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Research Letter

Characteristic Structural Features of Indolicidin: Effects of the *cis-trans* Isomerism on its Conformation

Balázs Leitgeb*

Institute of Biophysics, Biological Research Centre, Hungarian Academy of Sciences, Temesvári krt. 62, H-6726, Szeged, Hungary
**Corresponding author: Balázs Leitgeb, leitgeb@brc.hu*

Indolicidin is an antimicrobial peptide showing a broad spectrum of antibacterial and antifungal activities, and according to the *cis-trans* isomerism of three Xaa-Pro peptide bonds, eight different stereoisomers could be distinguished for this peptide. As the *cis-trans* isomerism about the Xaa-Pro peptide bonds was not considered in previous studies, the structural features of distinct stereoisomeric forms were not characterized in detail, so far. In this theoretical study, the influences of *cis-trans* isomerism on the conformation of indolicidin were investigated, as well as the typical structural properties of each stereoisomer were determined, focusing on the secondary structures and intramolecular interactions. Based on the results derived from the molecular dynamics simulations, it could be concluded that the eight different stereoisomeric forms of indolicidin adopted characteristic conformational features. Nevertheless, the appearance of various turn structures and intramolecular interactions proved to be dependent on the *cis* or *trans* nature of Xaa-Pro peptide bonds, indicating the relevant role of Pro amino acids in determining the three-dimensional structure of this peptide.

Key words: antimicrobial peptide, *cis-trans* isomerism, indolicidin, intramolecular H-bond, molecular dynamics, proline–aromatic interaction, turn structure

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Indolicidin (Indo) is an antimicrobial peptide (AMP) characterized by a remarkable primary structure (i.e. H-ILPWKWPWWPWRR-NH_2), consisting of five Trp and three Pro amino acids, as well as of basic (i.e. Lys and Arg) and apolar (i.e. Leu and Ile) residues. This AMP was isolated from the cytoplasmic granules of bovine neutrophils (1), and it shows a broad spectrum of antibacterial and antifungal activities, however, it exhibits also hemolytic and antiviral effects. As Indo contains three Pro amino acids, this peptide exists as an equilibrium mixture of different stereoisomeric forms; in accordance with the *cis-trans* isomerism about three Xaa-Pro (i.e. Leu^2-Pro^3, Trp^6-Pro^7, and Trp^9-Pro^{10}) peptide bonds, eight distinct stereoisomers of this AMP could be distinguished. Based on the data derived from previous structural investigations, it could be concluded that the relative proportions of the eight stereoisomeric forms of Indo could not be exactly determined. Moreover, in the course of earlier experimental and theoretical studies, the *cis-trans* isomerism of three Xaa-Pro peptide bonds mentioned above was not considered, and thus, the characteristic structural features of the different stereoisomers of this AMP were not examined in detail, so far. The previous structural investigations applying experimental or theoretical methods led to various observations concerning the backbone conformation and secondary structure of the solution conformations, as well as of the micelle- and membrane-bound conformations of Indo. These are as follows: (i) an unordered structure for water, buffer, and organic solvent (2–6); a combination of turn and random coil for buffer (7); (ii) 3_{10}– (5) and poly-proline II (2) helical structures; an ordered, but not α-helical conformation (4,6); extended structure (8); extended conformation with half-turns (9); boat-shaped structure (8,10); a combination of turn and random coil (7); turn conformation (11); in the case of micelles and lipid bilayers. As it can be seen, the above-mentioned earlier studies led to considerably diverse conclusions with regard to the characteristic backbone conformation, as well as to the typical secondary structural element of Indo.

In this study, the effects of the *cis-trans* isomerism of three Xaa-Pro peptide bonds on the conformational properties of Indo were investigated, as well as the characteristic structural features of all the stereoisomeric forms of this AMP were identified. In the course of this theoretical study, the presence of various types of the β-turn structures was examined, and furthermore, the occurrence of different intramolecular interactions was studied. The typical conformational properties were determined for the eight stereoisomers of Indo, and they were compared with one another.

Methods and Materials

To investigate the structural features of the stereoisomeric forms of Indo, molecular dynamics (MD) calculations were performed with the AMBER 9 software (12). For the MD simulations, the AMBER 99SB force field (13) and the GB/SA implicit solvent model (14–17) were used, as well as no

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cutoff was applied in the case of non-bonding interactions. For all stereoisomers of Indo, the starting structure was subjected to an initial energy minimization, to supply a reasonable conformation for the subsequent simulated annealing–molecular dynamics (SA-MD), as well as MD calculations. In the course of SA-MD simulations, the initial structures of stereoisomeric forms were first heated up to 2000 K for 1 ns, then they were equilibrated at the same temperature during 0.5 ns, and finally, they were cooled down to 275 K for 8 ns using a multistep (i.e. 40 × 0.2 ns), near-exponential cooling protocol. The aforementioned three dynamic stages (i.e. heating, equilibration, and cooling) were followed by a further MD calculation during 0.5 ns on 275 K. In addition to the SA-MD simulations, in the case of each stereoisomer, ten individual MD calculations were carried out for 50 ns on 300 K, applying the Langevin model for the temperature regulation. During these simulations, the time step was set to 2 fs, and all bonds including hydrogen atom were constrained by the SHAKE algorithm using a tolerance of 10^{-5} Å. These MD calculations were started from the same geometrically optimized conformation, with regard to each stereoisomeric form, respectively, and random initial velocities were applied for every single simulation. The trajectories of individual calculations were sampled in every 50 ps, resulted in 1000 conformational states in the case of a single simulation. Afterward, the structures derived from the ten MD trajectories were collected together, and thus, 10 000 conformations were obtained for all stereoisomers.

