Navigation and analysis of the energy landscape of small proteins using the activation–relaxation technique

Normand Mousseau¹, P Derreumaux² and G Gilbert¹

¹ Département de Physique et Regroupement Québécois sur les Matériaux de Pointe, Université de Montréal, Case Postale 6128, Succursale Centre-Ville, Montréal (Québec), H3C 3J7, Canada
² Laboratoire de Biochimie Théorique, UPR 9080 CNRS, Institut de Biologie Physico-Chimique and Université Paris 7, 13 rue Pierre et Marie Curie, 75005 Paris, France

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Abstract
The resolution of the protein folding problem has been tied to the development of a detailed understanding of the configurational energy or of the free energy landscape associated with these molecules. Using the activation–relaxation technique and a simplified energy model, we present here a detailed analysis of the energy landscape of 16-residue peptide that folds into a β-hairpin. Our results support the concept of an energy landscape with an effective topology consistent with a scale–free network.

1. Introduction

The protein-folding problem was first identified by Levinthal (1969). Also called Levinthal’s paradox, it refers to the unusual efficiency with which a protein can reach a well-defined organized native state starting from a denatured conformation. This paradox has attracted considerable attention and has been at least partially solved in the last decade using the concept of an energy landscape (see, for example, Onuchic et al. 1997).

Within this picture, it appears that the energy landscape of proteins can be considered as a high-dimensional funnel, with the native state at the bottom. This description is supported by a number of numerical results providing a good understanding of the overall topology of this landscape (Becker and Karplus 1997, Onuchic et al. 1997, Mortenson and Wales 2001, Krivov and Karplus 2002).

Conversely, this problem has helped in understanding the concept of an energy landscape. By focusing on a precise problem, researchers were able to develop many tools to characterize the landscape and extract a better understanding of the links between its topology and the associated dynamics. A detailed description of the type of analysis that can be done on energy surfaces, as well as an extensive bibliography, can be found in David Wales’ recent book on the topic (Wales 2003).

Energy landscapes are high-dimensional constructions, however, and even the very useful funnel picture is incomplete as it tries to encompass the dynamics of a point on a hypersurface embedded in a high-dimensional space. The properties of this hyperplane must therefore be characterized by many approaches, each of which can only address a few of its aspects.

In this spirit, we try here to understand the effect on folding trajectories of the choice of an accept/reject criterion in a Monte Carlo simulation. This shifts the focus from the general topology of the energy landscape, as it is generated through various methods, to its impact on the dynamics of folding in a spirit similar to that of Rao and Caflisch 2003, who proposed a general characterization of the protein folding network of a 20-residue antiparallel β-sheet peptide.

More precisely, we report here on simulations of the folding of a 16-residue β-hairpin using the activation–relaxation technique (ART) (Barkema and Mousseau 1996, Malek and Mousseau 2004, Wei et al. 2002). Comparison of ART-generated folding trajectories with extensive molecular dynamical (MD) simulations indicates that both approaches generate similar pathways. This result is somewhat counterintuitive as MD trajectories are controlled by barriers...
surrounding each local minimum while ART uses an accept/reject criterion based on the energy difference between two adjacent minima.

In this paper, we revisit this question by comparing the folding trajectories generated with ART using two different acceptance criteria: the standard one, based on the energy difference between two connected local minima, and one using the barrier height. We find that both types of trajectories visit the same minima along the folding trajectories, supporting the concept of an energy landscape in the form of a scale-free network with some highly connected nodes representing quasi-essential intermediates (Rao and Callisich 2002).

Before describing the simulations and our results, we first discuss our view on the energy landscape problem. Other perspectives are available in some of the papers published in this special issue.

2. The energy landscape problem

For an N-atom system, the configurational energy landscape is hypersurface embedded in a 3N-dimensional space defined by the atomic coordinates plus the energy. (To be exact, one has to subtract from this value the number of constraints on the system: total energy, etc.) Since each point on the landscape corresponds to a single set of coordinates, this surface can also be used to extract the thermodynamical and dynamical information. This extraction is not direct however; the configurational energy landscape does not include the momentum degrees of freedom, and it is generally necessary to perform some thermodynamical integration over the kinetic degrees of freedom.

