Molecular dynamics simulations of the conformational behaviour of the synthetic dipeptide PIDOTIMOD, a new immunostimulating agent

Vincenzo Villani*, Rachele Pucciariello

Dipartimento di Chimica, Universita' della Basilicata, Via N.Sauro 85, 85100 Potenza, Italy

Abstract

Molecular dynamics (MD) simulations of the conformational behaviour of the conformers of PIDOTIMOD, an interleukin-2 lymphocyte T receptor agonist dipeptide, have been carried out. MD simulations at constant temperatures of 300 and 400 K and constant total energy runs at high temperatures have been performed, and trajectories and statistical properties of motions investigated.

At 300 K two trans and one cis conformer were found to be dynamically stable. Two puckered forms of the oxo-prolyl ring in rapid equilibrium have been detected. The equilibrium conformations appear rather rigid and poor in H-bonds.

At 400 K both trans and cis conformers are thermally mixed. At high temperatures large fluctuations of the peptide bond between the oxo-prolyl and thyazolidin rings are observed. Nevertheless in the short time scale trans–cis interconversion does not occur.

Suggestions on the nature of the structure–activity relationship are made.

Introduction

PIDOTIMOD [3-(5-oxo-L-prolyl)-L-thyazolidin-4-carboxylic acid], a synthetic dipeptide [1], has been demonstrated to induce the functional activation of interleukin-2 (IL-2) lymphocyte T receptor, and could play an important role as an immunostimulating agent, improving the immuno defences in immunodepression [2].

As in our previous work [3], we have taken into account the conjugate base of PIDOTIMOD. In fact, with $pK_a = 3.03$, under physiological conditions (constant pH ≈ 7) PIDOTIMOD is present nearly completely in its ionized form.

In our previous paper [3] we studied the conformational features and harmonic-dynamic behav-iour of PIDOTIMOD by NMR, molecular mechanics, normal mode analysis and dynamics.

By NMR PIDOTIMOD, in aqueous solution at pH = 7 and 21°C, appears as an equilibrium mixture of trans and cis conformers, with respect to the peptide bond between the rings, with 0.55 and 0.45 conformational populations, respectively. At 75°C the coalescence of homologous peaks indicates a fast trans–cis isomerization. From theoretical calculations for the isolated molecule of the conjugate base of PIDOTIMOD, five conformers were determined, and the conformational populations at relative permittivity $\epsilon = 7$ perfectly agree with the experimentally determined ones. As far as the harmonic-dynamic behaviour is concerned the extent of the conformational fluctuations has been described for all conformers and evidence obtained that their behaviour is characterized by

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*Corresponding author.

the presence of a few normal modes which predominate over the other modes. The molecular mobility of the conformers is strongly determined by their H-bonds; the same H-bond patterns induce the same mobility. Lastly, significant correlations hold for the motion of the torsion angles.

In this paper a molecular dynamics (MD) study of the conformational behaviour of the conformers of PIDOTIMOD, previously determined by us [3], has been carried out. MD constant temperature simulations of 100 ps at 300 and 400 K have been performed. All five conformers have been considered for MD runs at 300 K; only the most stable conformer has been taken into account at 400 K with different conditions of thermalization (slow or fast heating). An MD adiabatic simulation, i.e. a microcanonical simulation at constant total energy, has been performed at high temperatures starting from 1000 K. Although such high temperatures are not physically meaningful, they are of interest for exploring conformational space in accordance with the philosophy from which simulated annealing was derived [4]. The use of high temperatures provides the kinetic energy necessary to activate (i.e. to surmount energetic barriers) the torsional degrees of freedom. Then, the conformational space is efficiently explored by means of transitions between different potential energy minima [5].

The behaviour of structural parameters as a function of time, the time-averaged properties of the motion and the structural fluctuations have been investigated, to describe the conformational, dynamic behaviour of the molecule. The results of MD simulations have been compared with analogous results obtained previously [3] from normal mode dynamics (harmonic approximation) and NMR experimental spectra.

Theoretical computations

The model force field

The AMBER program [6–9] was used in all calculations. The force field equation is of the form:

$$E_p = \sum_{bonds} k_r(r - r_{eq})^2 + \sum_{angles} k_\tau(\tau - \tau_{eq})^2$$

$$+ \sum_{dihedrals} V_n/2[1 + \cos(n\phi - \delta)]$$

$$+ \sum_{i<j} w(A_{ij}/R_{ij}^{12} - B_{ij}/R_{ij}^6 + 332.2 q_i q_j/\epsilon R_{ij})$$

$$+ \sum_{H\text{-bonds}} (C_{ij}/R_{ij}^{12} - D_{ij}/R_{ij}^{10})$$

The calculations were carried out on a VAX 8530 computer under the VMS 5.4 operating system.

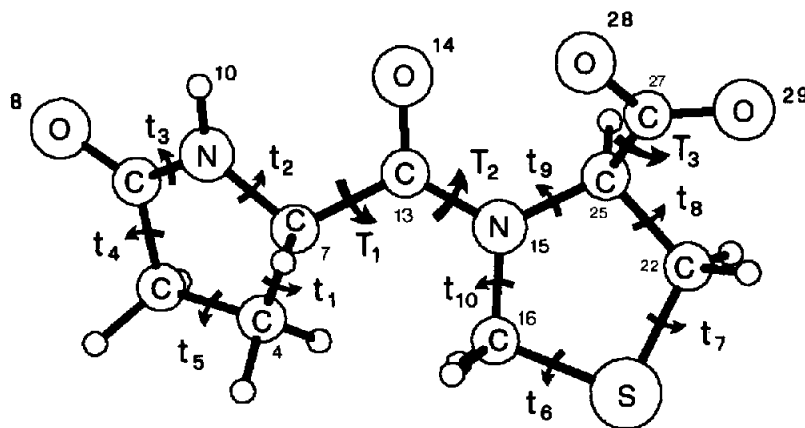In Fig. 1 the molecular structure with atom

Fig. 1. Molecular structure of the conjugate base of PIDOTIMOD with definitions of the torsion angles and numbering of the atoms which define the torsions T_i and of amidic hydrogen and oxygen atoms. Non-hydrogen atoms are also labelled.

numeration and the definition of the torsions is shown. The five conformers of PIDOTIMOD labelled as A_{trans}, B_{trans}, C_{trans}, D_{cis} and E_{cis} [3] have been taken into account. All the computations were performed in vacuo using the same all-atom force field [7] and net atomic charges previously used in molecular mechanics calculations [3].

Previously [3], in order to simulate roughly the molecule in the medium used and to tune the conformational populations from molecular mechanics calculations to the experimentally determined values in aqueous solution by NMR, the relative permittivity was systematically varied and the best agreement was obtained at $\epsilon = 7$. This value is then used in the present paper. This, of course, does not exhaust the detailed effects induced by the solvent molecules due to their local interactions with the solute molecules.

MD simulations

Newton's equations of motion were accurately integrated in Cartesian coordinates via the Verlet leap-frog algorithm [10] with an integration step of 10^{-3} ps and holonomic constraints of all bond distances using the SHAKE algorithm [11] with an error tolerance of 10^{-5} Å. An integration step of 10^{-4} ps was used in MD adiabatic simulations in order to ensure that the r.m.s. fluctuation of the total energy was less than 10^{-5} kcal mol^{-1} and the drift was removed (i.e. conservation of the total energy was obtained).

The five conformers of ref. 3, determined by molecular mechanics energy minimizations (EM) at $\epsilon = 7$, were used as starting points in MD simulations.

The simulations were run after assigning initial random values to the Cartesian components of the velocities of each atom chosen from a Maxwellian distribution at a given temperature.

In constant temperature MD simulations the velocities were scaled at each step by coupling the system to an external heat bath at fixed reference temperature using the time constant τ [12].

Particular care was taken over the thermalization and equilibration procedures [13].

As far as thermalization at 300 K is concerned, short dynamics runs (1 ps) were carried out at low temperatures below 300 K. Starting from 100 K, the temperature was increased in steps of 25 K and the final state of the previous run was used as the initial state for the following run. In these short MD runs $\tau = 0.02$ ps. After each simulation it was verified (from analysis of the torsion angles) that the conformation remains the same as the minimum energy conformation with a tolerance of 15° on each torsion. Once the temperature of 300 K was obtained, a simulation of 100 ps was carried out, resetting τ to 0.1 ps.