According to the *cis-trans* isomerism of three Xaa-Pro peptide bonds (i.e. Leu^2-Pro^3, Trp^6-Pro^7, and Trp^9-Pro^{10} peptide bonds) of Indo, eight different stereoisomeric forms could be distinguished for this AMP, and thus, the SA-MD and MD simulations were performed on each stereoisomer, respectively. The eight stereoisomeric forms of Indo were labeled by three letter codes (i.e. *ttt*-, *ctt*-, *tct*-, *ttc*-, *cct*-, *ctc*-, *tcc*-, and *ccc*-Indo), where the letters represented the certain isomer of Leu^2-Pro^3, Trp^6-Pro^7, and Trp^9-Pro^{10} peptide bonds, as well as the '*t*' and '*c*' letters indicated the *trans* and *cis* isomers, severally.

Results and Discussion

To study the effects of *cis-trans* isomerism on the conformational features of Indo, the appearing secondary structural elements and intramolecular interactions were identified. For the stereoisomeric forms, the presence of different β-turn structures (i.e. types I, III, and VI β-turns) was examined, which were determined based on the characteristic ranges of Φ and Ψ torsion angles with regard to the *i + 1th* and *i + 2th* residues of a certain tetrapeptide unit (18–20). As typical intramolecular interactions could play a relevant role in the stabilization of various turn structures mentioned above, the occurrence of two types of intramolecular interplays was investigated as follows: (i) the *i*←*i + 3* H-bonds formed between a backbone NH donor group of *i + 3th* and a CO acceptor group of *ith* residues; (ii) the proline–aromatic (i.e. Pro–Aro) interactions evolved between a pyrrolidine ring of Pro amino acid and an aromatic side chain of neighboring Trp residues. These intramolecular interplays were assumed to exist if the certain geometric parameters satisfied the following characteristic distance and angle criteria. In the case of *i*←*i + 3* H-bonds, the *N*····*O* distance between the N and O atoms of NH and CO groups was within 3.5 Å, and the *N*–*H*····*O* angle subtended at the H atom by the bond to the C atom, and the line joining the H and O atoms was larger than 120°. In the case of Pro–Aro interactions, the *C*····*centroid* distance between any C atom of the pyrrolidine ring and the centroid of aromatic ring was smaller than 4.5 Å (21,22), and the *C*····*centroid*····*normal* angle between the line joining the C atom of pyrrolidine ring to the centroid of aromatic ring and the normal to the plane of aromatic ring was smaller than 30° (21,23), and finally, the *C*–*H*····*centroid* angle subtended at the H atom by the bond to the C atom, and the line joining the H atom to the centroid of aromatic ring was larger than 120° (21,23). For the determination of Pro–Aro interplays, the six-membered ring of the indole group of Trp amino acids was considered.

For the stereoisomeric forms, the appearance of types I and III β-turns was investigated with regard to all the tetrapeptide sequences, as well as the occurrence of *i*←*i + 3* H-bonds was examined. Table 1 shows the populations of the above-mentioned two types of β-turn structures identified in certain tetrapeptide units of stereoisomers, as well as the populations of *i*←*i + 3* H-bonds, which were determined based on the conformations obtained by the MD simulations. The representative conformations of *ttt*-Indo characterized by one type I or III β-turn in certain tetrapeptide sequences are illustrated in Figure 1, while the Figure 2 shows the different types of *i*←*i + 3* H-bonds for the Indo.

Considering the tetrapeptide segments containing Pro amino acid in the second position (i.e. Leu^2-Pro^3-Trp^4-Lys^5, Trp^6-Pro^7-Trp^8-Trp^9, and Trp^9-Pro^{10}-Trp^{11}-Arg^{12} sequences), large populations of types I and III β-turns were observed in the case of *trans* isomers. These β-turn structures were also detected for the *cis* isomers, however, their amount was found to be much smaller as compared to those observed in the case of *trans* isomers. Taking into account the *i*←*i + 3* H-bonds, it could be concluded that they appeared with high frequency for the *trans* isomers, according to their important role played in the structural stabilization of types I and III β-turns. Nevertheless, these intramolecular interactions were completely absent in the case of *cis* isomers, indicating the occurrence of so-called open turn conformations without the stabilizing *i*←*i + 3* H-bonds.

