Effect of Conformational Constraints on the Topography of Complex Potential Energy Surfaces

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The extent to which structural modifications affect the topography of complex molecular energy landscapes is largely unknown. Through quantitative mapping of the potential energy surfaces of two related hexapeptides, we find that a conformational constraint can completely alter the character of the molecular energy landscape. The linear peptide exhibits a single-funnel potential topography, while its cyclic analog exhibits a shallow energy landscape with competing basins and unevenly distributed roughness. [S0031-9007(98)06713-1]

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Mesoscopic systems with many degrees of freedom, including liquids, glasses, and macromolecules, are under intensive study in physics, chemistry, and biology. In all cases the thermodynamic and dynamic properties of the system are determined by the nature of the underlying potential energy surface (PES), also known as "energy landscape" [1]. It is the potential energy surface that controls complex processes such as protein folding [2,3] or glass transitions [4]. An experimental evidence for the complexity of PES in a system with many degrees of freedom is the multiplicity of relaxation times [5] observed, for example, in the rebinding kinetics of CO to myoglobin [6,7]. Various studies led to the suggestion that the overall structure of molecular free energy surfaces, such as those controlling protein folding, is a multidimensional multitier "funnel" [7–9]. Recently, Becker [10] has demonstrated that such a multidimensional funnel indeed exists on the PES of an atomistic model of alanine-tetrapeptide.

It is well known that seemingly small structural modifications can change the activity of complex biomolecular systems. Examples are the attachment of small cofactor molecules to some enzymes and the dramatic effect of point mutations on protein function. Conformational constraints also play a central role in the context of rational drug design, where they often determine both the specificity and the potency of the drugs [11,12]. Nonetheless, to what extent and in what way do conformational modifications affect the topography of the underlying-energy landscape is still largely unknown. Insights into this process are expected to translate, for example, to more effective drug development processes.

Potential energy surfaces can be characterized by their local minima, which correspond to locally stable conformations, and by the transition regions connecting them. For large complex systems, however, it becomes practically impossible to accurately characterize the full multidimensional energy surface due to the multitude of local minima [13]. Nonetheless, such PES have been partially characterized through studies of conformation samples [13–18]. To better characterize the surface, barrier information, which is essential for understanding the system's kinetics, should be added. Only a small number of complex PES were so far (fully or partially) characterized in this way [14,16,19–22].

A new analysis method that characterized the overall topography of complex PES, named "topological mapping," was recently introduced by Becker and Karplus [23]. This method partitions conformation space into energy "basins" and highlights their interconnectivity. A topological "disconnectivity" graph, reflecting topographic features such as funnels [23], is obtained by following how superbasins break up as the system's energy E decreases. Applied to an atomistic model of alanine tetrapeptide [20,23], this analysis showed that the PES of this molecule has a complex funnel-like topography. By combining topological mapping and principal coordinate analysis, a visual three-dimensional projection of the PES was constructed [10,24]. Application of topological mapping to several Lennard-Jones clusters [25] revealed that these clusters have simple funnel topographies. Topological mapping, which focuses on the superbasin structure of the surface, bears resemblance to the Lid method developed independently by Schön, Sibani, and others for analyzing PES of clusters [26,27].

The topological mapping approach is part of a recent trend in theoretical studies of complex PES that shifts from simplified model systems to detailed analysis of fully atomistic models, allowing more realistic insights into the topography of the PES [16,22,28–30]. Since the number of locally stable structures (local minima), even for rather small systems, rapidly exceeds the capacity for exact enumeration, analysis of atomistic PES must rely on statistical samples. These are expected to capture the overall topography of the surface, although some details are inevitably lost.

In this Letter we use topological mapping to study the effect of conformation constraints on the topography of complex potential energy surfaces. This is the first quantitative study of this phenomenon in atomistic models. The energy landscapes of two related molecules are compared:

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linear alanine hexapeptide (Ala6) and its conformationally constrained counterpart—cyclic alanine hexapeptide (cyc-Ala6). For each molecule a sample of 500 conformations taken from a 500 ps molecular dynamics trajectory at 1000 K was used (each conformation was gradually cooled back to 300 K before minimization to the nearest local minimum) [21]. The CHARMM all atom force field [31] with a distance dependent dielectric constant was used. The terminal groups of Ala6 were set neutral to prevent the strong electrostatic attraction in the absence of explicit water.