The most stable conformer was submitted also to MD simulations of 100 ps at 400 K. Two different thermalizations were performed: (a) slow heating, with short dynamics runs (1 ps) at 325, 350 and 375 K; (b) fast heating, with the final set of atom positions and velocities from simulation at 300 K being used as the initial point at 400 K, and with $\tau = 0.4$ ps. For lower values of τ the structural variations were such that the structural requirements could not be satisfied with SHAKE. As far as the thermalization at 1000 K is concerned a short dynamics run (2 ps) with $\tau = 0.1$ ps was performed starting from the final state at 300 K. Then, 10 ps of MD adiabatic simulations were carried out.

During the simulation period, temperature, energies and coordinate sets were stored at every 10 steps. In Fig. 2 the histories of temperature and total, potential and non-bonded energies during the MD run at 300 K for the conformer A_{trans} are presented. At thermodynamic equilibrium the average value and r.m.s. fluctuation of temperature and energies are constant as a function of time: a gaussian distribution for the states of the ensemble [14]. Then, from the trend of the temperature and energy data sets, we could carefully define when the system was considered to be well equilibrated. Therefore at 300 K, we discarded the first 40 ps (transient stage) and only the data collected for the remaining 60 ps (equilibrium stage) were used for the statistical analysis.
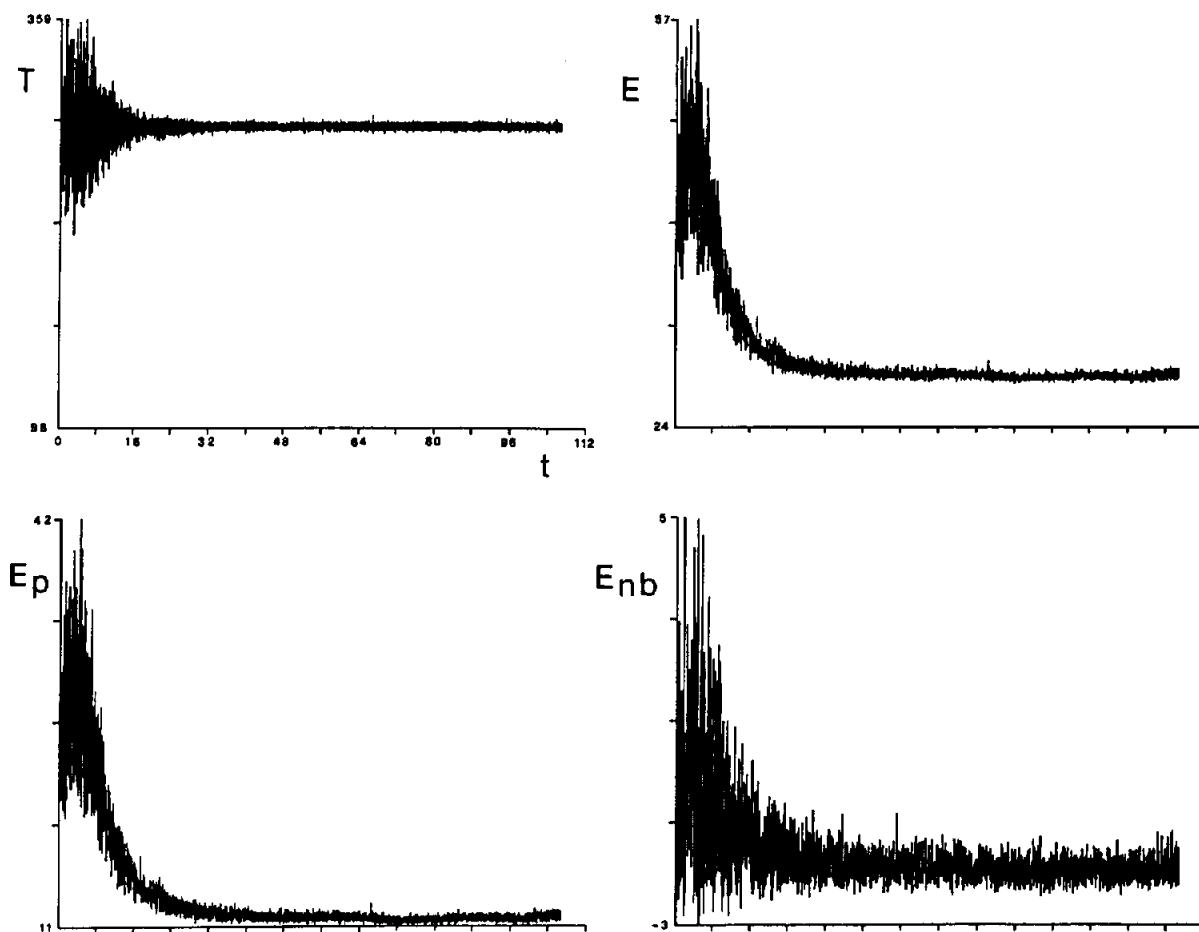
Fig. 2. The evolution in time of temperature, and total, potential and non-bonded energies, for conformation A_{trans} during MD at 300 K. Temperature is expressed in K, energies in $kcal\,mol^{-1}$ and time in ps.

Results and discussion

MD simulations at 300 K

In order to understand the dynamic behaviour of the molecule, we monitored the torsion angles outside the rings, T_1 (C_4–C_7–C_{13}–N_{15}), T_2, (C_7–C_{13}–N_{15}–C_{16}), T_3^1 (C_{22}–C_{25}–C_{27}–O_{28}) and T_3^2 (C_{22}–C_{25}–C_{27}–O_{29}), those inside the rings t_i ($i = 1, \ldots, 10$) and the interaction distances $d_i = {>}C{=}O_0 \cdots H_{10}N{<}$ ($i = 14, 28, 29$) during the MD simulations.

The conformers A_{trans}, B_{trans} and D_{cis} were found to be dynamically stable: during the equilibrium

stage the steady state fluctuates around the starting minimized structure. No conformational transitions are observed.

In Fig. 3 the trajectories of the torsion T_2 for the conformers A_{trans}, B_{trans} and D_{cis} are reported. We observed an average value of $|\langle T_2 \rangle| \approx 180°$ for conformers A_{trans} and B_{trans} and of $|\langle T_2 \rangle| \approx 0°$ for D_{cis}. Moreover the mobility (as the amplitude or r.m.s. of the fluctuation) of the cis conformation was higher than that of the trans both during the transient and the equilibrium stages.

In Fig. 4 the trajectories of the torsions T_1, T_3^1, T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} for the trans conformer A_{trans} are presented. The
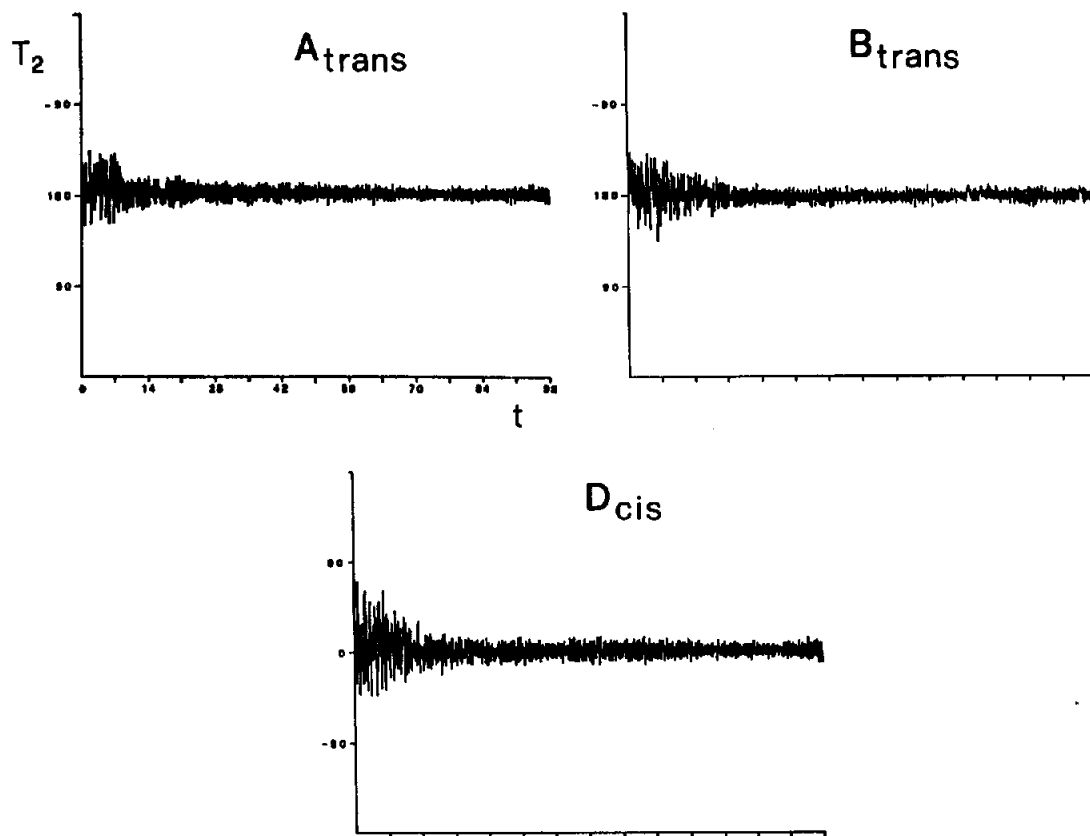
Fig. 3. Trajectories of the torsion T_2 for conformations A_{trans}, B_{trans} and D_{cis} during MD at 300 K. Angles are in deg.

