Pergamon

Biophysics, Vol. 41, No. 6, pp. 1187–1194, 1996

0006–3509/96 $24.00+ .00

PII: S0006-3509(97)00031-8

FEATURES OF THE STRUCTURE OF HYPERSURFACES OF THE CONFORMATIONAL ENERGY LEVELS AND THE DYNAMICS OF POLYALANINE*

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(*Received* 3 *March* 1995)

The elements of structure of hypersurfaces of the potential energy levels of tri- and deca-alanine are examined. The regions of attraction of the right α-helix in configurational space are studied. The results are discussed in relation to the calculations made here of the molecular dynamics of the process of destruction of the α-helix. © 1997 Elsevier Science Ltd. All rights reserved.

It is known that the functioning of proteins is connected with their conformational mobility [1–4]. The individual scenarios for fast conformational transitions in the picosecond and subnanosecond regions can be well traced by the methods of molecular dynamics [5–8]. Prediction of the possible scenarios and investigation of slower structural rearrangements require the development of new, including qualitative, approaches based on analysis of the structure of the hypersurfaces of the conformational energy levels (h.s.c.f.) and the regions of attraction of the local energy minima [4, 9–11]. It is known that the potential energy surface of rigid (quasicrystalline) systems is similar to a multidimensional paraboloid, while the hypersurfaces of the energy levels are topologically equivalent to a hypersphere [12]. For systems with conformational degrees of freedom and also for those with a large number of hydrogen bonds, i.e. where the interatomic potential functions have both local minima and local maxima, the structure of the hypersurfaces of the potential energy levels fundamentally differs from the case of rigid molecular systems. In the space of the configurations there are a large number of attracting loci joined by a network of tubes of lower dimensionality. Such a structure of the hypersurface of the potential energy level on which are played out the scenarios of the dynamic behaviour of the macromolecule is also crucial for its functioning [4, 9, 10]. Direct study of the structural features of the hypersurfaces of the conformational energy levels of macromolecules is at present poorly feasible and some experience needs to be accumulated by way of simpler systems (fragments of macromolecules) and the formulation on this basis of more detailed ideas on the h.s.c.f. of biomacromolecules.

Let us consider the n-dimensional configurational space of the internal degrees of freedom of the molecule $(g_1, g_2, g_3, \ldots, g_n)$. The set of points of the configurational space in which the molecule has a constant conformational energy $U(g_1, g_2, g_3, \ldots, g_n) = E$ forms the multi-

* *Biofizika*, **41**, No. 6, 1168–1175, 1996.

dimensional hypersurface of level E of conformational energy. This h.s.c.f. limits the classically available region of the configurational space of the molecule with the total energy E.

From the h.s.c.f. structure, one may predict the energetically possible conformational states and transitions and evaluate their probabilities by comparing them with the corresponding volumes of the configurational space [13]. Two-dimensional projections of the hypersurfaces of the conformational energy levels of the peptides without reference to the far atom–atom interactions on to the plane of the angles ϕ and ψ of one amino acid residue are well known — potential energy level maps or Ramachandran maps [1, 14–17]. Earlier, in plotting Ramachandran maps considerable simplification of the model was usually made, leaving out of account far van der Waals, hydrogen and electrostatic interactions playing a key role in the formation of the secondary and tertiary structures of proteins. Plotting of several modified potential energy maps has now been widely used. Some studies take into account not only near but also far interactions and also the interaction with the solvent. Naturally, different assumptions and simplifications are made, for example, in [18] the side radicals of the amino acids or the amino acid skeleton of a polypeptide (depending on the purpose of the calculations) are taken as rigid, not having internal degrees of freedom. However, it is essential that, within such an approach, the real representativeness of a particular region of configurational space determined by the relative time of presence in it of the system with spontaneous thermal movements is ignored.

