The range of the contact interactions and the kinetics of the Go models of proteins

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Abstract

We consider two types of Go models of a protein (crambin) and study their kinetics through molecular dynamics simulations. In the first model, the residue–residue contact interactions are selected based on a cutoff distance, \( R_c \), between the \( C_\alpha \) atoms. The folding times depend on the value of \( R_c \) strongly and non-monotonically due to the interplay between frustration and the free energy barrier for folding. This indicates a need for a physically determined set of native contacts that takes into account all the residual atoms. This can be accomplished by considering the van der Waals radii of the atoms and checking if they are found within a proper range of the van der Waals attraction. In the second model, non-native attractive contacts are added to the system. This leads to bad foldability. However, for a small number of such extra contacts there is a slight acceleration in the kinetics of folding.

keywords: protein folding; folding rate; molecular dynamics; chirality
INTRODUCTION

All-atom molecular dynamics (MD) simulations are not yet adequate tools to study kinetics of protein folding. Due to a large number of degrees of freedom involved the accessible time scales in MD are orders of magnitude too short compared to the experimental folding times which last for a millisecond or longer [1]. Thus theoretical studies of the kinetics must involve simplified models such as those in which each amino acid is represented by a single bead that is located at a position of the C\textsubscript{\textalpha} atom. This kind of a drastic simplification in turn requires a design of effective interactions between the beads. A determination of such interactions itself turns out not to be a simple task if one requires preservation of the chemical identities of the amino acids. Go models are a class of simplified systems that do not take into account the sequential specificity of the protein and yet have proved to be fairly realistic in terms of the kinetic properties of proteins [2, 3]. These models are built based merely on the knowledge of the experimentally determined native state. The interactions between the beads are made attractive if the pair of residues form a native contact and repulsive otherwise (to take the excluded volume into account).

The selection of what pair of residues is considered as forming a native contact is a basic ingredient of a Go-type model. A common procedure is to consider the distances, $r$’s, between the C\textsubscript{\textalpha} atoms in the native state, and to adopt a certain cut-off distance $R_c$ such that an $r$ smaller than $R_c$ gives rise to a native contact. The value of $R_c$ has been chosen in the literature quite arbitrarily, between 6.5Å and 8.5Å. Though in principle there should be some optimal choice of $R_c$ that reflects the size and structural details of the amino acids, the simple expectation is that the folding kinetics is not too sensitive to the specific value of $R_c$ within a reasonable range. In this paper, we examine in details the dependence of folding kinetics on $R_c$ and show that this dependence is actually rather strong and quite non-trivial. As an illustration we consider the Go-type models of crambin and find that the fastest folding time, as determined under optimal folding conditions, depends on $R_c$ non-monotonically, although the non-monotonicity is restricted to a rather narrow range. Generally, however, the bigger the $R_c$, the shorter the folding time and the bigger the stability because more and more interactions favor moving into the native state.
A more physical way to determine the native contacts involves taking all pairs of the non-hydrogen atoms in the two amino acids, assigning the van der Waals radii to them and checking if there is an overlap. The criterion for the overlap takes into account some softness in the potential and assigns a factor of 1.244 (the inflection point in the Lennard Jones potential) to the sum of atomic radii. This factor is again somewhat subjective but the whole procedure involves considering the actual sizes of the amino acids and the corresponding Go model will serve as a "realistic" reference system here. For crambin, the distribution of the resulting 137 contact distances is shown in Figure 1 and the structure file was taken from the PDB. The contact distances range from 4.1 to 9.5 Å. The $R_c$-based criterion would involve the van der Waals-determined contacts up to the distance of $R_c$ but also many additional pairs which are upgraded to contacts artificially. On comparing kinetics of the reference system to those corresponding to various $R_c$s one can find a value for which there is a close resemblance. For crambin, we find that the corresponding equivalent $R_c$ is near 7.5 Å.