behaviour of the torsion T_1 and of the distance d_{14} as a function of time are closely correlated which is evidence of the substantial rigidity of the oxo-prolyl ring and of the bridged peptide unit. The torsions T_3^1 and T_3^2 are perfectly correlated with "reverse phase" in the sense that when the former is reduced the latter is increased, confirming the rigid-body-like motion of the carboxyl group. The distances d_{28} and d_{29} fluctuate at higher values than H-bond interactions and with different amplitudes which is evidence of a non-equivalent orientation of the carboxylic oxygens with respect to the direction of the motion of the peptide hydrogen, substantially determined by the torsion T_1.

During the transient phase the structural parameters oscillate between a number of conformational states in an apparently erratic way

except the torsions T_3^1 and T_3^2 for which a clear transition to an equivalent conformational state is observed. Then, during the equilibration time interval, the molecule visits some metastable states but does not escape the minimum energy conformation structure.

In Fig. 5 the trajectories of the torsions T_1, t_1, t_2, t_4 and t_5 and of the interaction distance d_{14} for the trans conformer B_{trans} are presented. The behaviour of T_3^1, T_3^2 and d_{28}, d_{29} as a function of time is analogous to that of the conformer A_{trans} and will not be further discussed. The motion of the torsion T_1 and of the distance d_{14} were strictly correlated and showed small short-lived deviations from the average conformation: at about 45, 55 and 57 ps a state with values $\{T_1\} > \langle T_1 \rangle$ and $\{d_{14}\} > \langle d_{14} \rangle$ was observed. Moreover between 71 and 79 ps and at 95 ps a state with $\{T_1\} > \langle T_1 \rangle$ and
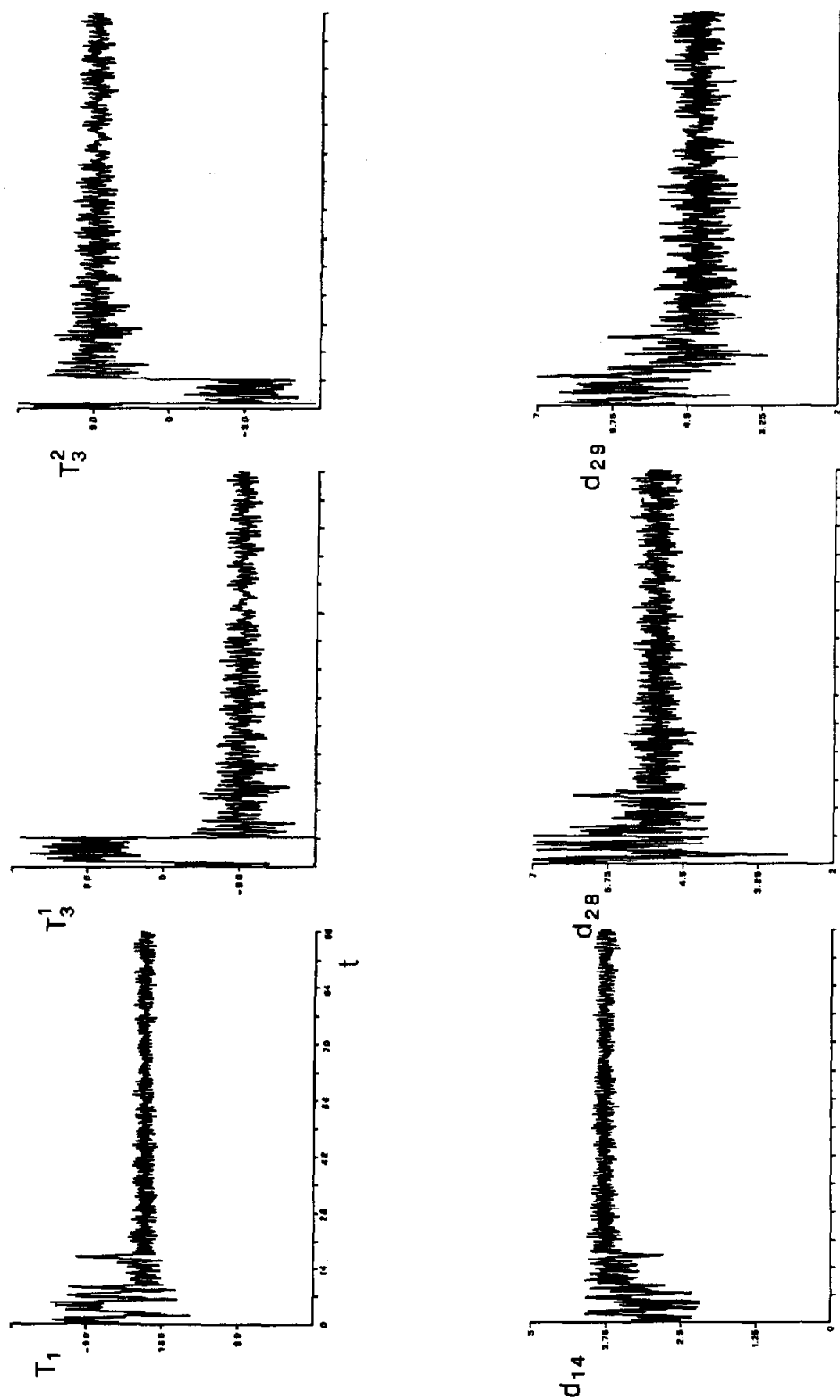
Fig. 4. Trajectories of the torsions T_1, T_3^1, T_3^2 and interaction distances d_{14}, d_{28} and d_{29} for the conformation A_{trans} during MD at 300 K. Angles are in deg and distances in Å.

Fig. 5. Trajectories of torsions T_1, t_1, t_2, t_4 and t_5 and interaction distance d_{14} for the conformation B_{trans} during MD at 300 K. Angles are in deg and distances in Å.

