
	$\phi$	$\xi$	$\psi$	$\omega$	$\chi_1$	$\chi_2$			$\chi_3$	N $\cdots$ H $\cdots$ O (deg)	
13/4	145.2	-62.0	130.8	180.0	175.0	180.0	-101.2	0.29	165.4	HEX <sup>c</sup> ; $a = 1.35$ nm, $c = 1.99$ nm	0.7
4/1	144.2	-86.9	148.5	180.0	-179.5	180.0	-128.8	0.29	168.6	TET; $a = 1.40$ nm, $c = 0.495$ nm	0.0

<sup>a</sup> After refining by LALS. Energies were evaluated with AMBER. Angle notations as given in Fig. 2.

<sup>b</sup> Relative energies.

<sup>c</sup> Actually a monoclinic lattice made of up-and-down chains.

The conformational energy in the GEMOX force-field is computed by adding the van der Waals and electrostatic non-bonding contributions to the energy associated with the torsion of the bonds. The van der Waals and the electrostatic energies were estimated using a 6-12 function and the atom-centered monopole approach, respectively. A Fourier series approach with three terms per dihedral angle was used to compute the torsional energy. Parameters previously developed for us for poly( $\beta$ -L-aspartate)s were used [7]. A scaling factor of 0.5 has been incorporated when dealing with 1-4 non-bonding interactions. This strategy allows to reduce the excess of repulsive energy provided by the 6-12 function for the shorter non-bonded interactions [30,31].

In order to evaluate the dependence of the results on the potential energy function, conformational energies were further calculated using the AMBER force-field [30,31]. Thus, for a given window of the transition path, the geometry of the side chains was minimized for all the structures generated by GEMOX while backbone dihedral angles were kept unchanged. These calculations were performed with the AMBER 3.0 Rev program [32] on an oligomer consisting of 24 residues blocked at the ends as N-acetyl and N-methyl groups.

### 3. Results and discussion

The energy surfaces obtained by the computational procedures described in the above section are shown in Fig. 4. Energies are referred to the most favored conformation which was proved to be the right-handed 4/1 helix by the two force-fields. Thus, GEMOX and AMBER force-fields predicted that the 13/4 helix is 0.3 and 0.7 kcal/mol-residue, respectively, unfavored with respect to the 4/1-helix. This is in agreement with previous molecular mechanics results which had revealed that attractive van der Waals intramolecular interactions between spatial neighboring residues for the tend to favour the 4/1 helix with respect to the 13/4 one. For each helix point of the reaction path the energy was determined using a weighted Boltzmann average of the conformational energies estimated for the structures generated by GEMOX. Both energy surfaces show the existence of an activation energy barrier for the transition process with a magnitude of about 6 kcal/mol-residue. However, it should be noted that this is an upper bound estimate since the dihedral angle  $\phi$  was fixed. This point was discussed by some authors who investigated [19] the differences in energy estimates obtained using the Jorgensen approach and other more sophisticated methods for the transition in helical peptides derived from  $\alpha$ -amino acids [20,23]. Conformational parameters and relative energies of the barrier species afforded by GEMOX and AMBER are summarized in Table 2.

The number of conformations energetically favored compatible with a given value of the transition coordinate is larger for the intermediate states than for the end point