The high dimensionality of the energy landscape also hides the presence of a large number of constraints, imposed by the three-dimensional reality of the system it represents and which can considerably restrict the effective dimensionality of this hypersurface.

These two limitations impact the picture we develop for protein dynamics and are discussed in some detail in this section.

2.1. The spatial connection

The high dimensionality of the energy surface immediately suggests a very high connectivity and a short shortest-distance path between any two points on this surface. But the systems we are interested in are not random networks and the high-dimensional hypersurface is strongly constrained by the fact that it must correspond to a physically possible three-dimensional (3D) structure. Many of the short pathways on the energy landscape require going over high-energy regions of this surface and are therefore thermally unaccessible. By restricting the energy landscape to physically relevant states, we find a surface that is more less connected and that reflects the real-space local nature of most displacements.

This is what is seen in all the bulk systems we have studied. These include amorphous semiconductors (Mousseau and Lewis 1997, Mousseau and Barkema 2000, Song et al 2000), silica glass (Mousseau et al 2000) and crystalline semiconductors (El Mellouhi et al 2004). The motion through the energy landscape takes place over energy barriers no higher than the value of forming one bond or so. All higher energy barriers can be relaxed into a series of less complex events with a barrier respecting this criterion. This does not imply that the events are all small in scope: some of the events found in a-Si (Song et al 2000) or g-SiO2 (Mousseau et al 2000) involve up to a hundred atoms and more than a half-dozen bond exchanges. At all time during the activated trajectory, however, the system ensures that a minimum number of bonds are broken.

In view of these results, the 3N-dimensional energy landscape is maybe better understood as an overlapping patchwork of lower dimensional hypersurfaces determined by the localization length of these events. For example, one can think of the energy landscape of a-Si as being embedded locally into a 60- to 120-dimensional space encompassing 20 to 40 atoms or so.

This result also implies that it is very unlikely that real (i.e., correlated) higher order activated events take place in bulk material. The higher-order saddle points found in recent molecular dynamics (Grieger et al 2002) are therefore much more likely to be composed of independent first-order transition states. Let us consider the example in figure I

In real space, it is obvious that the two events taking place in synchrony are independent even though, in the energy landscape picture, these two events appear as a second-order saddle point: they are both local in nature and occur at a large distance from each other. In this situation, the energy landscape is misleading and the physical meaning of this result can only be understood by also taking the spatial description into account.

The situation is similar in proteins. Even though system-wide events can occur, most moves involve displacements between 5 and 25 Å. Clearly, the spatial information remains important to characterize the energy landscape of these molecules.
2.2. Energy and free energy landscapes

For proteins, it is also important to assess the entropic contributions in the kinetics of protein folding. While a few researchers have calculated the free energy surface associated with protein folding (see, for example, the papers by Gsponer et al. [2003] and Shea et al. [2004]), most researchers have focused on the energy landscape or on some mixture of energy and free energy landscapes (such as we do, for example, since our interaction potential includes an implicit solvent contribution).

In spite of the various energy landscape models developed for proteins (Becker and Karplus [1994], Onuchic et al. [1997], Mortenson and Wales [2001], Krivov and Karplus [2001]), it is not clear how much one can neglect the entropic contributions to folding. Given the known importance of steric hindrance in protein dynamics, we suppose that this is not negligible. These contributions could turn out to dominate the dynamics, in which case the current energy funnel model of protein folding would need a considerable overhaul (Krivov and Karplus [2001]).

Solving this question is challenging, however, as contributions to entropy have both a local and a non-local origin. While it is relatively straightforward to compute the harmonic contributions, we still do not know how to evaluate properly the anharmonic and the configurational contributions. Some methods developed recently (Vink and Barkema 2002, Jacobs et al. 2003) could prove useful. But it is too early to tell.