As noted, the complete and correct construction of the hypersurfaces of the conformational energy levels of the macromolecule is very laborious in view of the computational difficulties. To construct hypersurfaces it is necessary to calculate the energy of a large number of points of configurational space. In fact let the molecule have n internal degrees of freedom, then its configurational space has the dimensionality n. If the value of each torsional angle is measured with an accuracy to 10°, then in the configurational space of the molecule there are 36^n points. Even for simple macromolecules, the number of degrees of freedom is above 10 and an enormous number of calculations are required. The calculations are somewhat simplified by the fact that the total volume of permitted regions of configurational space with an acceptable energy is far less than the volume of the whole configurational space.

In the case of a large number of degrees of freedom it is also of interest to construct the zones of the hypersurfaces of the conformational energy levels close to the local energy minima and calculate the regions of attraction of these energy minima. The region of attraction of the local energy minimum is a set of points of configurational space entering into which the molecule in the conditions of heavy friction will inevitably lead to this minimum. In this work we devised a method for studying the structure of the hypersurfaces of the conformational energy levels and the regions of attraction of the local energy minima of the biomacromolecules with reference to all existing interactions. This method, coupled with the molecular dynamics, is used to analyse the conformational mobility of polyalanine and the processes of formation of its secondary structure.

DESCRIPTION OF MODEL

Below, we regard the molecule as a mechanical system consisting of interacting material points (generalized atoms O, C or N together with H atoms bound to them). The valent bonds and valent angles are considered rigid and non-deformable. The molecule has internal mobility only through change in the torsional angles, i.e. the angles of rotation about the chemical bonds.

The potential energy of the molecule is calculated as the sum of the energies associated with the torsional angles, van der Waals and electrostatic interactions and hydrogen bonds, respectively:

$$U(r) = U_{\Phi} + U_{LJ} + U_{el} + U_{hb}. \quad (1)$$

The energy of the torsional angles is defined as

$$U_{\Phi} = \sum_{\Phi} K_{\Phi} [\cos(n\Phi - \delta) + 1], \tag{2}$$

where n is the multiplicity of the torsional barrier, δ is the phase shift, and the constants K_{Φ} determine the heights of the potential barriers of the dihedral angles Φ.

The van der Waals interactions of the atoms separated by three and more valent bonds are described with the aid of Lennard–Johns potentials:

$$U_{LJ} = \sum_{i<j} \left[\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}} \right]. \tag{3}$$

The parameters of the potential A and B depend on the types of atoms i and j involved in the interaction; r_{ij} is the distance between these atoms.

The electrostatic interactions are set by the Coulomb potential

$$U_{el} = \sum \frac{q_i q_j}{\varepsilon r_{ij}}, \tag{4}$$

where q_i, q_j are the partial charges on the atoms, and ε is the dielectric constant of the medium.

The functional form of the potential of the hydrogen bond is similar to the potential of the van der Waals interactions:

$$U_{hb} = \sum_{i<j} \left[\frac{A'_{ij}}{r_{ij}^{12}} - \frac{B'_{ij}}{r_{ij}^{10}} \right]. \tag{5}$$

The parameters of the potential A, B, A', B' are calculated from the formulae [6]:

$$B_{ij} = \frac{3}{2} \left(\frac{1}{4\pi\varepsilon_0} \right)^{1/2} \frac{ehm_e^{-1/2}\alpha_i\alpha_j}{(\alpha_i/N_i)^{1/2} + (\alpha_j/N_j)^{1/2}}, \tag{6}$$

$$A_{ij} = 1/2B_{ij}(R_i + R_j)^6, \tag{7}$$

$$A' = -5E_{\min}r_{\min}^{12}, \tag{8}$$

$$B' = -6E_{\min}r_{\min}^{10}, \tag{9}$$

where i, j are two atoms not bound by valent bonds and valent angles, ε_0 is the dielectric constant for vacuum, e is the electron charge, h is the Planck constant, m_e is the rest mass of the electron, α_i is polarizability, N_i is the effective number of electrons, R_i is the van der Waals radius, $E_{\min}$ is the minimal energy of the hydrogen bond, and $R_{\min}$ is the distance between atoms for the minimal energy of the hydrogen bond. The values α_i, N_i, $E_{\min}$, $R_{\min}$ are the partial charges on the generalized atoms and also the parameters of the valent distances, valent angles, the torsional angles were taken in line with the values used in the molecular dynamics complex [19]. All the calculations were made for the molecule *in vacuo*, i.e. the influence of the solvent was disregarded.