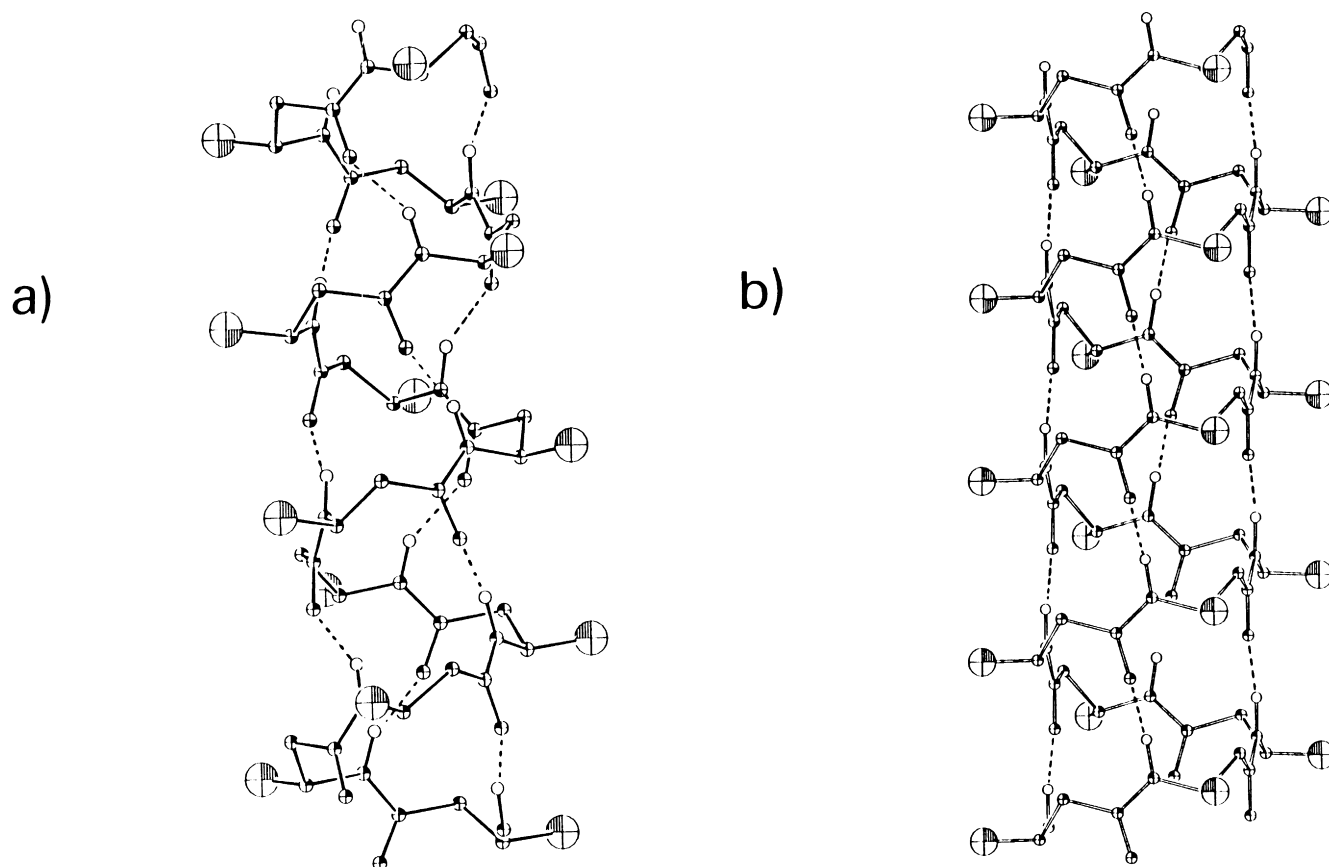


Fig. 3. Axial projections of the right-handed 13/4 (a) and 4/1 (b) helices of PAIBLA. In order to make clearer the view, side chains have been replaced by spheres.

structures. This trend can be explained in terms of hydrogen bonding geometries, which are almost ideal for the end points. Thus, the 13/4 and 4/1 helices are conformationally more restricted than the intermediate states. The maximum of possibilities is observed for those helical arrangements having about 3.5 residues per turn which is close to the point where the energy attains the top value. This result indicates

Table 2  
Conformational parameters and relative energies (in kcal/mol-residue) for the GEMOX and AMBER barrier species of PAIBLA<sup>a</sup>

	GEMOX	AMBER
Residues/turn	3.55	3.60
$\omega^b$	180.0°	180.0°
$\phi^b$	144.7°	144.7°
$\xi$	$-71.2^\circ \pm 1.0^\circ$	$-70.1^\circ \pm 1.0^\circ$
$\psi$	$145.6^\circ \pm 2.5^\circ$	$145.7^\circ \pm 3.0^\circ$
$\Delta E(13/4 \rightarrow \text{Barrier})^c$	$6.3 \pm 1.2$	$5.1 \pm 0.2$
$\Delta E(4/1 \rightarrow \text{Barrier})^d$	$6.6 \pm 1.2$	$5.8 \pm 0.6$

<sup>a</sup> Torsion angles in degrees.

<sup>b</sup> The angles  $\omega$  and  $\phi$  were fixed at 180° and 144.7° respectively (see text).

<sup>c</sup> Relative energy with respect to the 13/4 helix.