Fig. 6. Trajectories of the torsions T_1, T_3^1, T_3^2 and interaction distances d_{14}, d_{28} and d_{29} for the conformation D_{cis} during MD at 300 K. Angles are in deg and distances in Å.
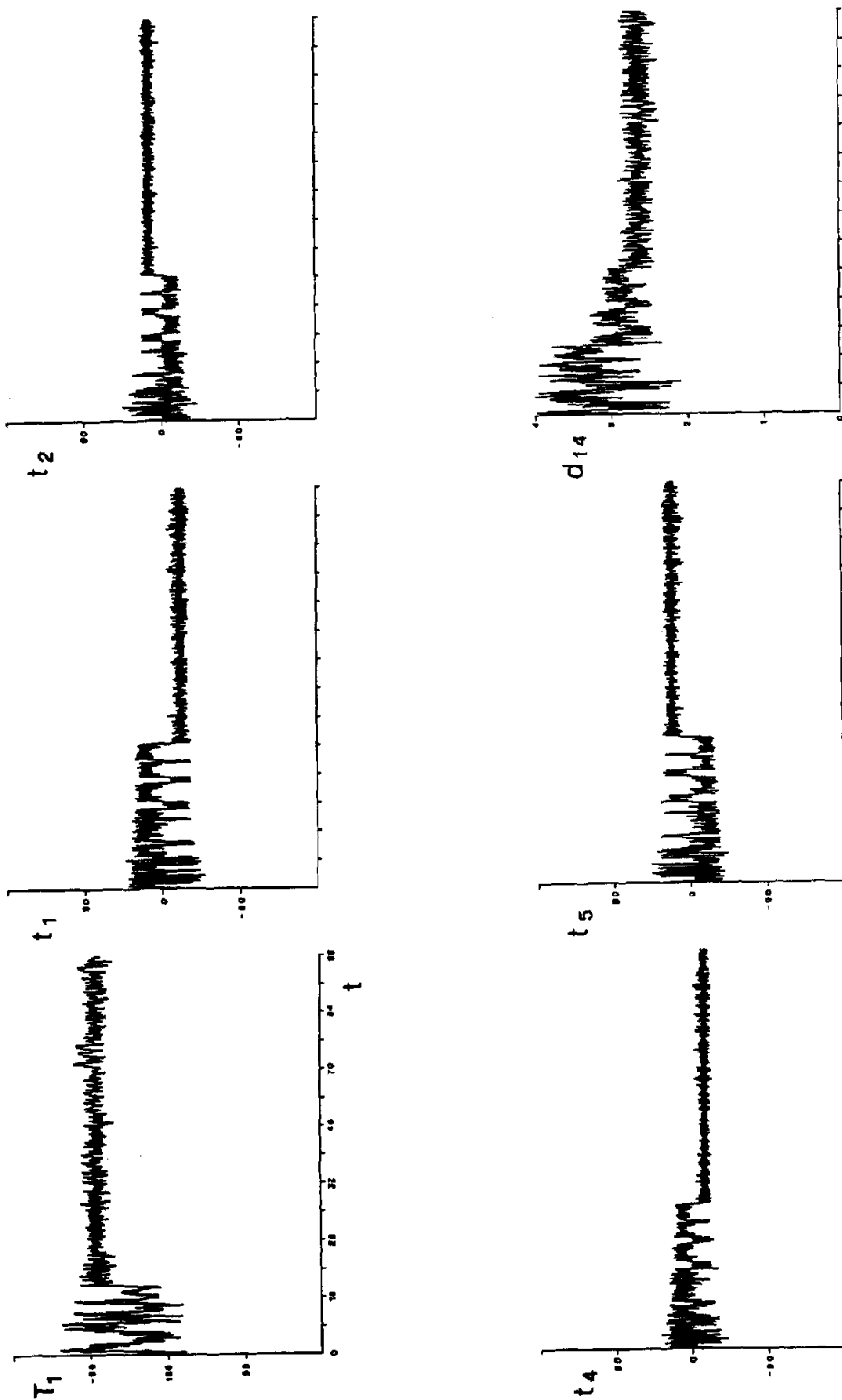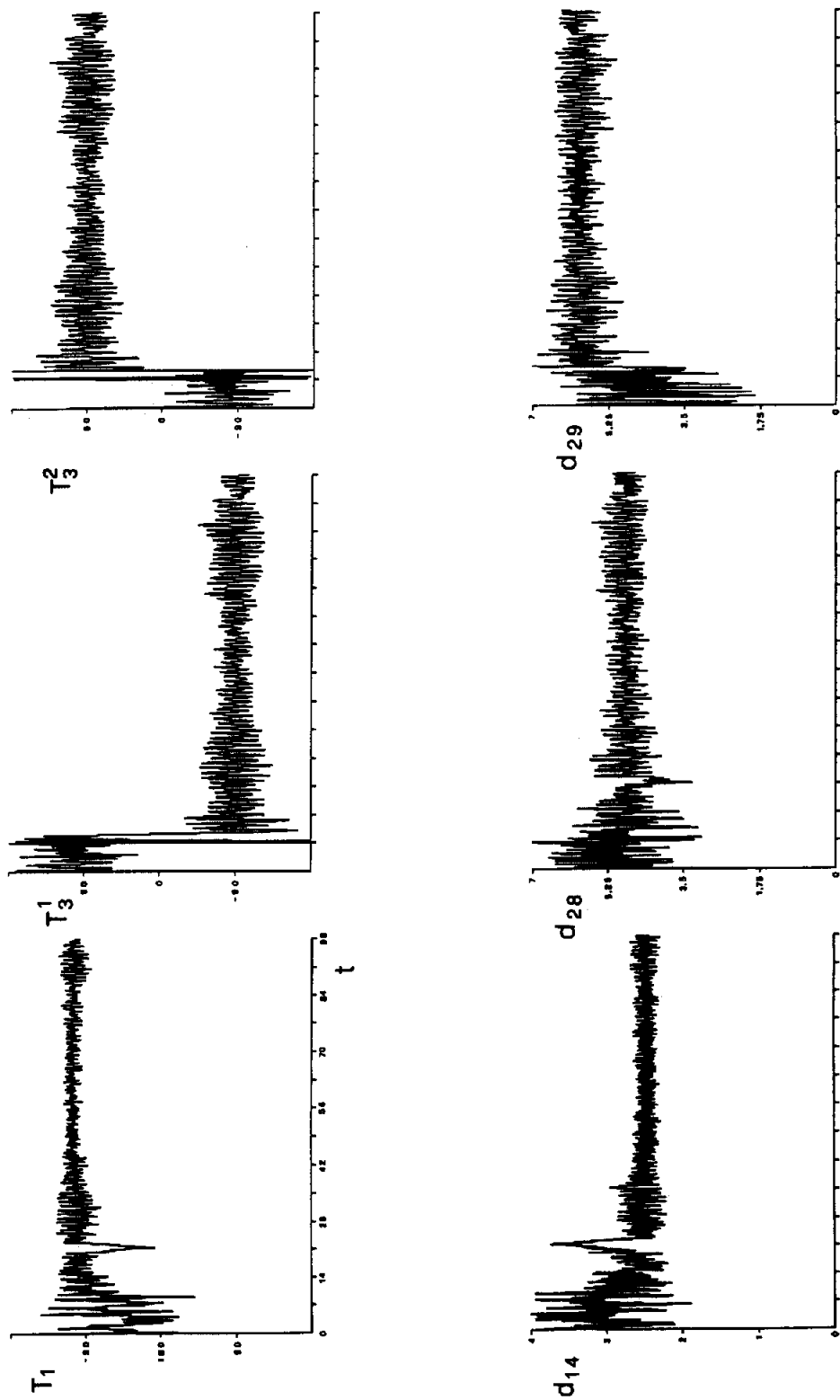
$\{d_{14}\} < \langle d_{14}\rangle$ was observed. Analogous oscillations were observed for the torsions $t_{i=1,5}$, producing evidence for a complex dynamic behaviour (i.e. involving many coupled degrees of freedom). In the equilibration phase, between 21 and 36 ps, the trajectory of the distance d_{14} was strongly coupled with the torsions t_i that show two fixed values around which the ring oscillates. We note that the adjacent torsions t_i (i.e. t_1 and t_2, t_1 and t_5, t_4 and t_5) are strongly anticorrelated, in fact when a torsion assumes a positive value, the second torsion assumes a negative one (see also the analogous behaviour described in ref. 3).

In Fig. 6 the trajectories of T_1, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} for the conformer D_{cis} are presented. The behaviour of these trajectories is characterized by a change in amplitude of fluctuation during the equilibrium stage showing the presence of coupled motions. In particular, the torsion T_1 and the distance d_{14} in the time interval from 40 to 90 ps show a lesser mobility than between 90 and 100 ps. This behaviour may be correlated with the formation of a strong H-bond $>C=O_{14}\cdots H_{10}N<$ during the first time interval, as confirmed by the high frequency of oscillation of the distance d_{14}, appreciable even qualitatively. The higher mobility during the second time interval seems to be correlated with the large fluctuation which occurs in the transient phase from 30 to 40 ps. The trajectories of the torsions T_3^1 and T_3^2 and distances d_{28} and d_{29} are perfectly correlated.