The effective temperature of the molecule was determined from the formula:

$$T = \frac{2<E>}{nk_B}, \tag{10}$$

where n is the number of degrees of freedom of the molecule and k_B is the Boltzmann constant.

Calculation methods

The projections of the hypersurfaces of the conformational energy levels were calculated by the following algorithm. Using a random number indicator, a point is arbitrarily chosen in the

n-dimensional configurational space of the internal degrees of freedom of the molecule. Thus, we have n numbers — the values of the torsional angles, which completely set the relative position of the atoms of the molecule, i.e. its conformation. Each conformation of the molecule has a definite potential (conformational) energy, a function of the torsional angles. To calculate the Cartesian coordinates of the atoms, the Eyring method was used [16], followed by minimization of the potential energy function, i.e. the search for the nearest local minimum. To minimize the potential energy function, we employed the method of the largest gradient. One minimization step consists in the successive minimization of the function for each variable.

If the conformational energy of the molecule in the local minimum is higher than the previously chosen value, the whole process begins anew. If the energy of the molecule is less than the fixed level, its value and the values of the corresponding torsional angles are memorized. Then a "random step" is made from the local minimum, i.e. the values of the torsional angles change arbitrarily, using the random number indicator. If after such a step the energy of the corresponding conformation of the molecule is less than the set value, the corresponding values of energy and torsional angles are also memorized. If not, the torsional angles assume the former values. After a certain number of such returns, the search for a new local minimum begins. To construct the projection of the potential energy hypersurface, the set of points obtained is projected on to the plane of the two chosen variables.

To construct the sections of the region of attraction of the local energy minimum, the points of the configurational space close to this minimum are reset, and minimization occurs from each such point. If after minimization the molecule enters the given minimum, then the point belongs to the region of attraction of this minimum.

In this work we calculated the regions of attraction of the right α-helix of deca-alanine. As criterion of the α-helix we used the relation:

$$d_1^2 + \frac{d_2^2}{4} < 0{,}4, \tag{11}$$

where d_1^2 is the mean square deviation of the torsional angles in radians from the corresponding values for the right α-helix, and d_2^2 is the mean square deviation of the distance between turns in ångströms from the optimal value. To study the dynamics of the process of destruction of the right α-helix, we used visual observation, using the PUMA program complex [19].

RESULTS

Figure 1 presents the projections of the hypersurfaces of the conformational energy levels on to the plane of torsional angles ϕ and ψ of the second amino acid residue of tri-alanine. Figure 2 indicates the region of attraction of the right α-helical conformation of deca-alanine when as initial we chose only conformations obtained for identical variations for all angles ϕ and ψ in monomer units and also the region of attraction in the case of unordered structures.

The sections of the region of attraction of the right α-helix of deca-alanine were also calculated for the case when all the torsional angles, apart from two, plotted along the axes, have values optimal for the α-helix. A typical section of the region of attraction in this case is depicted in Fig. 3 (bold line). The typical region of attraction of the right α-helix, when all the angles apart from those plotted along the axes have values close to the boundary of the region of attraction for unordered structures, is bounded by a broken line in Fig. 3. The continuous line in Fig. 3 indicates the boundaries of the region of attraction of the right α-helix when all the angles, apart from those plotted along the axes, have values over a distance of about 1/3 from the boundary of the region of