<sup>d</sup> Relative energy with respect to the 4/1 helix.

that barrier species are entropically favored with respect to the 13/4 and 4/1 helices which is consistent with the appearing of less favorable energetic components owing to either steric clashes or the loss of electrostatic interactions. Non-bonding energy contributions to the potential surfaces derived from GEMOX and AMBER calculations are given in Table 3. The inspection of the different terms reveals that the energy surplus appearing at the intermediate states is a combination of the van der Waals and electrostatic contributions since a destabilization of several kcal/mol-residue relative to the end points is observed for these two components.

The evolution of N...O hydrogen bond distances throughout the transition is displayed in Fig. 5a. As logically

Table 3  
Energy contributions (in kcal/mol-residue) for the 13/4 helix, barrier species and 4/1 helices of PAIBLA

Force-Field	Contribution	13/4 Helix	Barrier	4/1 Helix
GEMOX	Electrostatic	-96.3	$-94.9 \pm 0.5$	-97.5
	van der Waals	-6.3	$-1.6 \pm 0.8$	-5.3
AMBER	Electrostatic	-64.0	$-61.2 \pm 0.2$	-66.5
	van der Waals	-2.8	$-0.4 \pm 0.3$	-1.7

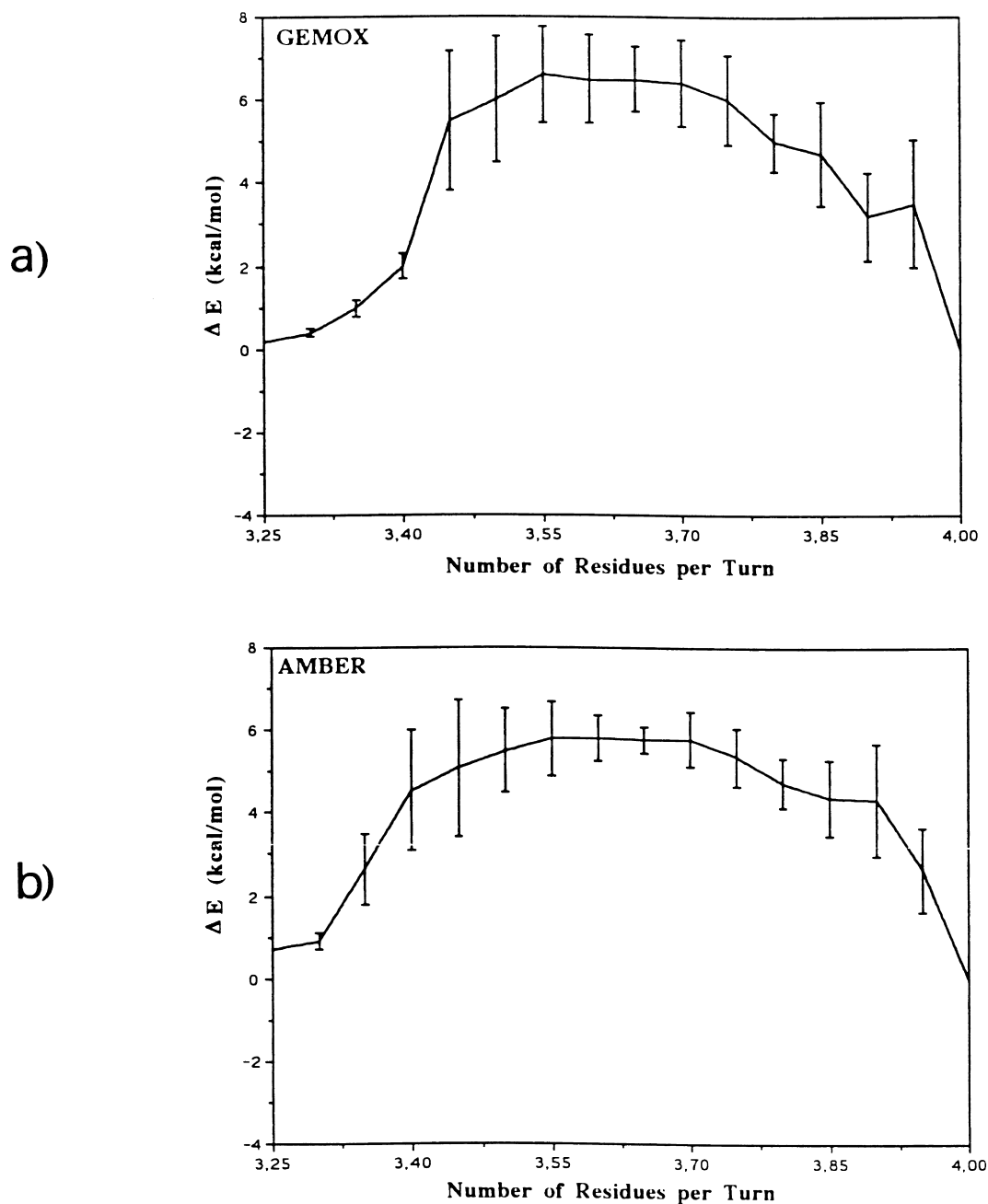


Fig. 4. Energy surfaces obtained from GEMOX (a) and AMBER (b) calculations. The error bars correspond to the standard deviations of the energies for the conformations found for each helix pitch.