The conformer C_{trans} was found to be dynamically unstable: during the equilibrium stage the conformational states fluctuated around average conformations, not corresponding to the starting minimized conformation. Conformational transitions were observed.

In Fig. 7(A) the trajectories of the torsions T_1, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29}, and in Fig. 7(B) the trajectories of the torsions t_1, t_2, t_4 and t_5, for the conformer C_{trans} are presented. During the equilibrium stage the trajectories in a first time interval, between 40 and 45 ps, fluctuate around the average conforma-

tion A_{trans}, then through a sharp conformational transition to the steady state of the conformer B_{trans}. The ring torsions, t_1, t_2, t_4 and t_5 (correlated as in Fig. 4) and the interaction distances d_{14} and d_{28} show a short-lived deviation from the average conformation B_{trans} in the time intervals between 51 and 53 ps and 81 and 86 ps which characterize a state B' isoenergetic with B, between which the oxo-prolyl ring oscillates.

The conformer E_{cis} is dynamically unstable: during the equilibration phase the molecule escapes the minimum energy conformation structure and in the equilibrium stage fluctuates around the average conformation D_{cis}.

From MD runs for the conformations C_{trans} and E_{cis} there are reasons to believe that the paths connecting C_{trans} to A_{trans} and B_{trans}, B_{trans} to B'_{trans}, and E_{cis} to D_{cis} have barriers near the kT value at 300 K (about $0.6\,kcal\,mol^{-1}$), and then a transparent barrier during conformational motions (thermally mixed).

The average values of the internal energy (sum of the kinetic and potential energies), the potential energy and various potential energy terms (bending, dihedral, non-bonded, electrostatic and H-bonding) are presented in Table 1, for 60 ps of the MD equilibrium stage for conformers A_{trans}, B_{trans} and D_{cis} which were found to be dynamically stable. All energy values are expressed as $\Delta E = \langle E\rangle - \langle E_0\rangle$ where $\langle E_0\rangle$ is the average energy corresponding to the most stable equilibrium conformation (i.e. the ensemble of the conformational states during the MD equilibrium stage).

The values of the kinetic energy are, as expected, conformation-independent (the kinetic energy in Cartesian coordinates is a function only of the momentum) so at constant temperature $\Delta E_{int} = \Delta E_p$.

The relative stability of the equilibrium conformations is evidence that the forms A_{trans} and B_{trans}, though slightly more stable, are practically isoenergetic with the form D_{cis}, confirming the results previously obtained for the corresponding conformers [3]. The primary source for the small
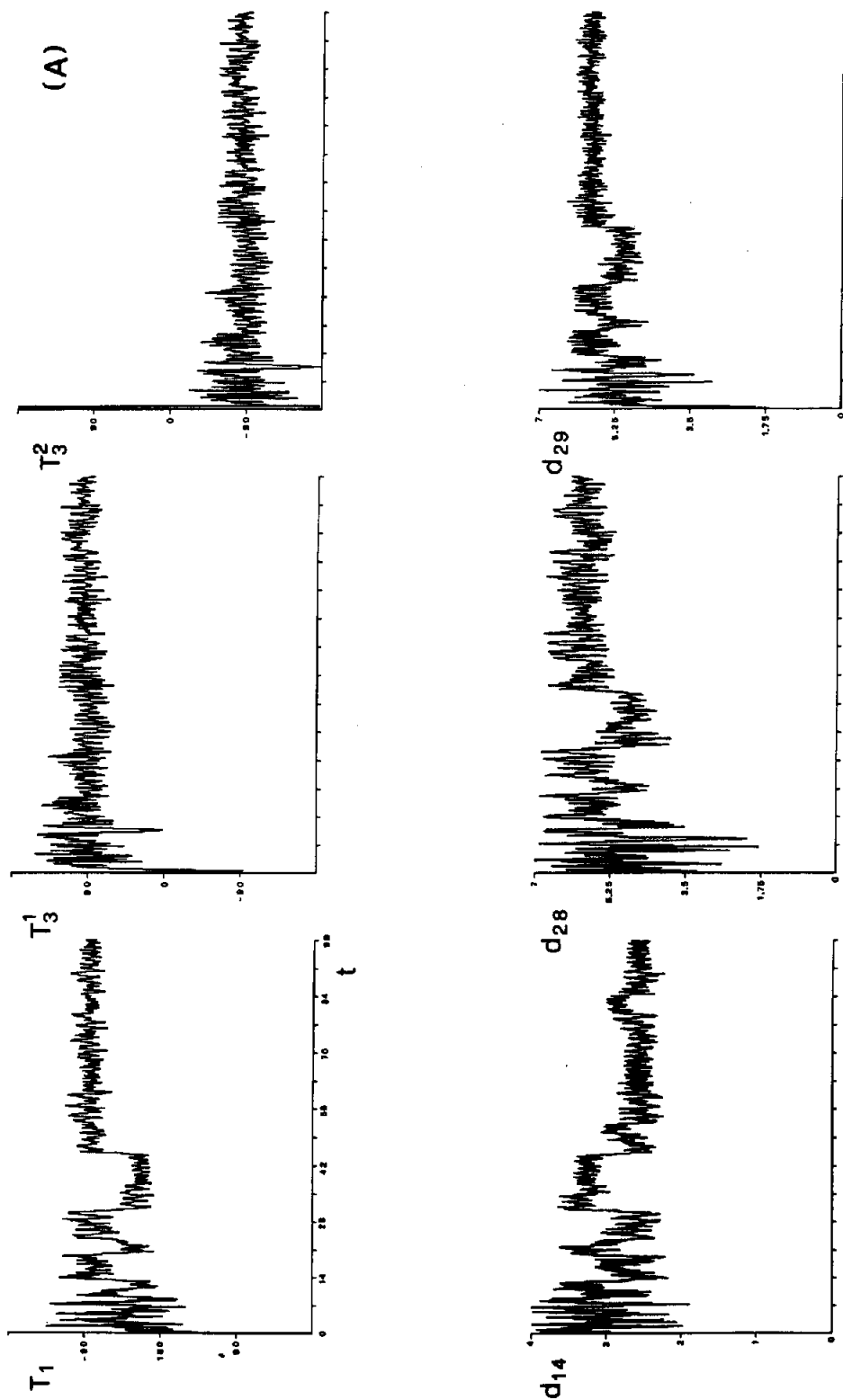
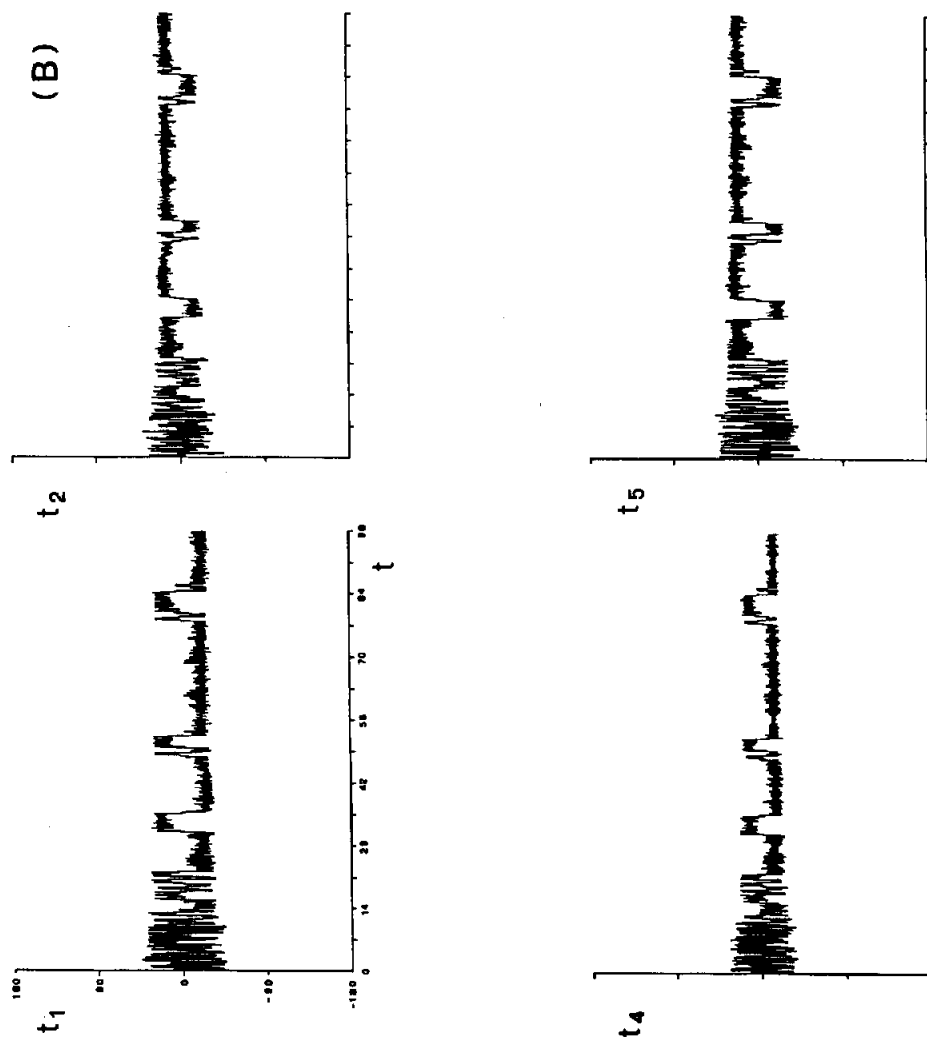Fig. 7. Trajectories of the torsions T_1, T_3^1, T_3^2 and interaction distances d_{14}, d_{28} and d_{29} (A), and of torsions t_1, t_2, t_4 and t_5 (B), for conformation C_{trans} during MD at 300 K. Angles are in deg. and distances in Å.