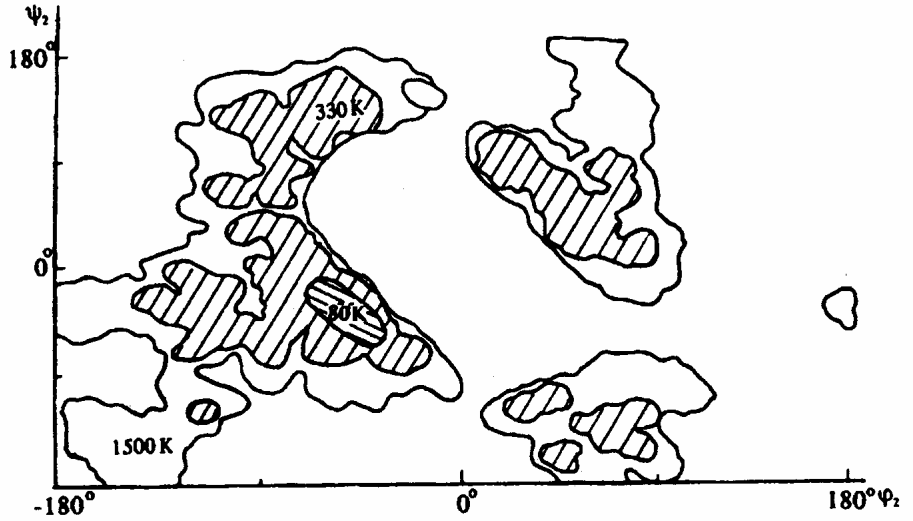

Fig. 1. Projections of the hypersurfaces of the conformational energy levels of tri-alanine on to the plane of the torsional angles ϕ and ψ of the second amino acid residue at the effective temperatures 80, 330 and 1500 K.

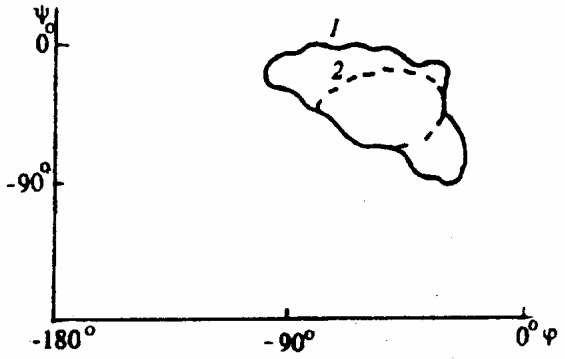

Fig. 2. Regions of attraction of the right α-helical conformation of deca-alanine *in vacuo*. Continuous line (*1*) indicates the region of attraction when as initial we chose conformations obtained for identical variations for all angles ϕ and ψ in monomer units. The broken line (*2*) bounds the region of attraction of the α-helical conformation with random variation of the torsional angles.

attraction bounded by the broken line in Fig. 2. Figure 4 illustrates the stages of destruction of the α-helical conformation of deca-alanine *in vacuo* at 700 K.

DISCUSSION OF RESULTS AND CONCLUSIONS

At low temperature, in the configurational space of tri-alanine, there is a single classically available or "permitted" region (Fig. 1). With rise in temperature (i.e. of the energy of the molecule) there are more "permitted" regions, new paths open up for the displacement of the figurative point in configurational space. At relatively low temperatures the figurative point shifts over one of the "permitted" regions of configurational space. However, the transition between the corresponding subspaces is impossible. With further rise in temperature, between the permitted regions a bridge appears, ensuring the corresponding conformational transition. Even at high temperatures the region close to the point (0°, 0°) remains forbidden in connection with steric