expected, hydrogen bonding distance between the  $i$  and  $i + 3$  amide groups occurring in the  $13/4$  helix increases with the advancement of the reaction coordinate whereas the reverse is found to happen for hydrogen bonds between the  $i$  and  $i + 4$  amide groups characteristic of the  $4/1$  helix. Moreover, whereas the variation is steady for the former case, an uneven trajectory is observed for the latter indicating the greater flexibility of the  $i - i + 3$  hydrogen bonding scheme. Note that if the hydrogen bond distance is retained below  $0.30$  nm, the  $3/4$  helix can be distorted up to  $+0.4$  residues/turn whereas a distortion of  $-0.25$  residues/turn is

the maximum allotted for the  $4/1$  helix. On the other hand, it is worth to note that bifurcated hydrogen bonds were not found to be compatible with any of the intermediate states. This is contrary to recent studies carried out on poly( $\alpha$ -amino acid)s reporting that three-centered hydrogen bonds are present throughout the whole transition coordinate. In the case of poly( $\beta$ -L-aspartate)s such a possibility is hindered by the existence of the additional methylene in the main chain.

Fig. 5b shows the variation profiles of the torsion angles  $\xi$  and  $\psi$  along the reaction coordinate generated by GEMOX. An almost linear function giving a total variation about  $30^\circ$