Table 1

ΔE^a (kcal mol^{-1}) of potential energy and bending, dihedral, non-bonded, electrostatic and H-bonding terms from 60 ps of the MD equilibrium stage at 300 K for conformations A_{trans}, B_{trans} and D_{cis}

	ΔE_{tot}	ΔE_k[b]	ΔE_p[c]	ΔE_{bend}[d]	ΔE_{dihed}[e]	ΔE_{nb}[f]	ΔE_{el}[g]	ΔE_{HB}
A_{trans}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
B_{trans}	0.17	0.00	0.17	−0.04	0.34	−0.071	−0.01	−0.083
D_{cis}	0.52	0.00	0.52	−0.63	0.23	0.277	0.73	−0.081

[a] $\Delta E = \langle E \rangle - \langle E_0 \rangle$.
[b] ΔE_k, kinetic energy.
[c] ΔE_p, potential energy.
[d] ΔE_{bend}, bending term.
[e] ΔE_{dihed}, dihedral term.
[f] ΔE_{nb}, non-bonded term.
[g] ΔE_{el}, electrostatic term.
[h] ΔE_{HB}, H-bonding term.

difference of 0.17 kcal mol^{-1} between the forms A_{trans} and B_{trans} is the dihedral angle energy term which is higher by 0.34 kcal mol^{-1} for the MD conformation D_{cis}. There are multiple sources for the difference of 0.52 kcal mol^{-1} between forms A_{trans} and D_{cis}. In fact, the bending term is lower by 0.63 kcal mol^{-1} for the cis form, but the dihedral, non-bonded and elecrostatic terms are higher by 0.23, 0.28 and 0.73 kcal mol^{-1} for the cis form compared with the trans, determining the higher stability of the latter.

In Table 2 the average values $\langle X \rangle$ and the r.m.s. fluctuations $\langle \Delta X^2 \rangle^{1/2} = \langle (X - \langle X \rangle)^2 \rangle^{1/2}$ for the torsions T_i and t_i and the interaction distances

Table 2

The average values $\langle X \rangle$ and the r.m.s. fluctuations $\langle \Delta X^2 \rangle^{1/2} = \langle (X - \langle X \rangle)^2 \rangle^{1/2}$ for the torsions T_i and t_i (deg), and interaction distances d_i (Å) from 60 ps of the MD equilibrium stage at 300 K for conformations A_{trans}, B_{trans} and D_{cis}

Torsions	A_{trans}		B_{trans}		D_{cis}	
	$\langle X \rangle$	$\langle \Delta X^2 \rangle^{1/2}$	$\langle X \rangle$	$\langle \Delta X^2 \rangle^{1/2}$	$\langle X \rangle$	$\langle \Delta X^2 \rangle^{1/2}$
T_1	−159.1	5.68	−97.3	7.06	−76.1	5.5
T_2	−177.8	3.94	178.4	3.51	1.3	4.4
T_3^1	−92.4	12.41	−83.6	9.60	−89.2	15.1
T_3^2	87.7	12.30	96.2	9.65	90.1	15.2
t_1	23.9	2.83	−20.8	4.09	−20.4	4.7
t_2	−16.0	2.74	13.4	3.29	13.2	3.8
t_3	1.2	2.71	0.3	2.52	0.3	2.6
t_4	14.2	2.80	−13.6	3.25	−13.3	3.4
t_5	−22.7	2.84	20.7	4.01	20.3	4.5
t_6	−24.9	3.29	−27.4	2.88	−25.6	3.2
t_7	34.8	2.22	34.9	2.11	34.9	2.3
t_8	−36.3	2.28	−33.9	2.71	−35.8	2.6
t_9	18.7	3.59	14.2	3.79	17.8	3.8
t_{10}	7.5	4.28	12.2	4.02	8.6	4.2
H-bonds						
$O_{12} \cdots H_{10}$	2.64	0.014	2.64	0.014	2.64	0.013
$O_{14} \cdots H_{10}$	3.77	0.075	2.80	0.092	2.62	0.069
$O_{28} \cdots H_{10}$	4.95	0.186	5.78	0.138	4.99	0.253
$O_{29} \cdots H_{10}$	4.33	0.264	5.70	0.237	5.91	0.271

$>C=O_i\cdots HN<$, d_i, for the 60 ps MD equilibrium stage of the dynamically stable conformers are reported. In all cases the torsions T_3 have the highest mobility: the r.m.s. values are in the angular range from 10 to 15°. The mobility of the torsion T_1 is small; in fact the r.m.s. values are in the range 5.5 to 7°. The mobilities of the torsions T_2 and t_i are lower: the r.m.s. values are in the range 2.1 to 4.7°. Then, from analysis of the torsion angles, it was found that the MD conformations are rather rigid structures, characterized by a particular steric arrangement of the rings with the functional groups vibrating slightly around their equilibrium positions. In contrast, the carboxyl group has a large mobility.

The average values of the structural parameters for the equilibrium conformations have been analysed and compared with the analogous values for the corresponding conformers [3]. For torsions and H-bond distances $>C=O_i\cdots HN<$ deviations of at most 8° and 0.13 Å, respectively, occur, showing the strong structural similarity. From analysis of the values $\langle T_i \rangle$ it results that the torsion T_1 is stable at $-159°$ (A_{trans}) and is in the angular range -97 to $-76°$ (B_{trans} and D_{cis}); the torsion T_2 assumes only $|\langle T_2 \rangle| \approx 180°$ (A_{trans} and B_{trans}) and $|\langle T_2 \rangle| \approx 0°$ (D_{cis}) values; the torsions T_3 have only the one value of about $\pm 90°$. The torsions t_i have average values near to 0° with small positive or negative deviations.

The equilibrium conformations A_{trans} and B_{trans} differ in the values of the torsions T_1 and t_1, t_2, t_4 and t_5, the last ones showing deviations from 0° of opposite sign; the MD conformations B_{trans} and D_{cis} differ, substantially, only in the torsion T_2. Then we hypothesize that the conformational equilibria trans ⇌ trans (between the forms A_{trans} and B_{trans}), through the rotation of T_1, and trans ⇌ cis, according to NMR results [3], (between the forms B_{trans} and D_{cis}), through the rotation of T_2, appear possible if the necessary activation energy is available.