2.3. Characterizing the landscape through activated trajectories

As discussed above, the characterization of the energy landscape can be done in a number of ways. Here, we focus on understanding the folding trajectories of proteins and the main intermediate steps towards the native state, in an effort to tie the structure of the landscape to real-space states. To do this, we use the activation–relaxation technique and apply it to the second β-hairpin of domain B1 of protein G.

3. Details of the simulations

3.1. The activation–relaxation technique

The folding trajectories for the peptide are generated using the activation–relaxation technique (ART nouveau) (Malek and Mousseau 2000, Wei et al. 2002), with the OPEP potential (Derreumaux 2000, Forcellino and Derreumaux 2001). ART nouveau generates events that can be considered as steps in Metropolis Monte Carlo simulations, for example.

ART was developed to study the configurational space of systems with dynamics dominated by activated events. Neglecting thermal fluctuations, it focuses on identifying transition states directly connected to a local minimum and, from there, defines a trajectory of local minima separated by an intermediate transition state (Barkema and Mousseau 1996). In its latest implementation, ART nouveau (Malek and Mousseau 2000), an event is generated through the following steps:

(i) Starting in a local minimum, a conformation is deformed in a random direction until a direction of negative curvature appears on the energy surface. At each step during this phase, the value of the lowest curvature is obtained using the Lanczos algorithm. The force perpendicular to this random direction is also crudely minimized after each iteration in order to avoid physical conformations.

(ii) Once the direction of negative curvature is identified, the conformation is then pushed along it, while its energy is minimized in the other directions, converging onto a first-order saddle point (the transition state). The lowest eigenvalue of the Hessian, and its corresponding eigenvector, are also computed using the Lanczos algorithm. At the saddle point, all forces are zero; it is therefore possible to converge with any desired precision onto this point. If during this stage the lowest eigenvalue moves above zero the iteration is stopped and we start again at the previous step, with a different initial direction.

(iii) From the transition state, the conformation is pushed away from the local minimum and it is relaxed into a new local-energy minimum, generating an event. In some cases, the conformation falls back onto the original minimum, suggesting that the transition point found is simply a shoulder inside the local minimum–energy basin.

Two other methods, proposed by Doye, Munro and Wales (Doye and Wales 1999, Munro and Wales 1999) and Henkelman and Jónsson (1999), also implement a very similar algorithm but with slight differences in selecting the escape direction. Recent tests have shown that ART nouveau is the least biased and most efficient algorithm for sampling activated events in high-dimensional systems (Olsen et al. 2004). It has been applied with success to a wide range of materials, including amorphous semiconductors (Barkema and Mousseau 1996, Mousseau and Lewis 1998, Mousseau et al. 2000), Lennard-Jones clusters (Malek and Mousseau 2000) and proteins (Wei et al. 2003, 2004, Santini et al. 2004).

3.2. The optimized-peptide potential

The energy surface of the peptide is modeled by the OPEP energy function (Optimized Potential for Efficient peptide-structure Prediction in solution) which uses a reduced off-lattice protein representation. All amino acids are represented by their N, H, Cα, C and O atoms and each side chain is modeled by one sphere with a van der Waals radius appropriate to the hydrophobic/hydrophilic character and the geometry with respect to the main chain. As fully described in Derreumaux (2000) and Wei et al. (2002), OPEP includes solvent effects explicitly and is not biased to any specific topology. OPEP includes terms to maintain stereochemistry (bond lengths, bond angles, improper angles of the peptide bonds and of the side chains), avoid steric clashes between all particles, allow for the formation of possible secondary structures with backbone two-body and four-body hydrogen bonding interactions, and treat side chain–side chains interactions (using a 20 amino acid alphabet) with a 12-6-potential if the interactions are hydrophobic in character or involve opposite charges, and a 6-potential otherwise.
3.3. Generating the trajectories