Among the tetrapeptide units, which possess Pro residue in the first position, types I and III β-turns, as well as *i*←*i + 3* H-bonds, were observed for two segments (i.e. Pro^3-Trp^4-Lys^5-Trp^6, and Pro^{10}-Trp^{11}-Arg^{12}-Arg^{13} sequences), however, they were not detected for one seg-

Table 1: Populations (in%) of the types I and III β-turns identified in various tetrapeptide units of the stereoisomeric forms of Indo, and the populations (in%) of the different types of $i \leftarrow i + 3$ H-bonds

	Type I β-turns							
	ttt-Indo	*ctt*-Indo	*tct*-Indo	*ttc*-Indo	*cct*-Indo	*ctc*-Indo	*tcc*-Indo	*ccc*-Indo
Tetrapeptide units								
Ile^1-Leu^2-Pro^3-Trp^4	1.30	0	2.22	1.70	0	0	2.43	0
Leu^2-Pro^3-Trp^4-Lys^5	71.17	11.76	62.71	36.52	0.99	7.10	30.94	0.35
Pro^3-Trp^4-Lys^5-Trp^6	46.34	15.10	3.61	31.34	1.83	8.52	2.55	1.23
Trp^6-Pro^7-Trp^8-Trp^9	72.81	64.68	7.93	50.36	4.56	56.13	5.13	7.80
Trp^9-Pro^{10}-Trp^{11}-Arg^{12}	74.81	77.34	82.19	1.51	84.06	1.36	5.28	0.70
Pro^{10}-Trp^{11}-Arg^{12}-Arg^{13}	24.69	28.35	27.25	4.57	17.66	3.21	5.21	1.85
	Type III β-turns							
	ttt-Indo	*ctt*-Indo	*tct*-Indo	*ttc*-Indo	*cct*-Indo	*ctc*-Indo	*tcc*-Indo	*ccc*-Indo
Tetrapeptide units								
Ile^1-Leu^2-Pro^3-Trp^4	3.27	0	2.87	2.45	0	0	3.17	0
Leu^2-Pro^3-Trp^4-Lys^5	54.19	10.65	34.57	35.21	0.75	10.51	18.07	0.17
Pro^3-Trp^4-Lys^5-Trp^6	22.32	16.11	3.22	20.39	1.19	10.94	2.48	1.07
Trp^6-Pro^7-Trp^8-Trp^9	50.36	47.40	4.43	31.40	2.93	32.74	4.60	4.76
Trp^9-Pro^{10}-Trp^{11}-Arg^{12}	46.93	57.60	55.43	2.20	56.82	2.11	6.04	1.32
Pro^{10}-Trp^{11}-Arg^{12}-Arg^{13}	14.75	11.75	17.95	3.35	11.10	2.54	2.54	1.87
	Intramolecular H-bonds							
	ttt-Indo	*ctt*-Indo	*tct*-Indo	*ttc*-Indo	*cct*-Indo	*ctc*-Indo	*tcc*-Indo	*ccc*-Indo
$i \leftarrow i + 3$ H-bonds								
Ile^1←Trp^4	2.75	0	2.44	2.23	0	0	2.87	0
Leu^2←Lys^5	62.98	0	57.94	33.13	0	0	28.18	0
Pro^3←Trp^6	37.90	14.15	2.34	28.40	1.74	10.37	1.99	1.14
Trp^6←Trp^9	58.05	50.85	0	37.17	0	42.01	0	0
Trp^9←Arg^{12}	72.46	71.96	76.07	0	80.59	0	0	0
Pro^{10}←Arg^{13}	19.34	23.19	20.60	4.24	14.69	2.74	3.97	1.52

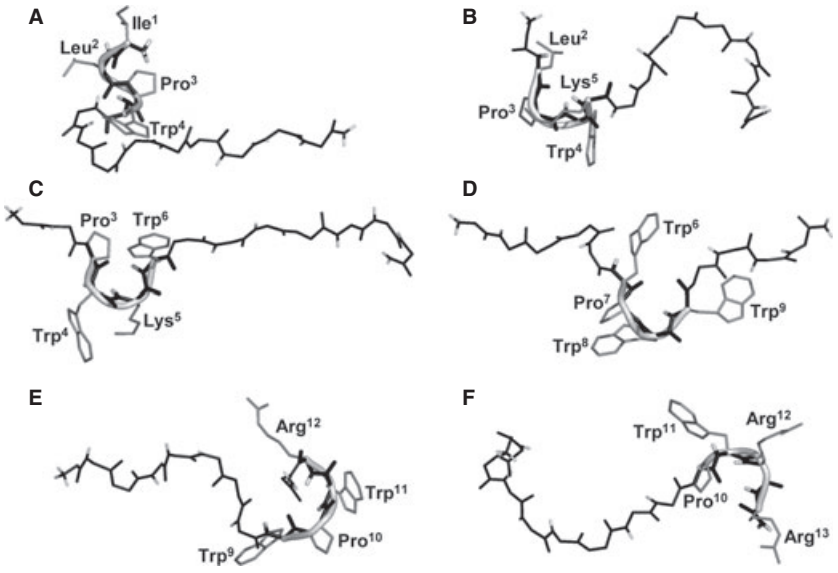

Figure 1: Representative conformations of the *ttt*-Indo characterized by one type I or III β-turn in certain tetrapeptide units as follows: (A) β-turn for the Ile^1-Leu^2-Pro^3-Trp^4 sequence, (B) β-turn for the Leu^2-Pro^3-Trp^4-Lys^5 sequence, (C) β-turn for the Pro^3-Trp^4-Lys^5-Trp^6 sequence, (D) β-turn for the Trp^6-Pro^7-Trp^8-Trp^9 sequence, (E) β-turn for the Trp^9-Pro^{10}-Trp^{11}-Arg^{12} sequence, and (F) β-turn for the Pro^{10}-Trp^{11}-Arg^{12}-Arg^{13} sequence. The peptide backbones are represented by both sticks and ribbon for the tetrapeptide segments containing β-turn structures and by lines for the remaining parts of peptides, as well as the side chains of amino acids are represented by lines.