In principle, a topological map is generated based on information regarding "direct barriers," i.e., barriers connecting neighboring minima [23]. When using conformation samples, however, the definition of direct barrier has to be extended. To compensate for missing data, barriers between sample points that are not strictly neighboring (taken as the highest energy along the connecting least energy path [32]) must also be included. Fortunately, not all pairwise barriers (about 125 000 barriers in our samples) have to be calculated, and the number of barrier evaluations can be significantly reduced without affecting the overall results. This is done by a twofold process. First, the conformation sample is "diluted," removing conformations that are very similar (both structurally and energetically) to other conformations, leaving only dissimilar conformations in the sample. Second, a distance criterion is imposed to refrain from calculating barriers between minima that are very far away on the PES. Reevaluating the alanine-tetrapeptide system [10,23], we found that a correct "disconnectivity" graph is reproduced when as little as the nearest 50% of the pairwise barriers is retained [21]. Applying this combined procedure to the hexalanine analogs resulted in about 20 000 barrier evaluation for generating the topological map of Ala6 and about 5500 barrier evaluation for the topological map of cyc-Ala6.

Figure 1 shows the disconnectivity graph of linear Ala6. Each node in the graph represents a superbasin on the potential energy surface, defined as a set of local minima connected by barriers not higher than the energy level at which the node is situated. For example, the basin represented by a node at level $E = 16$ kcal/mol includes minima connected by barriers at energy levels not higher than 16 kcal/mol. The lines stretching downward from the nodes extend to the energy of the individual conformations. Since the graph reflects connectivity (and not structural similarity), the horizontal dimension is not quantitative and was chosen in a way that helps visualize the connectivity. The single node at the top of the graph represents the (ergodic) basin in which all 280 distinct minima are connected. Moving 1 kcal/mol down the energy scale, two (high energy) minima are detached from the main basin. Continuing downward more minima become disconnected from the main basin. The lack of further branching indicates that the branched-off local minima are essentially isolated (not forming subbasins). The repeated branching pattern in Fig. 1 reflects an almost ideal "funnel" topography [23], namely, one dominant basin, centered over the global minimum, that "shrinks" as the energy decreases. Almost all low energy minima are located at the core of this funnel connected by barriers not higher than 14 kcal/mol (with a single exception of a low energy minima that branches off at $E = 21$ kcal/mol). The PES topography of Ala6 is simpler than the previously described topography of tetra-alanine, where competing basins could be identified [10,23], possibly due to the more coarse sampling procedure.

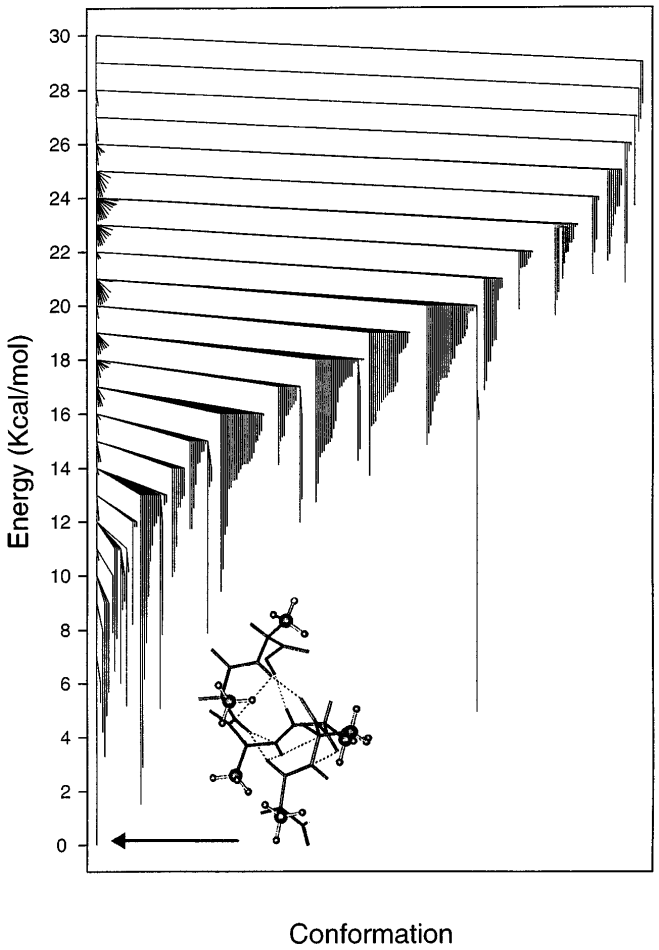

FIG. 1. The topological disconnectivity graph of linear alanine hexapeptide (Ala6) based on a sample of 500 conformations. Each node represents a "superbasin" on the potential energy surface, defined as a set of local minima connected by barriers not higher than the energy level at which the node is situated (levels of the graph are separated by $\Delta E = 1$ kcal/mol). The vertical lines extend to the energy of the individual conformations. The horizontal dimension is chosen in a way that helps visualize the connectivity (it is not quantitative). The inset shows the structure of the lowest energy conformation (hydrogen bonds denoted by dashed lines).