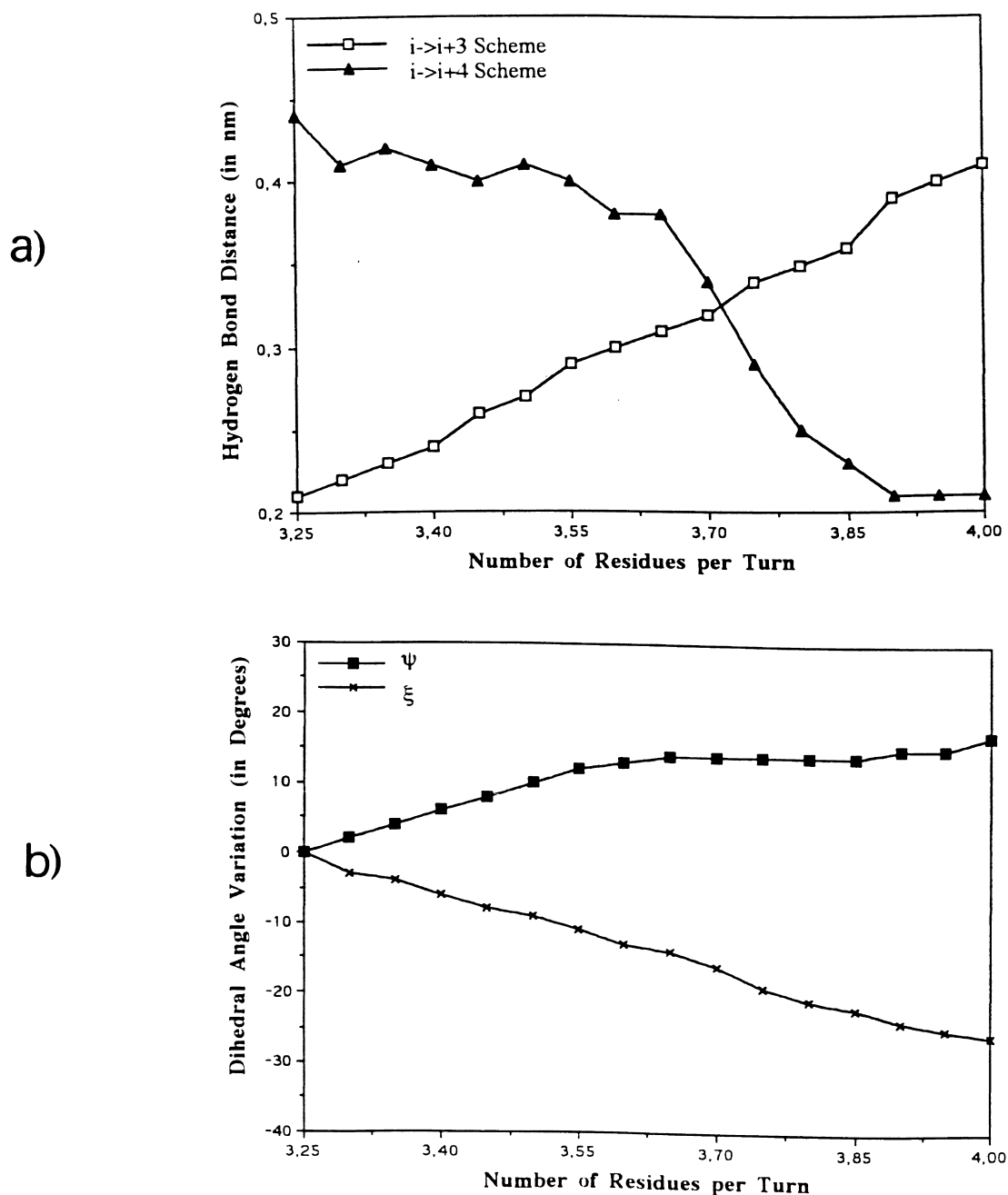


Fig. 5. Variation of the hydrogen bond N...O distance (a) and  $\xi$  and  $\psi$  dihedral angles (b) with the advancement of conversion of the 13/4-helix into 4/1-helix.

is predicted for the former. Conversely, the angle  $\psi$  presents a linear variation within the 3.25–3.65 residues/turn range and remains essentially unchanged for larger values of the reaction coordinate. These results suggest that the conformational transition occurs through a two-steps process. In the first step there is a concerted movement of angles  $\xi$  and  $\psi$  driving the 13/4 helix to the barrier structure. Secondly the angle  $\xi$  continues changing to drive the barrier species to the 4/1 helix while the angle  $\psi$  remains at the value attained in the first step.

Fig. 6 shows the equatorial projections of the lowest energy conformations found by GEMOX for a selection

of values of the reaction coordinate which includes the initial and final states as well. Comparison of projections of the 13/4 and 4/1 helices clearly shows that the central cavity is notably larger in the latter structure. However it is worthy to remark that the size of the hole does not change significantly in the earlier stages of the transition, being at the end of the process when a significant increasing in the diameter of the helix is produced. It can be concluded therefore that the enlarging of the hole should be associated with the variation of angle  $\xi$  taking place during the second step of the transition.