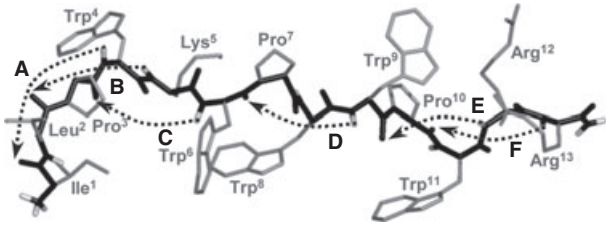

Figure 2: Different types of the *i*←*i* + *3* H-bonds for the Indo: (A) Ile[1]←Trp[4] H-bond, (B) Leu[2]←Lys[5] H-bond, (C) Pro[3]←Trp[6] H-bond, (D) Trp[6]←Trp[9] H-bond, (E) Trp[9]←Arg[12] H-bond and (F) Pro[10]←Arg[13] H-bond. The intramolecular H-bonds are indicated by dashed lines.

ment (i.e. Pro[7]-Trp[8]-Trp[9]-Pro[10] sequence). Considering the Pro[3]-Trp[4]-Lys[5]-Trp[6] unit, a decreasing tendency could be found, regarding the populations of β-turns and *i*←*i* + *3* H-bonds, which could be correlated with the following order of stereoisomers: (*ttt*- and *ttc*-Indo) → (*ctt*- and *ctc*-Indo) → (*tct*- and *tcc*-Indo) → (*cct*- and *ccc*-Indo). These data indicated that, concerning the β-turn structures and *i*←*i* + *3* H-bonds, the largest populations could be detected for the stereoisomeric forms containing *trans* Leu[2]-Pro[3] and *trans* Trp[6]-Pro[7] peptide bonds, while the smallest ones for the stereoisomers with *cis* Leu[2]-Pro[3] and *cis* Trp[6]-Pro[7] peptide bonds. Nevertheless, these results suggested that the appearance of types I and III β-turns, as well as of *i*←*i* + *3* H-bonds, seemed to be dependent not only on the *cis* or *trans* nature of Leu[2]-Pro[3] peptide bond, but also on the isomerization state of Trp[6]-Pro[7] peptide bond. Taking into account the Pro[10]-Trp[11]-Arg[12]-Arg[13] unit, it could be concluded that the stereoisomeric forms containing *trans* Trp[9]-Pro[10] peptide bond showed a larger propensity to form types I and III β-turns in comparison with the stereoisomers with *cis* Trp[9]-Pro[10] peptide bond. Accordingly, for the former stereoisomeric forms, larger amount of stabilizing *i*←*i* + *3* H-bonds was observed than for the latter ones. Considering the Pro[7]-Trp[8]-Trp[9]-Pro[10] sequence, neither β-turn structures nor *i*←*i* + *3* H-bonds were detected, which could be explained by the presence of Pro amino acid in the fourth position, namely, its N atom could not participate in the formation of any intramolecular H-bond as a donor group.

Among three tetrapeptide segments containing Pro residue in the third position, types I and III β-turns, as well as *i*←*i* + *3* H-bonds, were found only for one unit (i.e. Ile[1]-Leu[2]-Pro[3]-Trp[4] sequence). Taking into account this tetrapeptide segment, small populations of β-turn structures, as well as of *i*←*i* + *3* H-bonds, were observed in the case of *trans* isomers, while these β-turns and H-bonds were completely absent in the case of *cis* isomers. Interestingly, for the other two units (i.e. Lys[5]-Trp[6]-Pro[7]-Trp[8] and Trp[8]-Trp[9]-Pro[10]-Trp[11] sequences), any aforementioned types of turn structures and H-bonds were not found, considering all the stereoisomeric forms.

Beside the types I and III β-turns, the appearance of type VI β-turns was examined for three tetrapeptide segments mentioned above, because these turn structures could be detected in tetrapeptide units possessing a Pro amino acid in the *i* + *2th* position, as well as a *cis* peptide bond between the *i* + *1th* and *i* + *2th* residues. As the Pro–Aro interactions could usually contribute to the structural stability of type VI β-turn structures, the presence of these intramolecular interplays was investigated between the Pro residues and their neighboring Trp amino acids. Table 2 represents the populations of type VI β-turns identified in three tetrapeptide units of stereoisomers, as well as the populations of Pro^{i}-Aro^{i-1} and Pro^{i}-Aro^{i+1} interactions, which were determined based on the conformations derived from the MD trajectories. The representative conformations of *ccc*-Indo characterized by one type VI β-turn in the tetrapeptide segments (i.e. Ile[1]-Leu[2]-Pro[3]-

Table 2: Populations (in%) of the type VI β-turns identified in three tetrapeptide units of the stereoisomers of Indo, and the populations (in %) of Pro^{i}-Aro^{i-1} and Pro^{i}-Aro^{i+1} interactions

