Can protein unfolding simulate protein folding?

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Since the discovery that the native structure of a globular protein can fold spontaneously (Anfinsen et al., 1961), numerous experimental and theoretical efforts have been made to elucidate folding pathways, intermediates, transition states, etc. (for recent reviews, see Bryngelson et al., 1995; Fersht, 1995, 1997; Karplus and Szabo, 1995; Pritsyn, 1995; Miranker and Dobson, 1996; Shakhnovich, 1997). Protein folding pathways are of great interest not only in themselves, but also because understanding them is important for both protein structure predictions (one has to know what to search for; the most stable chain fold or the one resulting from a rapid folding pathway) and for de novo protein design (one has to know what to design: a stable fold only or a pathway to this fold also).

Molecular dynamics (MD) simulations aim to provide the most detailed description of the behavior of ‘realistic’ atomic models of proteins during their folding or unfolding processes. In practice, however, these simulations are used mostly to study the unfolding from the native state (Caflisch and Karplus, 1994, 1995; Daggett and Levitt, 1994; Hunenberger et al., 1995; Daggett et al., 1996; Li and Daggett, 1996; Williams et al., 1997).

The reason is simple. Current MD simulations, even with the most powerful computational techniques, can barely explore more than a few nanoseconds for an all-atom model of a protein and its solvent environment. On the other hand, the folding or unfolding experiment takes not less than hundreds of microseconds (Huang and Oas, 1995; Schindler et al., 1995; Eaton et al., 1997), and usually milliseconds, seconds or minutes (Segawa and Sugihara, 1984; Radford et al., 1992; Itzhaki et al., 1994; Eaton et al., 1997; Roder and Colón, 1997). Thus, one has to accelerate the events in simulations. This can be done with high temperature, but a high temperature corresponds to unfolding conditions. Therefore, unfolding is much more tractable than folding for molecular dynamics.

To observe anything interesting on the nanosecond simulation time-scale, MD has to operate under extreme unfolding conditions where the protein virtually explodes: usually, a temperature of 500–600 K is used (Caflisch and Karplus, 1994, 1995; Daggett and Levitt, 1994; Hunenberger et al., 1995; Daggett et al., 1996; Li and Daggett, 1996) to surmount the unfolding activation barrier over 1 ns. Such a high temperature must accelerate the processes by about six orders of magnitude (Karplus and Szabo, 1995) compared with room temperature but, certainly, it allows an investigation of the protein unfolding process.

So far, to the author’s knowledge, no-one has suggested an effective way to accelerate the folding process, while unfolding can be accelerated in different ways. To avoid an unrealistically high temperature of 500–600 K, some investigators have used low pH (strong electrostatic repulsion) combined with a moderately high temperature of 360 K (Caflisch and Karplus, 1995), extremely high pressure (Hunenberger et al., 1995) or even artificial ‘radial’ forces swelling the protein globule (Hunenberger et al., 1995).

In this issue of Protein Engineering, a new MD-like approach is described (Williams et al., 1997) which avoids high temperatures yet still obtains visible unfolding over 1 ns. This approach is based on the artificial pressing of a water molecule into any pore arising in a protein globule during the unfolding. Although at first sight this algorithm may resemble Maxwell’s demon, it actually mimics an artificially accelerated diffusion of water molecules under the implicit assumption that they are very strongly attracted by protein from the outside to the inside (because this diffusion proceeds only ‘in’ and not ‘out’). This means that water virtually behaves as a very strong denaturant.

The fact is, to accelerate unfolding by many orders of magnitude one has to mimic extreme denaturing conditions in one way or another. Of course, one additional ‘denaturant’ is implicitly present in all MD simulations and in all energy calculations in general, namely energetic errors. They are unavoidable even for all-atom simulations which give a maximally precise reproduction of the motions and energetics of protein–solvent systems. Errors diminish or even destroy an energy gap between the native fold and its competitors (Finkelstein et al., 1995) and thus ‘destabilize’ this fold (an MD simulation control that shows only a small drift of the native structure over nanoseconds at 300 K can prove only a local stability of the structure but it certainly cannot be taken as serious evidence that the computations preserve the thermodynamic stability of this structure: even a small drift over nanoseconds can result in protein unfolding over seconds). Since stability is the main or even the only marker of the native fold (Finkelstein and Badredinov, 1997; Shakhnovich, 1997), the errors make it virtually impossible to find the native structure by a folding simulation starting with the unfolded chain or by any other molecular computations in the absence of additional artificial restraints (Finkelstein et al., 1995; Finkelstein, 1997). This is one more reason why the ‘realistic’ simulations of unfolding are feasible while those of folding are not.