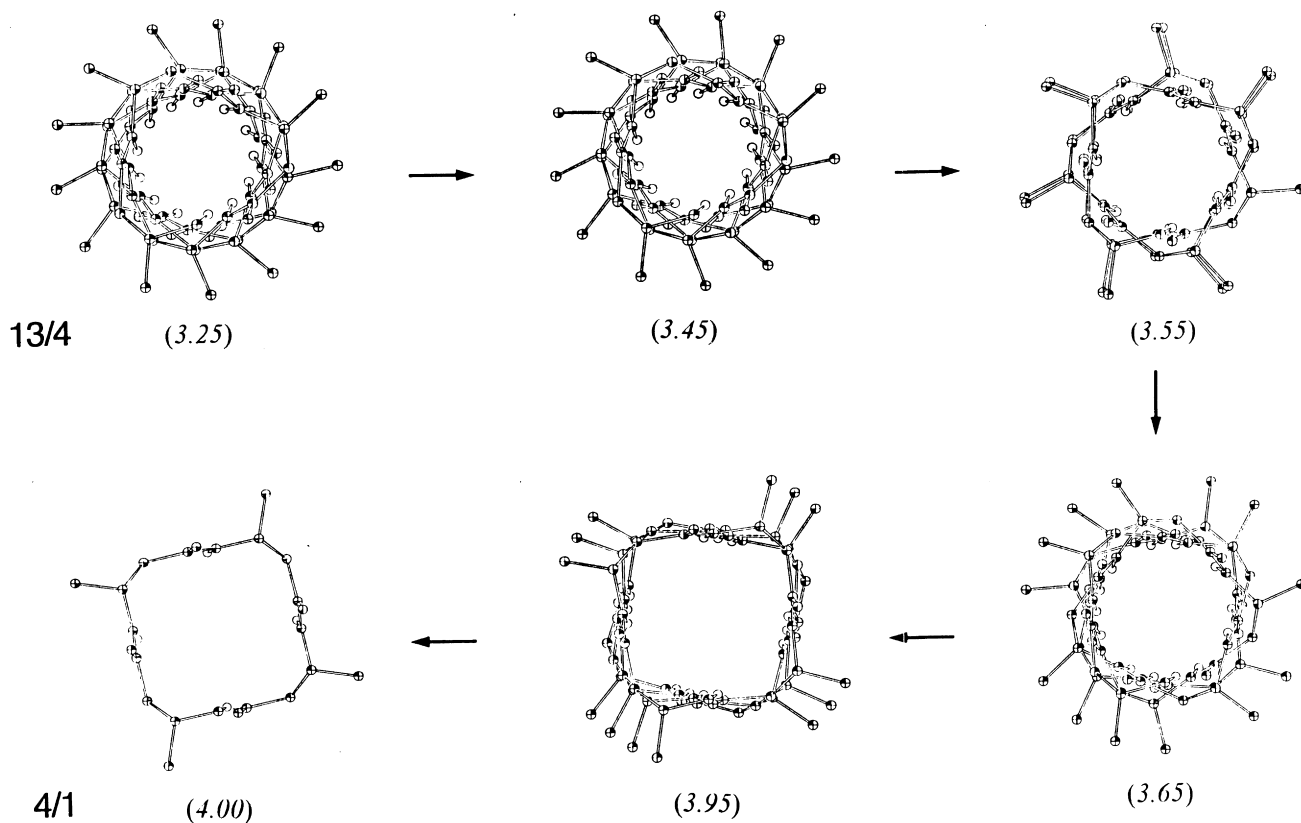


Fig. 6. Molecular mechanism for the conversion of the 13/4-helix into the 4/1 helix of PAIBLA. The lowest energy conformations for a selection of points of the transition coordinate are represented. Equatorial projections are obtained from chains consisting of 13 residues. Side groups have been replaced by spheres for clarity.

### 3.1. Implications in the hexagonal-to-tetragonal crystal transition

The conversion of the hexagonal structure of right-handed 13/4 helices into the tetragonal form made of right-handed 4/1 helices has been observed in powder, fibers and single crystals of PAIBLA. The process has been closely followed by electron microscopy which unveiled the existence of a definite crystallographic relationship between the original hexagonal lattice and the tetragonal phase that emerges as a result of the heating treatment. A schematic description of the crystal transition based on the shifting of parallel planes made of adjacent chains was given on the basis of such observations [12].

Although no evidence are available to support directly the mechanism lying behind the transition at the molecular level, the atomistic model presented in this work is consistent with most of available experimental data. It is perfectly compatible indeed with the model proposed by Muñoz-Guerra et al. [12] accounting for the change in the crystal lattice taking place at transition. According to observations, no intermediate helical arrangements with diameter out of the range 1.35–1.40 nm are involved in the mechanism. As it should be reasonably expected from the topological simi-

larities existing between the two end helices, breaking and remaking of hydrogen bonds take place without requiring an extensive rearrangement of the whole molecule. Energy data indicate also that barrier species are considerably favored from an entropy point of view with respect to the end points. This means that the entropy term would result in a more favorable contribution when temperature increases lowering the energy barrier for the transition.

There are firm evidences that the hexagonal form converts rapidly in tetragonal when soaked in slight alcohols. As the structure adopted by PAIBLA depends largely on the experimental conditions used for crystallization, it is not surprising that the transition can be promoted by certain solvents. A theoretical study on the effect of polar solvents on the conformational transition between 13/4 and 4/1 helices has been recently reported [33]. In such study an intermediate specie with 3.5 residues/turn was assumed to exist between the two end points. The relative stability of this intermediate structure was found to increase with the polarity of the environment as a result of the weakening of the hydrogen bonds occurring in both 13/4 and 4/1 helices. These results are consistent with the analysis presented in this work which reveals that barrier species are unable to form well-defined hydrogen bonded structures.

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