Applying the same methodology to the conformationally constrained hexapeptide, cyclic-Ala6, resulted in the topological map (i.e., disconnectivity graph) depicted in Fig. 2. The marked difference between Fig. 2 and Fig. 1

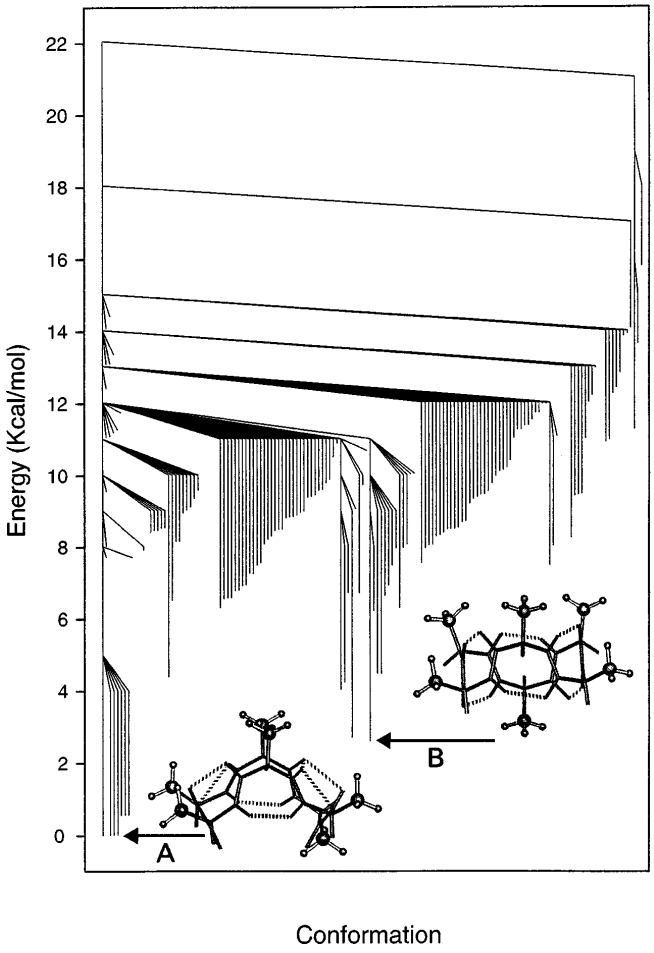

FIG. 2. The topological disconnectivity graph of cyclic alanine hexapeptide (cyc-Ala6) (levels of the graph are separated by $\Delta E = 1$ kcal/mol). The insets show the structure of the lowest energy conformation in each of the two basins *A* and *B* (hydrogen bonds denoted by dashed lines).

indicates that the two PES's are dissimilar in terms of connectivity, range, and roughness.

(1) *Connectivity.*—Unlike the PES of linear Ala6, which has a simple funnel topography, the PES of cyc-Ala6 splits into two basins at barrier height $E = 12$ kcal/mol. One basin continues down to the global minimum while the other reaches a group of minima about 2.5 kcal/mol higher in energy. These two basins show different internal connectivity and are related to very different molecular conformations. The first basin includes a group of minima connected by 5 kcal/mol barriers, and separated from the rest of the system by barriers in the range of 10–12 kcal/mol. The second basin shows a more gradual splitting pattern with (relative) barriers of 7–10 kcal/mol.

(2) *Range.*—The PES of cyc-Ala6 is significantly shallower than the PES of Ala6. Local minima in Ala6 cover a 30 kcal/mol range while in cyc-Ala6 they cover a much smaller range of about 17 kcal/mol.