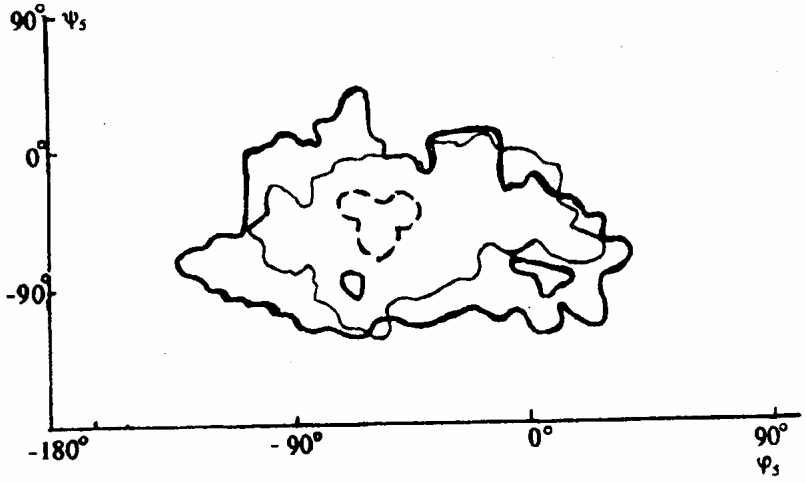

Fig. 3. Regions of attraction of the right α-helical conformation of deca-alanine *in vacuo*. The bold line denotes the region of attraction when all the torsional angles, apart from two, (ϕ_5 and ψ_5), plotted along the axes, have values optimal for the α-helix; continuous line when the angles, not shown in the figure, in the vicinity of the region of attraction have random values lying, on average, on 1/3 of the radius of the region of attraction (Fig. 2, *2*). The broken line bounds the region of attraction when all the angles, apart from two, have random values close to the boundary of the region of attraction corresponding to the unordered structure (Fig. 2, *2*).

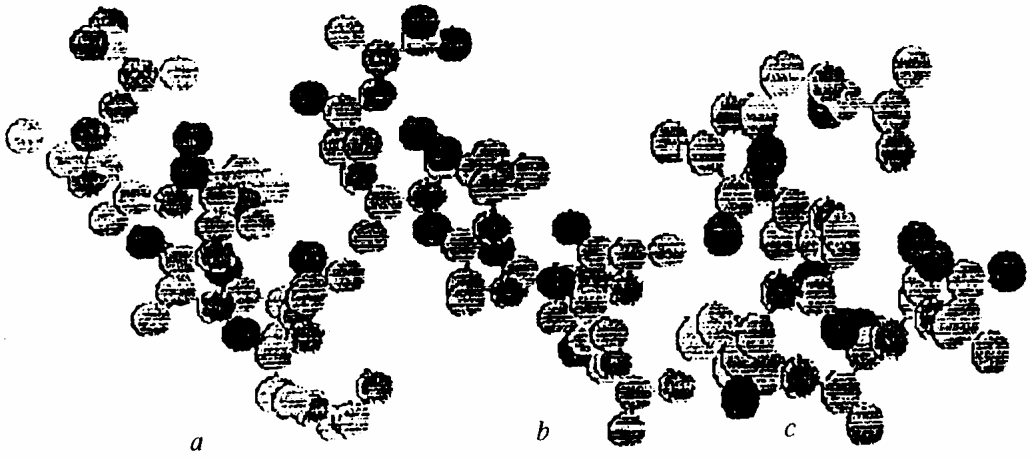

Fig. 4. Stages of destruction of the right α-helical conformation of deca-alanine in vacuo: *a*, initial α-helical conformation; *b*, "melted" state; *c*, irreversibly destroyed α-helix.

hindrances. The deepest minimum in the map of tri-alanine is in the region corresponding to the α-helix, although tri-alanine is a short peptide and cannot form a complete turn of the α-helix. Apparently, the right α-helical conformation *in vacuo* is stabilized not only by the hydrogen bonds between turns, but also by the van der Waals and electrostatic interactions within the turns of the α-helix.