In this work, we generate two sets of 20 trajectories starting from the initial extended conformation. The first set (asymmetry) uses the traditional accept−reject Metropolis criterion based on the energy difference between the final and initial minimum in an event: \( P_{\text{accept}} = e^{-\frac{\Delta E}{kT}} \); this is the criterion normally used in ART simulations. The second set (barrier) uses the energy difference between the conformations at the saddle point and at the initial minimum. To ensure similar acceptance rates, the Metropolis temperature is set at 0.6 kcal mol\(^{-1}\) for the first series and at 1.8 kcal mol\(^{-1}\) for the second. Each simulation is run for about 5000 events with an acceptance rate of 50% for the asymmetry-based criterion and of 15% for the barrier one.

4. Results and discussion

In a recent study, we have shown that the β-hairpin can fold into its native state following three distinct pathways: (1) the protein folds at the turn and the bond network propagates from the turn to the endpoint of the strand; (2) the protein folds by favoring contacts between the two endpoints, the bond network then propagates from the endpoint to the turn; (3) finally, the protein can also fold in an asymmetric conformation, forming non-native hydrogen bonds. Step by step, one strand walks over the other, in a repetitive move. The first two mechanisms were already identified by various numerical techniques. The third mechanism involving the repetition of one strand over the other was presented for the first time for the isolated β-hairpin and was also observed in the aggregation pathway of small dimers and trimers (Santini et al. 2004). Krivov and Karplus (2004) have also shown that the free energy landscape associate with this peptide has more than many funnels, each leading to a different structure.

Given this richness in the landscape, all folding trajectories will differ from each other in the details. To analyze these trajectories, we first compare the distributions of energy barriers and atomic displacement for the asymmetry and the barrier simulations. In order to assess the importance of the closeness to the native conformation in the results, we first regroup the events by the value of the energy at the initial minimum. Here, we focus on two ranges of energies: from \(-29\) to \(-32\) kcal mol\(^{-1}\), i.e., in the energy range just above the native state (which is at about \(-33\) kcal mol\(^{-1}\)), and from \(-23\) to \(-25\) kcal mol\(^{-1}\), which is on the way to the native state but can accommodate a wide range of possible conformations. To help place these energies in context, we plot the conformational energy as a function of an attempted event for two trajectories with different accept/reject criteria (figure 2).

Figures 3 and 4 show the distribution of the energy barriers as measured from the final minimum to the barrier for all attempts and only the accepted events using both accept/reject criteria. We choose to focus on this side of the barrier because we do not know the bias of ART on the forward barrier height. Recent work has shown that the barrier as measured from the final minimum was a more meaningful quantity (Valiquette and Mousseau 2003).

![Figure 2](image2.png)

**Figure 2.** Evolution of the energy for two trajectories folding into the native state. The solid line represents the evolution for simulation 17 which uses the energy difference between the final and the initial local minima in its Metropolis criterion; the dashed line shows data from simulation 3, which uses the barrier height in its accept−reject step. Simulation 17 has an acceptance rate of about 50% while simulation 3 has an acceptance of about 12%.

![Figure 3](image3.png)

**Figure 3.** Normalized distribution of energy barriers as measured from the final minimum to the saddle point using both accept/reject criteria and all attempted events in two energy windows. A Gaussian filter with a width of 0.1 kcal mol\(^{-1}\) is applied to all distributions. There are, respectively, 500 and 2500 points for the top and bottom barrier distributions, and 5000 to 9000 points for the asymmetry distributions.

We see first that the distribution of the reverse barrier energies is the same using either accept/reject criteria when looking at all events. The energy landscape visited by either method has therefore a similar structure. If we consider only the accepted events, we see a certain bias towards lower asymmetry with the barrier criterion reflecting the simplest correlation between the forward and the backward barriers:
Figure 4. Normalized distribution of energy barriers as measured from the final minimum to the saddle point for both accept/reject criteria in two energy windows and restricted to accepted events. A Gaussian filter with a width of 0.1 kcal mol$^{-1}$ is applied to all distributions. There are, respectively, 100 and 400 points for the top and bottom barrier distributions, and 4000 to 6000 points for the asymmetry distributions.