	Type VI β-turns							
	ttt-Indo	*ctt*-Indo	*tct*-Indo	*ttc*-Indo	*cct*-Indo	*ctc*-Indo	*tcc*-Indo	*ccc*-Indo
Tetrapeptide units								
Ile[1]-Leu[2]-Pro[3]-Trp[4]	0	36.30	0	0	37.35	33.04	0	34.59
Lys[5]-Trp[6]-Pro[7]-Trp[8]	0	0	58.88	0	39.04	0	62.14	51.29
Trp[8]-Trp[9]-Pro[10]-Trp[11]	0	0	0	65.13	0	57.54	40.81	44.17
	Proline-aromatic interactions							
	ttt-Indo	*ctt*-Indo	*tct*-Indo	*ttc*-Indo	*cct*-Indo	*ctc*-Indo	*tcc*-Indo	*ccc*-Indo
Pro^{i}-Aro^{i-1} interactions								
Pro[7]-Trp[6]	0.29	0.41	35.78	0.55	24.57	0.39	43.01	34.72
Pro[10]-Trp[9]	1.47	1.02	0.03	44.70	1.25	48.44	38.41	41.91
Pro^{i}-Aro^{i+1} interactions								
Pro[3]-Trp[4]	22.31	36.27	19.39	15.31	32.11	29.76	19.72	30.61
Pro[7]-Trp[8]	15.57	10.38	39.46	22.25	27.77	20.67	51.06	33.89
Pro[10]-Trp[11]	20.27	16.33	16.84	34.49	21.80	34.41	36.71	36.62

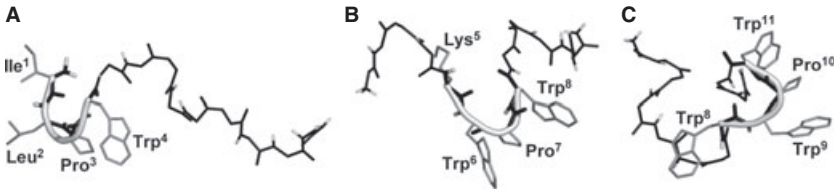

Figure 3: Representative conformations of the *ccc*-Indo characterized by one type VI β-turn in certain tetrapeptide units as follows: (A) type VI β-turn for the Ile^1-Leu^2-Pro^3-Trp^4 sequence, (B) type VI β-turn for the Lys^5-Trp^6-Pro^7-Trp^8 sequence and (C) type VI β-turn for the Trp^8-Trp^9-Pro^{10}-Trp^{11} sequence. The peptide backbones are represented by both sticks and ribbon for the tetrapeptide segments containing type VI β-turns and by lines for the remaining parts of peptides, as well as the side chains of amino acids are represented by lines.

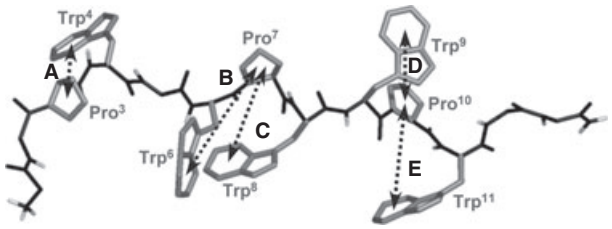

Figure 4: Different types of the proline–aromatic interactions for the Indo: (A) Pro^3-Trp^4 interaction, (B) Pro^7-Trp^6 interaction, (C) Pro^7-Trp^8 interaction, (D) Pro^{10}-Trp^9 interaction, and (E) Pro^{10}-Trp^{11} interaction. The proline–aromatic interactions are indicated by dashed lines.

Trp^4, Lys^5-Trp^6-Pro^7-Trp^8, and Trp^8-Trp^9-Pro^{10}-Trp^{11} sequences), respectively, are displayed in Figure 3, whereas the Figure 4 shows the various types of Pro–Aro interactions for the Indo. These results pointed out that type VI β-turns appeared with high frequency in all three above-mentioned tetrapeptide segments for the *cis* isomers. Taking into account the Pro^i-Aro^{i-1} interactions, large populations of these interplays were observed in the case of *cis* isomers, indicating their relevant role played in the stabilization of type VI β-turn structures. In contrast, the Pro–Aro interactions formed between the Pro amino acids and the preceding Trp residues appeared with very low frequency for the *trans* isomers. Considering the Pro^i-Aro^{i+1} interactions, it could be concluded that these interplays were observed in the case of both *cis* and *trans* isomers. Nevertheless, the populations of Pro–Aro interactions evolved between the Pro amino acids and the following Trp residues were found to be larger for the *cis* isomers than for the *trans* isomers, suggesting that these interplays could also contribute to the structural stability of type VI β-turns in the case of *cis* isomers.