The structure of the region of attraction of the right α-helix of deca-alanine is characterized by extended regions (or valleys), drawn out in directions determined by the identical variations of the angles ϕ and ψ in several monomer units (Fig. 2). Comparison of the set of different projections of the regions of attraction of the right α-helix showed that this region has a highly complex

geometry (Figs 2 and 3). The region of attraction of the right α-helix in the configurational space is drawn out into valleys or tubes, entering which the system inevitably folds into an α-helix.

This result is interesting in terms of the mechanisms and dynamics of folding of the polypeptide chain. In fact, the point here is that there appears to be, as a minimum, two mechanisms of formation of the α-helix for the short peptide. The first is associated with entry of the figurative point on random migration in the configurational space of the oligopeptide into a very narrow region (Fig. 2). The second is linked with the far faster and effective process of folding from a very extensive group of specially prepared states. The dimensionality of the set of these states is smaller than that of the configurational space. However, if the system migrating intersects the hypersurface mentioned above (Fig. 2), then it, in fact, enters a gap running to the right α-helix. This result correlates with the calculations of folding of a model polypeptide chain [20], in which it has been shown that the Levinthal paradox may be overcome through passage of the system to a relatively extensive set of states, from which the native conformation rapidly and clearly forms.

The conclusions on the complex structure of the region of attraction of the right α-helix are also confirmed by the results of modelling the dynamics of destruction of this structure. Modelling of the internal mobility of deca-alanine *in vacuo* showed that the destruction of the α-helix occurs as follows: at first, there is melting (Fig. 4*a*, *b*). At this stage the molecule retains a stretched helical form and the process of destruction is still reversible. Then comes an irreversible stage — sharp fracture of structure and formation of new hydrogen bonds (Fig. 4*c*).

Calculation of a section of the region of attraction when all the torsional angles, apart from two, have values optimal for the α-helix showed that *in vacuo*, it is far more stable on "fracture" than on "melting" (Fig. 3, bold line). The region of attraction is also quite large when all the angles, apart from two, have values close to the boundary of the region of attraction of the α-helix (Fig. 3, continuous line). Consequently, simultaneous "fracture" and "melting" of the α-helix practically do not enhance the destructive action of each other.

Thus, the irreversible destruction of the α-helix occurs on formation of hydrogen bonds not peculiar to the α-helical conformation. The molecule enters a new local potential energy minimum. Consequently, for the final destruction of the α-helical conformation, its "fracture" must take place. The deca-alanine molecule *in vacuo* is quite stable on "fracture" and for it to happen, the molecule must overcome the energy barrier. This barrier has two components associated with the energies of the hydrogen bonds and the tension of the torsional angles. The stability of the system of hydrogen bonds in the α-helix is related to their cooperativeness: rupture of one of the hydrogen bonds is accompanied by destruction of some further neighbours. As long as the hydrogen bonds are not destroyed, the values of the torsional angles cannot significantly change because of geometric constraints. On the other hand, even if the hydrogen bonds are destroyed, the α-helical conformation stabilizes through torsional interactions. Therefore, for the α-helix to be destroyed, its "melting", must first occur. The hydrogen bonds between the turns are partially destroyed with change in the distance between the turns (with increase in the length of the hydrogen bond by 1 Å, its energy falls about 7 times). The α-helical conformation at this stage is stabilized, as noted, through the energies of the torsional angles. If the temperature of the system is sufficiently high, then such a melted state is quite probable and change in the values of at least some torsional angles becomes possible, also resulting in "fracture" of the α-helix. Then new hydrogen bonds rapidly form, which, in fact, signifies the completion of the process of irreversible destruction of the α-helical conformation.

In conclusion, the authors wish to thank the Russian Foundation for Basic Research and the Siberian Commercial Bank for financial support (grant No. 95-04-12197a). The authors are also

grateful to N. K. Balabayev for assistance in the molecular-dynamic calculations and the participants of the seminar of the Department of Biophysics of the Biology Faculty of Moscow State University for useful discussions.

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