Figure 5. Normalized distribution of energy asymmetry between the final and initial energy minima for both accept/reject criteria in two energy windows and restricted to accepted events. A Gaussian filter with a width of 0.1 kcal mol$^{-1}$ is applied to all distributions. There are, respectively, 100 and 400 points for the top and bottom barrier distributions, and 5000 to 9000 points for the asymmetry distributions.

The higher the forward barrier is, the easier for the backward one to be also high.

We can also consider the distribution of energy difference between the final and initial states (the energy asymmetry) for the accepted events (figure 5). Interestingly, the distributions are very similar for both accept/reject criteria, even though the barrier-based simulations do not imply any direct information regarding the asymmetry. Since the acceptance rate is much lower with the barrier criterion, this result suggests that low barriers mean relatively low asymmetry while the opposite is not true.

Finally, we can also look at the distribution of the total atomic displacement for accepted events (figure 6). While the numbers are relatively small for the accepted events with the barrier criterion at the lowest temperature, we can see that the distributions overlap with each other suggesting, again, that the accepted events are of similar size (because of the low acceptance rate with the barrier criterion, statistics are not sufficient to give significance to the peak at 5 Å in the barrier distribution). We also note that the total displacement can be considerable with ART, reaching values as high as 20 Å with a peak at around 5 Å.

4.1. Intermediate states

The results presented in the previous section indicate that the local energy landscape environment seen with both accept–reject criteria is similar and that the trajectories using an asymmetry or a barrier criterion describe the same folding pathways. To assess this point, we consider all accepted conformations that are within 0.02 kcal mol$^{-1}$ of each other with a RMSD between them less than 2.5 Å. We find overlap between trajectories at all energies, with many hundreds of conformations similar between the 20 folding trajectories with the asymmetry and the 20 others with the barrier criteria between $-16$ and $-30$ kcal mol$^{-1}$ (at lower energies, the number of overlapping conformations increases significantly because of their closeness to the native state.) Some of these conformations are plotted in figure 6.
These results demonstrate that the same regions of the energy landscape are sampled using both criteria, suggesting that the number of paths leading to a native state is relatively small.

5. Conclusions

The description of the energy landscape picture for protein folding is not an easy task. This landscape exists in a high-dimensional space, but its thermally relevant parts are strongly constrained by real-space requirements. Many approaches have been used to characterize this landscape (Becker and Karplus 1997, Onuchic et al. 1997, Mortenson and Wales 2001, Shea et al. 2002, Gsponer et al. 2003, Krivov and Karplus 2004), and all present a different set of properties. Here, we focus on the more restricted set of conformations involved in the folding process of a small 16-residue peptide known to fold into a β-hairpin.

This hairpin has been studied by a number of groups, including ours. We found three different folding mechanisms, including a repetition move by which one strand walks on the other until full symmetry is achieved, leading to the native state (Wei et al. 2004). We also noticed that ART-generated trajectories, based on a Metropolis accept–reject move considering the energy difference between the initial and the final state of each event, have similar intermediates to molecular dynamics simulations. This correlation between dynamical trajectories, influenced primarily by the barriers surrounding each minimum-energy basin, and Monte Carlo trajectories constructed only of local-energy minima, is somewhat unexpected and represents the main topic of this paper.

Comparing the visited conformations during folding using both the standard energy difference Metropolis and the energy barrier based Metropolis criteria, we find that the generated trajectories are very similar both statistically and in the details: at all energies, the same conformations are visited in both cases. Moreover, similar events are sampled along the way, even though the accepted ones are not the same. While it is difficult to extract from these results a precise topology, they clearly indicate a high degree of connectivity between the various regions of the phase space in agreement with the protein folding network presented by Rao and Caffi (2004).

While the analysis presented here confirms the close relationship between dynamics and ART-generated trajectories, it further remains to be done in order to understand fully the topological constraints on the energy landscape of proteins.

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