On the basis of all above-mentioned results, it could be concluded that the eight different stereoisomeric forms of Indo adopted typical conformational features, concerning their secondary structural elements and intramolecular interactions. Among the stereoisomers, for example, the *ttt*-Indo was characterized by types I and III β-turns, as well as by $i \leftarrow i+3$ H-bonds (see Figure 5), while for the *ccc*-Indo, type VI β-turns and Pro–Aro interactions were considered as the typical structural properties (see Figure 5). Nevertheless, taking into account all the stereoisomeric forms, data represented in Tables 1 and 2 indicated that different tendencies could be observed with

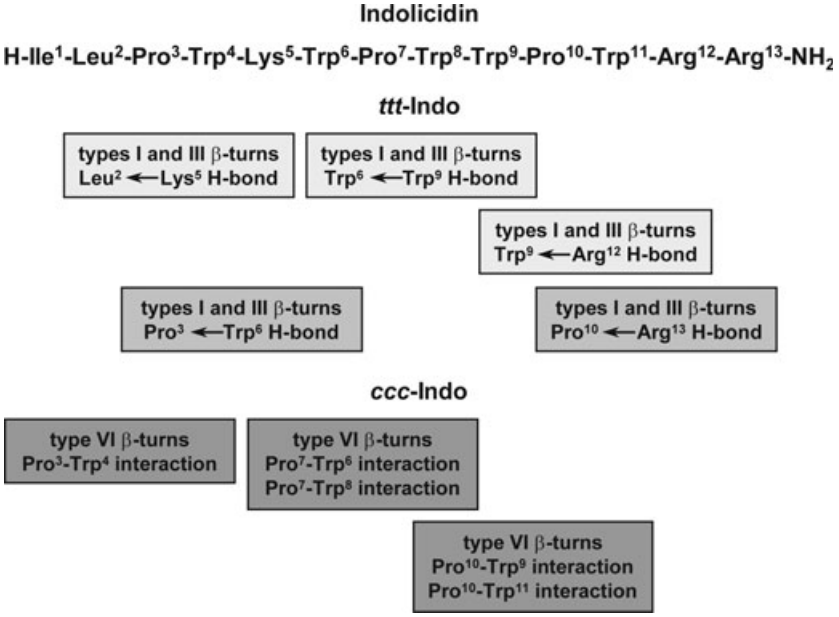

Figure 5: Characteristic turn structures and intramolecular interactions for the *ttt*-Indo and *ccc*-Indo.