(3) *Roughness.*—In topological disconnectivity graphs surface roughness is reflected by the number of minima that branch off (from the main branch) at a given energy level. Figures 1 and 2 indicate that while the PES of Ala6 has a fairly constant roughness over a very broad range of energy levels, the roughness of cyc-Ala6's PES is restricted to a narrow energy range. Figure 3 compares the roughness density of the PES of both molecules as a function of the energy level from which they split. The qualitative difference between the two surfaces is evident. Surface roughness in Ala6 is spread over a 20 kcal/mol energy range, while surface roughness in cyc-Ala6 is limited to less than a 10 kcal/mol range. Also, in cyc-Ala6 the roughness peaks at about 12–13 kcal/mol above the global minimum (near its basin split), while in Ala6 the roughness peaks around 20 kcal/mol above its global minimum, indicating a deeper global funnel.

To conclude, while it was expected that cyclization will affect the energy landscape of the peptide, the extent of the transformation is surprising. Ala6 has an almost ideal single-funnel topography (a single deep funnel, with evenly distributed roughness, centered over the global minimum), adding yet another proof to the existence of funnel topographies in polypeptides. On the other hand, cyc-Ala6, which has a much more restricted conformation space [21], has a shallow PES characterized by two competing basins and an uneven distribution of roughness. Namely, the constraining effect of the cyclization is twofold: first, reduction of the available volume in conformation space (reflected by the shallowness of the landscape [21]), and second, further localization into narrow sub-basins. Both effects are not unique to a cyclization constraint and may appear with other constraining factors as well (e.g., proline residues).

The characteristic low energy conformations on the two surfaces (insets in Figs. 1 and 2) can yield a molecular interpretation of the difference in landscapes. The helical structure of the lowest conformation of Ala6 (Fig. 1) corresponds to its funnel topography, indicating that starting from this conformation the molecule can undergo small continued changes as it climbs the energy scale. On the other hand, the two conformations characterizing the cyc-Ala6 basins (Fig. 2) indicate that the split in the PES is due to a large-scale concerted "ring buckling" transition required to move from one conformation to the other. Namely, the constraints introduced by the ring topology forces the system to adopt a concerted transition pattern reflected by a barrier that splits the PES.

In a broader context, this study shows that conformation constraining perturbations, in this case backbone cyclization, may have profound effects on the PES to the extent of completely altering the energy landscape. This dramatic effect may be suggestive of the role of chemical modifications in larger biomolecular systems. Adding cofactors, binding substrates, and other types of modification may result in similar dramatic effects on the topography

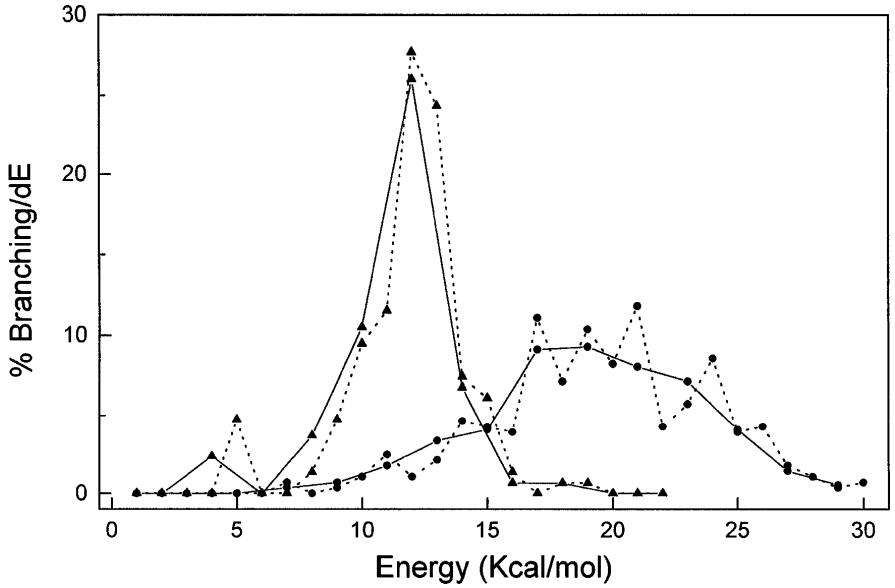

FIG. 3. Roughness density of the potential energy surface of both molecules, Ala6 (circles) and cyc-Ala6 (triangles), as a function of the energy level, for graphs constructed with $\Delta E = 1$ kcal/mol (dashed lines) and $\Delta E = 2$ kcal/mol (solid lines). Roughness density is measured as the percent of minima that branched off at a given energy level divided by ΔE.

of the corresponding PES, changing the folding process or causing an inactive system to become functional.

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