The next issue which we study here is how the folding kinetics are affected when attractive interactions are added to the non-native contacts. Recently, Plotkin has argued that, as he put it in the title of his paper, "a little frustration sometimes helps", i.e. adding some small noise to non-native interactions may actually accelerate the kinetics of folding up to several times. In Plotkin’s model the non-native contacts are assigned with a random energy with some small variance. The models we studied are in a similar spirit but in our models non-native attractions are assigned to only few non-native contacts while their energies are the same as those of the native ones. This is done by first generating the reference (van der Waals-based) Go model and then by randomly selecting $n_a$ extra pairs with the sequence distance of at least 4. These extra pairs are endowed with the repulsive potential in the reference model and now they acquire a potential which is attractive. In agreement with what proposed by Plotkin, the acceleration of the folding kinetics is also observed in our models but we find it to be rather weak and it happens only when $n_a$ is small. After some threshold, the basic effect of increasing the $n_a$ is to turn the systems into poorer and poorer folders.
MODELS AND METHODS

The Hamiltonian

An input for the construction of the Go model is a PDB file \cite{6} with the
coordinates of all atoms in the native conformation. The coordinates are
used to determine the length related parameters of the model.

There are many variants of the Go models depending, for instance, on
the choice of the functional form of the attractive potentials in the native
contacts. The 10-12 potentials (with the \(r^{-12}\) repulsion and \(r^{-10}\) attraction)
are a common choice, see for instance \cite{8,4}. Another, used here, is based
on the Lennard Jones form. We follow the procedure as described in our
previous papers \cite{9,10,11,12,13} and especially in \cite{14}. The Hamiltonian
consists of the kinetic energy and of the potential energy, \(E_p(\{r_i\})\), which is
given by

\[
E_p(\{r_i\}) = V^{BB} + V^{NAT} + V^{NON} + V^{CHIR}.
\]

The first term, \(V^{BB}\) is the harmonic potential

\[
V^{BB} = \sum_{i=1}^{N-1} \frac{1}{2}k(r_{i,i+1} - d_0)^2,
\]

which tethers consecutive beads at the equilibrium bond length, \(d_0\), of 3.8Å.
Here, \(r_{i,i+1} = |r_i - r_{i+1}|\) is the distance between the consecutive beads and
\(k = 100\epsilon/\text{Å}^2\), where \(\epsilon\) is the energy scale characterizing the native contacts.

\(V^{NAT}\) corresponds to the Lennard-Jones interactions in the native
contacts and is given by

\[
V^{NAT}_{0-12} = \sum_{i<j}^{NAT} 4\epsilon \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right],
\]

where the sum is taken over all native contacts and \(\epsilon\) is common to all con-
tacts. The parameters \(\sigma_{ij}\) are chosen so that each contact in the native
structure is stabilized at the minimum of the potential, and \(\sigma \equiv 5\text{Å}\) is a typ-
ical value. For each pair of interacting amino acids, the two potentials have
a minimum energy of $-\epsilon$. The non-native interactions, $V^{NON}$, are purely repulsive and are necessary to reduce the effects of entanglements. They are taken as the repulsive part of the Lennard-Jones potential that corresponds to the minimum occurring at 5Å. This potential is truncated at the minimum and shifted upward so that it reaches zero energy at the point of truncation.

The last term in the Hamiltonian, $V^{CHIR}$, favors the native sense of the chirality at each location along the backbone. A chirality of residue $i$ is defined as

$$C_i = \frac{(v_{i-1} \times v_i) \cdot v_{i+1}}{d_0^2},$$

where $v_i = r_{i+1} - r_i$. A positive $C_i$ corresponds to right-handed chirality. Otherwise the chirality is left-handed. $V^{CHIR}$ is given phenomenologically by

$$V^{CHIR} = \sum_{i=2}^{N-2} \frac{1}{2} \kappa C_i^2 \Theta(-C_i^{NAT}),$$

where $\Theta$ is the step function (1 for positive arguments and zero otherwise), $C_i^{NAT}$ is the chirality of residue $i$ in the native conformation, and $\kappa$ is taken to be equal to $\epsilon$. The role of $V^{CHIR}$ is primarily to punish energetically any deviations from the non-native sense of chirality.