From examination of the values $\langle d_i \rangle$ of $>C=O_i\cdots HN<$ distances, only two H-bond patterns occur: (a) $>C=O_{12}\cdots H_{10}N<$, and (b)

$>C=O_{12}\cdots H_{10}N<$ together with $>C=O_{14}\cdots H_{10}N<$. The first one is characteristic of the trans MD conformation A_{trans}, the second of B_{trans} and D_{cis}. The carboxyl oxygens are never involved in H-bonding, the opposite occurs with EM calculations [3]. This is what ought to occur in aqueous solution with the polar group solvated by the solvent. The absence of H-bonds involving the carboxyl oxygens may be due to the high mobility (entropic effect) of this group and could determine the dynamic instability of the EM folded conformers C_{trans} and E_{cis}.

The MD and normal-mode r.m.s. fluctuations for the torsions T_i and t_i and the H-bond distances have been compared. The order of magnitude and the observed trends are analogous in both cases; nevertheless we observed that the MD fluctuations are less than the corresponding normal mode ones in all cases, except those for T_3 in the conformation D_{cis}. Then, while the goodness of the harmonic approximation is verified, it also provides evidence that it overestimates the extent of the fluctuations of the internal degrees of freedom. Analysis of the r.m.s. fluctuations $\langle \Delta d_i^2 \rangle^{1/2}$ gives further information about the comprehension of the molecular mobility. In fact, while the torsional fluctuations are relative to the individual mobility of each torsion, the $\langle \Delta d_i^2 \rangle^{1/2}$, being a complex function of the fluctuations of the internal variables which separate the interacting couple $O\cdots H$, give global information on the molecular mobility, besides that for each individual interaction. The lower $\langle \Delta d_i^2 \rangle^{1/2}$ ($\approx 10^{-2}$ Å) corresponds to the interaction $O_{12}\cdots H_{10}$, as the mobility of the peptide unit is reduced by the presence of the oxo-prolyl ring. The values corresponding to the interaction $O_{14}\cdots H_{10}$ show the small mobility of the torsion T_2. Lastly, the large fluctuations (> 0.2 Å) of the interactions $O_{28}\cdots H_{10}$ and $O_{29}\cdots H_{10}$ are evidence of the large mobility of the carboxyl group.

MD simulations at 400 K

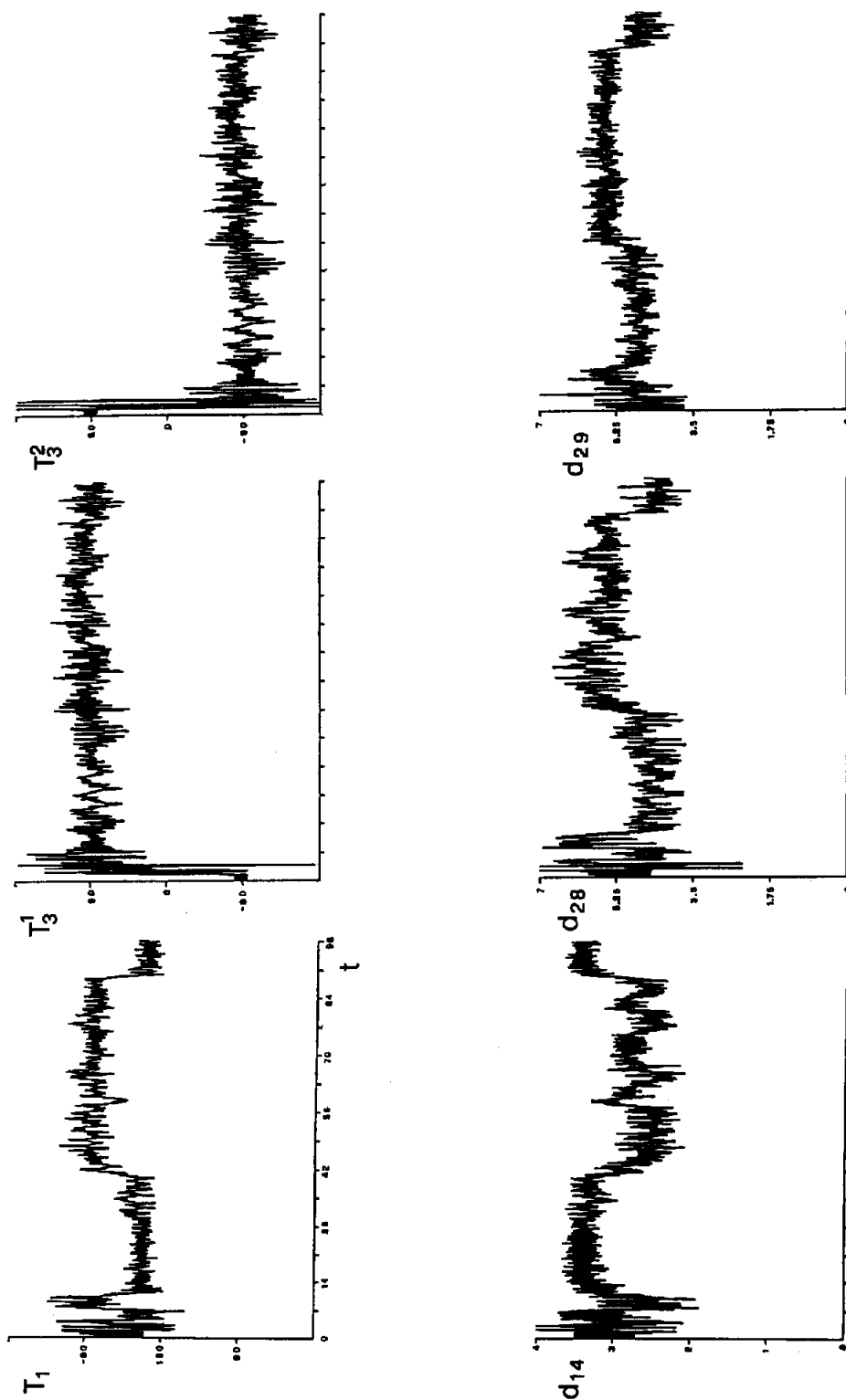The most stable conformer A_{trans} has been taken

Fig. 8. Trajectories of the torsions T_1, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} at 400 K starting from the final state of A_{trans} at 300 K.

Fig. 9. Trajectories of the torsions T_1, T_2, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} starting from the final state of A_{trans} at 300 K during adiabatic simulations at high temperature.
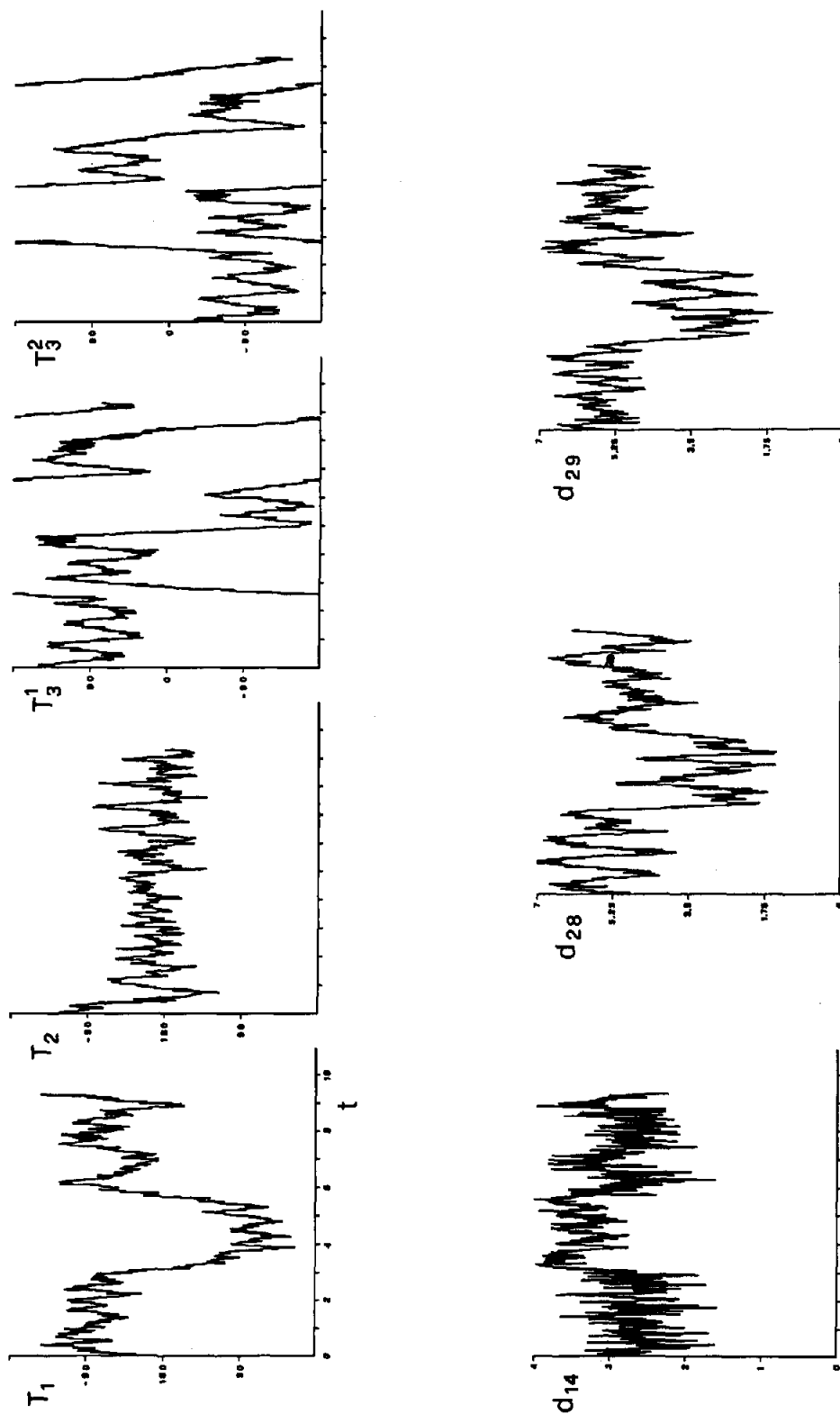
into account at 400 K and different conditions of thermalization (fast or slow heating) have been used. In run a, the final set of atom positions and velocities of the simulation at 300 K has been considered as the starting condition at 400 K (fast heating). In run b, a careful procedure of thermalization has been used (slow heating, see Theoretical Computations).