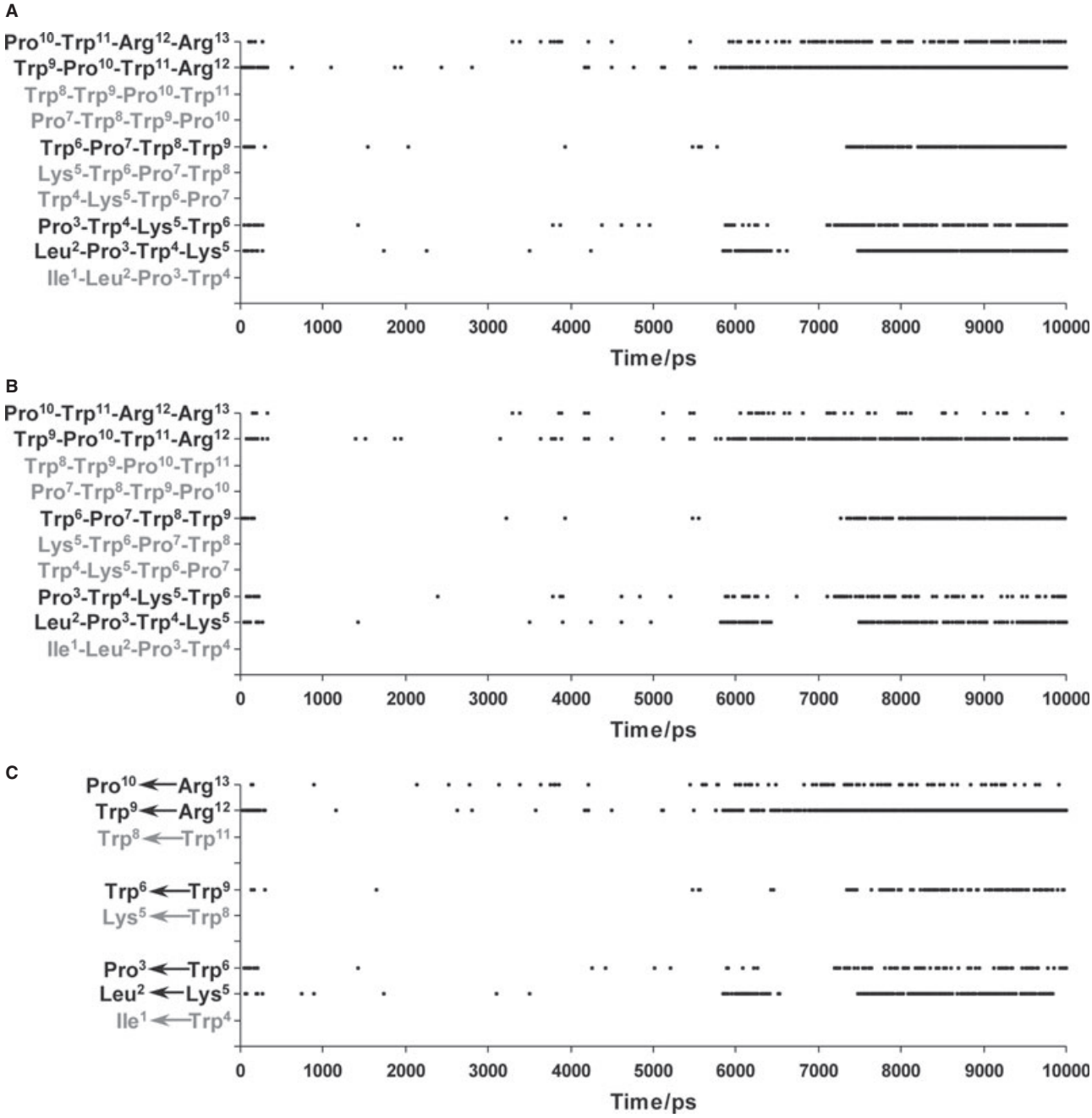

Figure 6: Appearance of (A) type I and (B) type III β-turns, as well as of (C) $i \leftarrow i + 3$ H-bonds, for the *ttt*-Indo as a function of time.

regard to the turn structures and intramolecular interplays. On the one hand, the cumulative populations of types I and III β-turns, as well as of $i \leftarrow i + 3$ H-bonds, showed a decreasing tendency as a function of the increasing number of *cis* Xaa-Pro peptide bonds. On the other hand, the overall amount of type VI β-turns and Pro–Aro interactions became more dominant as the proportion of *cis* Xaa-Pro peptide bonds increased. Considering the conformational features of each stereoisomer, the results obtained by the SA-MD calculations proved to be similar to the aforementioned observations derived from the MD simulations. Among the stereoisomeric forms, the appearance of types I and III β-turns, as well as of $i \leftarrow i + 3$ H-bonds, for the *ttt*-Indo is illustrated in Figure 6 as a function of time, based on the SA-MD calculation. As it can be seen, types I and III β-turns could be observed for five tetrapeptide units (i.e. Leu^2-Pro^3-Trp^4-Lys^5, Pro^3-Trp^4-Lys^5-Trp^6, Trp^6-Pro^7-Trp^8-Trp^9, Trp^9-Pro^{10}-Trp^{11}-Arg^{12}, and Pro^{10}-Trp^{11}-Arg^{12}-Arg^{13} sequences), and according to the occurrence of these turn structures, their stabilizing $i \leftarrow i + 3$ H-bonds could be also detected. The results presented in Figure 6, as well as data showed in Table 1 indicated that for the *ttt*-Indo and

certain other stereoisomers, several conformational states could be found, in which two or more types I and III β-turns appeared at the same time either consecutively or in separated tetrapeptide units. Similarly to these cases, for the stereoisomeric forms containing two or three *cis* Xaa-Pro peptide bonds, conformational states could be observed, in which two or three type VI β-turns appeared simultaneously. Among these multiple turn conformations, Figure 7 shows three different representative structures characterized by two type VI β-turns, as well as one representative structure possessing three type VI β-turns, for the *ccc*-Indo. In the case of these conformational states, both Pro^{i}-Aro^{i-1} and Pro^{i}-Aro^{i+1} interactions are represented, which play a relevant role in the structural stabilization of type VI β-turns. For all four representative conformations, Pro–Aro interplays could be observed between the Pro residue and the aromatic side chains of preceding and following Trp amino acids simultaneously, resulting in a compact sandwich structure, which contribute to the stability of type VI β-turns.

As it was previously mentioned, the *cis-trans* isomerism of three Xaa-Pro peptide bonds of Indo was not considered in earlier experimental and theoretical studies. Furthermore, the previous structural investigations led to considerably diverse conclusions regarding the backbone conformation of Indo. Nevertheless, these studies provided only a few information about the turn structures and intramolecular interactions for this AMP, as follows. The NMR measurements revealed that for the TFE, two turns were found in the Trp^{4}-Lys^{5}-Trp^{6}-Pro^{7} and Trp^{9}-Pro^{10}-Trp^{11}-Arg^{12} tetrapeptide units, as well as the Trp^{9}←Arg^{12} H-bond was detected, while for the DPC and SDS micelles, two half-turns were observed in the Trp^{4}-Lys^{5}-Trp^{6} and Pro^{7}-Trp^{8}-Trp^{9} tripeptide segments (6). Moreover, various contacts between the side chains of Pro and Trp residues were found, such as the Trp^{6}-Pro^{7}-Trp^{8} and Trp^{9}-Pro^{10}-Trp^{11} contacts in the case of TFE, as well as the Trp^{9}-Pro^{10}-Trp^{11} contact for the DPC micelles, and the Trp^{8}-Pro^{10} contact for the SDS micelles. Based on the results derived from other NMR study, it was concluded that the aforementioned two half-turns could be detected in the Trp^{4}-Lys^{5}-Trp^{6} and Pro^{7}-Trp^{8}-Trp^{9} tripeptide units for the DPC micelles, whereas the first half-turn was not well-defined, and the second half-turn was absent for the SDS micelles (9). Taking into account the contacts between the side chains of Pro and Trp amino acids found in the case of DPC and SDS micelles, this structural investigation led to similar observations as described in the other NMR study. Beside the turn structures mentioned above, it was also suggested that type VI β-turns could be formed in the Ile^{1}-Leu^{2}-Pro^{3}-Trp^{4}, Lys^{5}-Trp^{6}-Pro^{7}-Trp^{8}, and Trp^{8}-Trp^{9}-Pro^{10}-Trp^{11} tetrapeptide segments, respectively, which could be stabilized by Pro–Aro interactions (11). Comparing the results obtained by the earlier experimental studies with those derived from the present structural investigation, it can be seen that my theoretical study supplied a more detailed structural characterization of the stereoisomeric forms of Indo, concerning the turn structures and intramolecular interactions. Additionally, although it was previously proposed that type VI β-turns could be formed in the case of three tetrapeptide units for the Indo, my computational study provided conclusive evidences with regard to the existence of type VI β-turns in these tet-

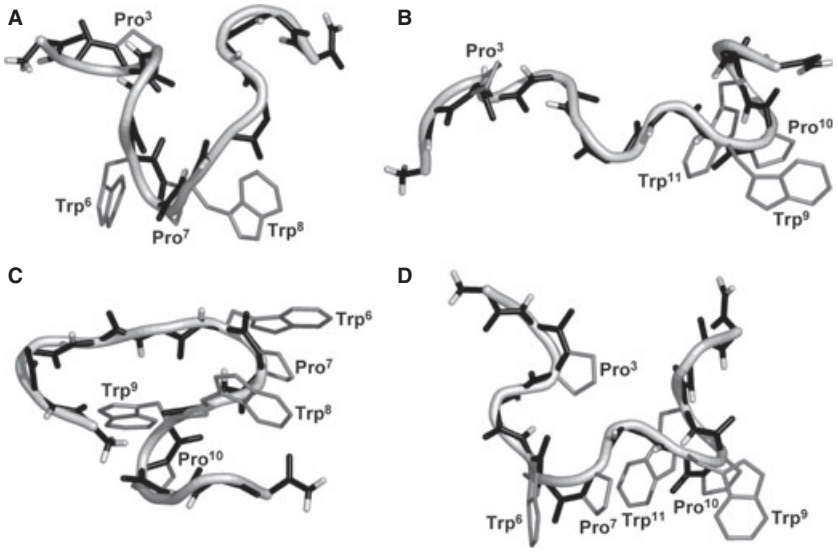

Figure 7: Representative structures for the *ccc*-Indo, in which two or three type VI β-turns appeared simultaneously. (A) A conformation characterized by two type VI β-turns for the Ile^{1}-Leu^{2}-Pro^{3}-Trp^{4} and Lys^{5}-Trp^{6}-Pro^{7}-Trp^{8} tetrapeptide units; (B) a conformation containing two type VI β-turns in the Ile^{1}-Leu^{2}-Pro^{3}-Trp^{4} and Trp^{8}-Trp^{9}-Pro^{10}-Trp^{11} tetrapeptide segments; (C) a conformation possessing two type VI β-turns in the Lys^{5}-Trp^{6}-Pro^{7}-Trp^{8} and Trp^{8}-Trp^{9}-Pro^{10}-Trp^{11} tetrapeptide units; (D) a conformation characterized by three type VI β-turns for the Ile^{1}-Leu^{2}-Pro^{3}-Trp^{4}, Lys^{5}-Trp^{6}-Pro^{7}-Trp^{8}, and Trp^{8}-Trp^{9}-Pro^{10}-Trp^{11} tetrapeptide segments. The peptide backbones are represented by both sticks and ribbon, while the side chains of Pro and Trp residues are represented by lines.

rapeptide units (i.e. Ile^{1}-Leu^{2}-Pro^{3}-Trp^{4}, Lys^{5}-Trp^{6}-Pro^{7}-Trp^{8}, and Trp^{8}-Trp^{9}-Pro^{10}-Trp^{11} sequences) of this AMP.

Conclusions

In this theoretical study, the characteristic structural features of the stereoisomeric forms of Indo were investigated, as well as the effects of *cis-trans* isomerism on the conformational properties of this peptide were examined. Based on the results obtained by the MD simulations, it could be concluded that the different stereoisomers of Indo could be characterized by typical structural features, with regard to their secondary structural elements and intramolecular interactions. The appearance of these conformational properties proved to be dependent on the *cis* or *trans* nature of three Xaa-Pro peptide bonds, pointing out that the Pro residues played an important role in the determination of the three-dimensional structure of Indo. As it was previously mentioned, distinct β-turn structures and intramolecular interactions could be considered as typical structural properties for the *cis* and *trans* isomers, such as, in the case of *trans* Xaa-Pro peptide bonds, types I and III β-turns, as well as $i \leftarrow i + 3$ H-bonds, were found; in the case of *cis* Xaa-Pro peptide bonds, type VI β-turns and Pro–Aro interactions were observed. Nevertheless, the occurrences of different intramolecular interactions were in agreement with the presence of various secondary structural elements. On the one hand, the $i \leftarrow i + 3$ H-bonds played a role in the stabilization of types I and III β-turns, and on the other hand, the Pro–Aro interactions contributed to the stability of type VI β-turns. On the whole, this computational study provided a detailed structural characterization of all the stereoisomeric forms of Indo, as well as led to relevant observations concerning the influences of the *cis-trans* isomerism of Xaa-Pro peptide bonds on the conformation of this AMP.

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