The attractive non-native contacts, when built in, are given by the Lennard Jones attraction with the minimum at 5 Å.

The time evolution

The time evolution of unfolded conformations to the native state is simulated by MD as described in [9, 10]. The beads representing the amino acids are coupled to Langevin noise and damping terms that provide thermostating at a temperature $T$. The equations of motion for each bead are

$$m\ddot{r} = -\gamma \dot{r} + F_c + \Gamma,$$

where $m$ is the mass of the amino acids represented by each bead. The specificity of masses has turned out to be irrelevant for kinetics [12] and it is sufficient to consider masses that are uniform and equal to the average amino acidic mass. $F_c$ is the net force due to the molecular potentials and
external forces, $\gamma$ is the damping constant, and $\Gamma$ is a Gaussian noise term with dispersion $\sqrt{2\gamma k_B T}$. For both kinds of the contact potentials, time is measured in units of $\tau \equiv \sqrt{m\sigma^2/\epsilon}$, where $\sigma$ is 5Å. This corresponds to the characteristic period of undamped oscillations at the bottom of a typical Lennard-Jones potential. For the average amino acidic mass and $\epsilon$ of order 4kcal/mol, $\tau$ is of order 3ps. We have found that the folding times, $t_{fold}$ depend on $\gamma$ linearly so going to more realistic larger values of viscosity, as controlled by $\gamma$ involves simple rescaling. The equations of motion are solved by means of the fifth order Gear predictor-corrector algorithm \cite{gear} with a time step of 0.005$\tau$.

The folding time is calculated as the median first passage time, and is estimated based on at least 201 trajectories. $T_{min}$ is defined as a temperature at which $t_{fold}$ has a minimum value when plotted vs. $T$. For short proteins, the U-shaped dependence of $t_{fold}$ on $T$ may be very broad and then $T_{min}$ is defined as the position of the center of the U-shaped curve. The simplified criterion for an arrival in the native conformation to be declared is based on a simplified approach in which a protein is considered folded if all beads that form a native contact are within the cutoff distance of $1.5\sigma_{ij}$.

The stability temperature $T_f$ is determined through the nearly equilibrium calculation of the probability that the protein has all of its native contacts established. $T_f$ is the temperature at which this probability crosses $\frac{1}{2}$. The calculation is based on at least 5 long trajectories that start in the native state. A protein is considered as a good folder if $T_f$ is found within a range of temperature at which folding is relatively fast, i.e. preferably close to $T_{min}$ \cite{gear}.

\section*{Dependence of Folding on $R_c$}

In our studies of the effects of the cut-off distance $R_c$ in the Go models of crambin (the PDB code is 1crn), we consider the range from 6 Å to 11 Å in which the number of native contacts varies between 75 and 379 (if one excludes the peptide bond interactions between the successive beads then there is a total of 990 possible contacts since the sequence length is 46). The 6Å case is borderline because it corresponds to one amino acid (the 36’th
along the sequence) which does not belong to any contact and the whole structure is stabilised by the remaining contacts.

Figure 2 shows the median values of $t_{fold}$ as a function of temperature, $T$. The top panel is for the reference system, considered to be the "true" model of crambin whereas the bottom panel is for three values of $R_c$. In each case, the $T$-dependence has the common U-shaped form but the shape itself depends on $R_c$ strongly. It becomes very broad for $R_c$ at and above 8.75 Å. This suggests that the nature of the model changes profoundly around 8.75 Å. Furthermore, as indicated by the top panel of Figure 2, $R_c$ of near 7.5 Å provides the closest, but by no means perfect, representation of the true model. 7.5 Å can be then considered as an equivalent value but this quantity is protein dependent (7.5 Å appears too big for the domain of titin [4]).