Although carried out under different (but always extreme) conditions, the simulations show a concurrent picture of early events in protein unfolding. The rupture of tight packing and liberation of side chains occur during the initial unfolding phase and then the water molecules penetrate inside the core and destroy, partly or completely, the secondary structure. The transition state for unfolding is found to be closer to the native than to the unfolded state. As a result of the early unfolding events, a protein usually adopts a collapsed, somewhat structured, molten globule-like state. This picture is more or less similar to that displayed or suggested by many experiments done under weakly unfolding and even folding conditions (Fersht, 1995; Pritsyn 1995) and predicted (for decay of the native state) by a thermodynamic theory of cooperative

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transitions in protein molecules (Finkelstein and Shakhnovich, 1989; Shakhnovich and Finkelstein, 1989). However, molecular dynamics present more detail than the experiments normally attempted, or general theory; it shows weak points where the native structure decay begins and where the cavities are formed; it shows which helix, sheet or loop is more distorted and which is less distorted; it also shows which residues are involved in the unfolding transition state. The latter can be verified experimentally (Daggett et al., 1996), as well as the non-uniform expansion of the globule (Caflisch and Karplus, 1994, 1995) and the ‘unlocking’ of two lysozyme domains at the first step in its unfolding (Williams et al., 1997).

However, one should be careful not to overestimate the ability of molecular dynamics to simulate protein folding. Molecular dynamics operate and can operate with nearly folded protein only, while a number of recent experimental studies point out in a very suggestive way that the most essential step of folding is the formation of a relatively small critical folding nucleus in the unfolded phase (Jackson et al., 1993; Fersht, 1995, 1997; López-Hernández and Serrano, 1996).

Theoretical studies aimed at elucidating the principal features of the folding process have shown that nucleation is operational also in the folding of model protein chains (Abkevich et al., 1994; Gütin et al., 1996). Further, they have given estimates of the time necessary for a real protein chain to achieve its most stable structure starting from the coil state (Finkelstein and Sadretdinov, 1997) and provided an explanation as to why nucleation makes it possible to reach this structure without an exhaustive search of billions and billions of competing conformations (Abkevich et al., 1994; Gütin et al., 1996; Finkelstein and Sadretdinov, 1997; Shakhnovich, 1997). Nucleation has provided a solution to the long-standing Levinthal (1968) paradox.

A discovery of a rather small folding nucleus (Jackson et al., 1993; Fersht, 1995) contradicts the earlier experimental evidence that the rate-limiting step of folding is near the native state (Semisotnov et al., 1987; Creighton, 1988). The origin of this contradiction is closely connected with the presence of metastable folding intermediates under the conditions of Creighton’s and Semisotnov et al.’s experiments and their absence in the experiments of Fersht et al. The transition between the denatured and the native state is of the ‘all-or-none’ type at equilibrium (Privalov, 1979), which means that no intermediates are present in observable quantities in the mid-transition; however, these intermediates can arise under strong folding or unfolding conditions (Figure 1), i.e. far from the equilibrium point [cf. Privalov (1979) and Radford et al. (1992)].

Protein folding in the vicinity of mid-transition occurs in the most simple and universal way. Therefore, as a theorist, this author prefers to consider this region of the transition. However, there is little to study here in kinetic experiments except the transition state (Fersht, 1995, 1997) and its changes under the action of mutations (Matouschek et al., 1995) and under modifications of folding/unfolding conditions (Silow and Oliveberg, 1997). Therefore, most experimentalists prefer to work far from the mid-transition; here proteins display a great variety of kinetics, intermediates, pathways, dead-ends and other interesting peculiarities (Creighton, 1988; Radford et al., 1992; Pritsyn, 1995; Roder and Colón, 1997). Taken together, these data, in principle, allow the experimentalist to follow the folding and unfolding pathways in great detail.

A threat to the interpretation of such studies is that the folding pathway under strong folding conditions can differ from the unfolding pathway studied under strong unfolding conditions. According to the principle of detailed balance, a folding process must proceed via the same pathway(s) as the reverse unfolding process when both of them are held at the same conditions, but the processes held under different conditions are not obliged to follow the same route (Figure 2). Therefore, one must be cautious in the projection of MD results (corresponding to extreme unfolding conditions) to protein folding.

A good physical reason for such caution has been pointed out by O.B.Pritsyn and E.I. Shakhnovich (personal communication). Consider, for example, the decay of a water droplet at 300 and 650 K, that is, below and above the critical point. The decay scenario under these two conditions will be entirely different. At 300 K there will be a steady evaporation with sharp segregation of the liquid and gas phases. At 650 K there will be an explosive change without phase separation since there is no division into gas and liquid above the critical temperature. Hence an increase in temperature may actually change the process rather than simply accelerating the same process.

Molecular dynamics studies of protein folding have not yet come of age. It is hoped they will pay off in the future but, so far they have not given unexpected insights into folding or even unfolding mechanisms, but have rather presented detailed pictures of the processes already known ‘in general’. Nevertheless, the all-atom simulations of protein unfolding [which are complementary to the studies performed with simplified models that fold and unfold much more rapidly (Karplus and Šali, 1995; Shakhnovich, 1997)] can present interesting movies
which awaken our imagination and give rich and valuable information on the possible transition states and intermediates on the unfolding and possibly even the folding pathway(s).

References