From MD run a conformers A_{trans}, B_{trans} and B'_{trans} are found to be dynamically stable and in equilibrium with each other: the trajectories of the structural parameters fluctuate around the average conformations A_{trans}, B_{trans} and B'_{trans} through which they oscillate through clear conformational transitions.

In Fig. 8 the trajectories of the torsions T_1, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} for the conformer A_{trans} are presented. Conformation A_{trans} predominates for $t < 40$ ps (during the transient phase) and $t > 90$ ps. For $40 < t < 90$ ps the conformations B_{trans} predominate, in a fast equilibrium between each other.

From MD run b the conformer A_{trans} is found to be dynamically unstable: during the equilibration phase the molecule escapes the conformation A_{trans} and in the equilibrium stage fluctuates between the trans conformations B_{trans} and B'_{trans}.

MD runs at 400 K do not give an unequivocal answer (i.e. a sensible dependence from the starting conditions) about the most dynamically stable conformation of PIDOTIMOD. Nevertheless, as previously shown from MD runs at 300 K, it clearly emerges that the conformations A_{trans}, B_{trans} and B'_{trans} are perfectly isoenergetic and appear to be connected by reaction paths with barriers equal to or less than the kT value at 400 K (about 0.8 kcal mol^{-1}) then in dynamic equilibrium at this temperature.

MD adiabatic simulations at high temperatures

The final state (set of atom positions and velocities) from the simulation at 300 K for the conformer A_{trans} has been used as the starting point for

the MD adiabatic simulation at 1000 K. After the high-temperature thermalization, the molecule has been subjected to 10 ps simulation (see Theoretical Computations Section).

In Fig. 9 the trajectories of the torsions T_1, T_2, T_3^1 and T_3^2 and of the interaction distances d_{14}, d_{28} and d_{29} are presented. The trajectory of the torsion T_2 shows a large rotational flexibility of the bridged peptide bond. Nevertheless, no transitions are observed and the bond fluctuates around the average trans conformation. The trajectories of the torsions T_3^1 and T_3^2 provide evidence for the rigid-body-like motion of the carboxyl group, characterized by large fluctuations around the equilibrium position $T_3^1 = -T_3^2 = \pm 90°$ and overall rotations with exchange of the equivalent oxygens. Examination of the trajectory of T_1 and of the distances d_{14}, d_{28} and d_{29} provide evidence of a complex dynamic behaviour: the conformers A_{trans}, B_{trans} and C_{trans} are present and interconvert during the simulations. In the time interval from 0 to 3 ps the conformer B_{trans} appears to predominate, although short deviations to conformer A_{trans} are observed; during clear conformational transitions in the interval from 3 to 6 ps conformer C_{trans} predominates; from 6 to 10 ps conformers A_{trans} and B_{trans} appear to coexist and to rapidly interconvert. In all cases the stabilizing conformations largely fluctuate. We note that conformer C_{trans}, with the highest energy and which is dynamically unstable at 300 K, is well sampled during the MD simulation as, at high temperatures, the barriers are more easily crossed and the propensity for lower-energy states is diminished.

In conclusion, the torsions T_1 and T_3 are rotationally activated and T_2 excited (large fluctuations), but are of the trans type. The conformationally stable states compatible with trans-type T_2 are efficiently sampled during the high-temperature MD simulations producing evidence for the ergodic properties of trajectories within the trans torsional subspace $\{T_1, T_3, \ T_2 = 180°\}$ and confirming the utility of the approach in conformational searches [5].

Conclusions

PIDOTIMOD is an IL-2 T-lymphocyte receptor agonist. Nevertheless, up to now nothing is known about its structure–activity relationship and interactions of the ligand with the receptor binding site. The present results have helped to elucidate the conformational dynamics of the isolated molecule of the conjugate base of PIDOTIMOD. The five conformers previously determined by us [3] have been considered for MD simulations. MD of 100 ps at constant temperature at 300 and 400 K, and 10 ps adiabatic runs at high temperatures have been performed.

The trans–cis isomerization frequency at 350 K is about 200×10^6 Hz [3] with a free activation energy $\Delta G^{\ddagger} = 7.3$ kcal mol^{-1} from the Eyring equation of the transition state theory [15]. The corresponding transition period of 5000 ps is too long to observe a trans–cis interconversion during our MD simulations.

The trans conformers A_{trans} and B_{trans} and the D_{cis} with extended structures were found to be dynamically stable (equilibrium conformations). A new stable conformation, B'_{trans}, different from B_{trans} in the puckering of the oxo-prolyl ring but thermally mixed with it, has been detected. In contrast, the thyazolidin ring is rigid in only one arrangement.

The trans and cis conformers at the highest energies (C_{trans} and E_{cis} respectively) with folded structures due to H-bonds with the carboxyl group were found to be dynamically unstable (metastable states). The paths connecting C_{trans} to A_{trans} and B_{trans}, and E_{cis} to D_{cis} have barriers near the kT value at 300 K (about 0.6 kcal mol^{-1}) crossed during conformational motion.

The conformational preferences of the functional groups have been shown to be as follows: (a) both the oxo-prolyl and the thyazolidin rings show only two of the possible orientations as a function of torsions T_1 and T_2, respectively; (b) for the oxo-prolyl ring two puckering forms are observed; (c) the carboxylic group shows only one orientation. In all cases the conformational moiety is elongated with the planes of the rings being almost perpendicular. We think that these invariant features may play a key role in the biological activity of PIDOTIMOD. The equilibrium conformations are poor in H-bonds; the carboxyl oxygens are never involved (as expected, however, in aqueous solution with the polar group solvated by the solvent). Moreover, the stable conformations appear rather rigid and only the carboxyl group has a large mobility.

At 400 K the conformations A_{trans}, B_{trans} and B'_{trans} are thermally mixed showing reaction paths with barriers of about 0.8 kcal mol^{-1}.

During the high-temperature MD simulations the trans conformers are rotationally activated and rapidly interconvert. The trans and cis conformations are never thermally mixed; nevertheless the short time scale (10 ps) and the large fluctuations of the torsion T_2 at high temperatures do not give a definitive conclusion about this point.

In conclusion, the actual results on the conformational dynamics of PIDOTIMOD confirm the exhaustiveness of the previous conformational analysis and may represent a starting point for comprehension of the pharmacologically active conformation and its mechanism of action.

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