It is expected that adding more and more contacts that tie the native structure stronger and stronger should have a dual effect: one is that the thermodynamic stability should get enhanced and the other is that the kinetic links with the native state multiply which should accelerate the folding. Figure 3 suggests, however, that this picture, though generally correct, is more subtle: the fastest folding time, as determined at $T_{min}$, is non-monotonic around $R_c$ of 8.75 Å even though the number of contacts, $n_{nat}$, remains monotonic throughout. This can be explained by the argument that while the free energy barrier for folding decreases on increasing $R_c$ the effects of frustration also get enhanced. The range of $R_c$ where $t_{fold}$ increases corresponds to the situation where the increment in frustration is the winning factor.

Whatever the value of $R_c$ up to 11 Å, $T_f$ stays in the temperature range corresponding to fast folding, i.e. on cooling down, the sequence acquires appreciable probability of staying near the native basin before the glassy effects set in. This is seen in Figure 2 and also in Figure 4. The latter figure shows the values of $T_f$, $T_{min}$, and $T_{g2}$ as a function of $R_c$. $T_{g2}$ is defined as the temperature at which the folding time is twice as high as the fastest time, on the low temperature side of the U-curve. For broad U-curves, $T_{g2}$ is a better measure of when the glassy effects set in than $T_{min}$. Each of these quantities grow monotonically with $R_c$ (and become straighter when plotted vs. $n_{nat}$) and $T_f$ stays between (or near) $T_{g2}$ and $T_{min}$ which indicates good foldability.

Interestingly, $T_f$ of the reference system agrees with a $T_f$ obtained for $R_c$
between 7 and 7.5 Å whereas $T_{\text{min}}$ of the reference system is equivalent to $R_c$ slightly higher than 6 Å which suggests that the true system cannot really be represented by any uniform cutoff value in the contact range.

Figure 5 shows the specific heat, as obtained by the weighted histogram method [17], which provides an additional characterization of the equilibrium properties. The positions of the maxima grow with $R_c$ and so do the maximal values. The temperature width, however, remains more or less constant. Note that even though $R_c$ of 7.5 Å provides a reasonable fit to the plots of the folding time vs. $T$ for the reference system, the specific heat is off more noticeably but the peak position is adequate. It should be noted that the peak positions in the specific heat are higher than both $T_f$ and $T_{\text{min}}$ for all cases. The emergence of a single maximum in the specific heat is a signature of an equilibrium folding transition when the affinity towards the native basin starts to dominate the free energy. The fact that $T_f$ is smaller than the temperature of the specific heat’s peak indicates that below the folding transition temperature the entropy associated with the chain is still significant and not all of the native contacts are established. Complete folding seems to be more accurately associated with $T_f$, which is defined in reference to a single micro state – the native conformation.

THE EFFECTS OF NON-NATIVE ATTRACTIVE CONTACTS

We now consider the second model in which $n_a$ attractive non-native contacts are added to the reference Go model of crambin. We consider just one realization for the contact addition for each $n_a$ and a system corresponding to a larger value of $n_a$ incorporates contacts generated at any smaller value of $n_a$.

We have checked that for $n_a \leq 90$ the native state does not change its nature and it remains accessible kinetically. Its stability, however, decreases with an increasing $n_a$. Figure 6 shows the U-shaped curves of the folding time vs. $T$. In contrast to what happens in the previous model, the curves become narrower and narrower on adding contacts. Furthermore, $T_f$ drifts towards lower and lower temperatures, eventually leaving the region of fast folding. For $n_a$ greater than $\sim 60$, the systems are bad folders.
The fastest folding time is shown in Figure 7 as a function of $n_a$. It is interesting to note that for small values of $n_a$ there is a weak decrease in $t_{fold}$ compared with the case of $n_a$ of 0. Though this decrease is small, it is in a qualitative agreement with Plotkin’s argument which suggests that non-native interactions involved in the collapsed phase may lower the free energy barrier for folding [7]. Beyond $n_a$ of about 25, there is a steady and nonlinear growth of $t_{fold}$ with $n_a$.

Figure 8 shows $T_f$, $T_{min}$, and $T_{g2}$ and a function of $n_a$. Their relative positioning indicates a flow from good to bad folding properties and bad native stability. $T_f$ becomes smaller than $T_{g2}$ at $n_a$ of about 50. For a sufficiently large $n_a$, $T_f$ is expected to disappear.

The specific heat for several values of $n_a$ is shown in Figure 9. In contrast to the picture shown in Figure 4, which also deals with a growing number of contacts, an increase in $n_a$ results in a decrease in the specific heat’s maximum and in shifting it towards lower temperatures. A remarkable change is observed at $n_a$ of 75 and higher, where the specific heat curve acquires a double humped shape. These two peaks can be interpreted as corresponding to two transitions: of folding and of collapse with the former appearing at the lower temperature. The emergence of these two transitions indicates conditions of bad foldability. Notice also that in accordance with what was discussed in the previous section, $T_f$ is always smaller than the temperature corresponding to the peak in the specific heat (the smaller peak if there are two).

**CONCLUSIONS**

In this paper, we demonstrated that the selection of the proper contact range has a strong effect on the folding kinetics in Go models and is not at all innocuous. It is thus important to have a physical scheme, such as based on the van der Waals radii of the atoms, that allows for an amino acid by amino acid determination of whether they make a native contact or not. Adding non-native attractive contacts leads eventually to bad foldability but adding just a few attractive non-native contacts slightly accelerates the folding process.
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FIGURE CAPTIONS

Figure 1. The distribution of the effective contact lengths in crambin as determined by the procedure which is based on the van der Waals radii of the atoms. The shaded region corresponds to the contacts that would not be included if the cutoff of 7.5 Å was adopted.

Figure 2. The dependence of the folding time on temperature for various Go models of crambin. The top panel is for the contacts determined through the criterion that involves the van der Waals radii of the residual atoms. The bottom panel is for contacts determined by the cutoff based criterion. The corresponding values of $R_c$ are indicated. The crosses on the top panel correspond to $R_c$ of 7.5 Å. The error bars are of order of the size of the symbol representing the data points. The arrows indicate values of the folding temperature $T_f$.

Figure 3. The folding time at $T_{\text{min}}$ as a function of the cutoff distance $R_c$. The error bars are less than double the circular symbol size. The stars represent the numbers of the native contacts corresponding to a given value of $R_c$.

Figure 4. The values of the characteristic temperatures $T_{\text{min}}$ (open squares), $T_f$ (solid circles), and $T_{g2}$ (triangles) and positions of the maximum in the specific heat (stars) for the Go models of crambin for various values of $R_c$.

Figure 5. The plots of specific heat as a function of $T$ for several values of $R_c$, as indicated. The solid line (denoted by VdW) corresponds to the reference model in which the native contacts are determined based on the van der Waals radii of the atoms.

Figure 6. The dependence of the folding time on temperature for Go models of crambin in which $n_a$ extra non-native attractive contacts are added. The values of $n_a$ are indicated. The arrows show values of $T_f$.

Figure 7. The folding time as a function of $n_a$. The dotted line indicates the value corresponding to $n_a=0$. 
Figure 8. Similar to Figure 4 but for the model with the added non-native attractive contacts.

Figure 9. Specific heat as a function of $T$ for the indicated values of $n_a$. 
Figure 1:
Figure 2:
Figure 4:
Figure 6:

- $n_a = 0$
- $n_a = 12$
- $n_a = 50$
- $n_a = 75$

$t_{\text{fold}} / 1000\tau$ vs $T$
Figure 7:
Figure 8:
Figure 9: