THE RANGE OF THE CONTACT INTERACTIONS AND THE KINETICS OF THE GO MODELS OF PROTEINS

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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. The folding times strongly depend on the value of R_c and nonmonotonically. This indicates a need for a physically determined set of native contacts. One may accomplish it by considering the van der Waals radii of the residual atoms and checking if the atoms overlap. 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.

1. Introduction

All-atom molecular dynamics (MD) simulations are not yet an 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 in the orders of magnitude too short compared to the experimental folding times of 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_α 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 and the corresponding three-dimensional structure provides an input to the model. 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^{α} 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 6.5 Å and 8.5 Å. 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 nontrivial. 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 nonmonotonically, although the nonmonotonicity 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[4] to determine the native contacts involves taking all pairs of the nonhydrogen atoms in the two amino acids, assigning the van der Waals radii[5] 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 Fig. 1 and the structure file was taken from the
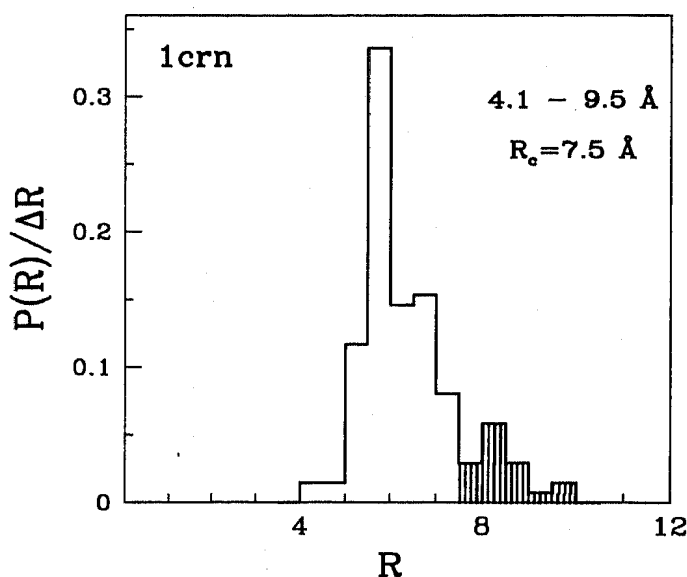

Fig. 1.   The distribution of the effective contact lengths in crambin as determined by the procedure which is based on the van der Waals radii of the atoms. The shaded region corresponds to the contacts that would not be included if the cutoff of 7.5 Å was adopted.

PDB[6]. 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 many additional pairs which are upgraded to contacts artificially. On comparing kinetics of the reference system to those corresponding to various R_cs 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[7] 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 has been 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.

2. Models and Methods

2.1. *The Hamiltonian*

An input for the construction of the Go model is a PDB file[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 are a common choice.[4,8] The other, used here, is based on the Lennard–Jones form. We follow the procedure as described in our previous papers[9–13] and especially in Ref. 14. The Hamiltonian consists of the kinetic energy and of the potential energy, $E_p(\{\mathbf{r_i}\})$, which is given by:

$$E_p(\{\mathbf{r_i}\}) = V^{\mathrm{BB}} + V^{\mathrm{NAT}} + V^{\mathrm{NON}} + V^{\mathrm{CHIR}} . \tag{1}$$

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

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

which tethers consecutive beads at the equilibrium bond length, d_0, of 3.8 Å. Here, $r_{i,i+1} = |\mathbf{r}_i - \mathbf{r}_{i+1}|$ is the distance between the consecutive beads and $k = 100\epsilon/\text{Å}^2$.

V^{NAT} corresponds to the Lennard–Jones interactions in the native contacts and is given by:

$$V^{\text{NAT}}_{6-12} = \sum_{i<j}^{\text{NAT}} 4\epsilon \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right], \tag{3}$$

where the sum is taken over all native contacts and ϵ is the energy unit that is common to all contacts. The parameters σ_{ij} are chosen so that each contact in the native structure is stabilized at the minimum of the potential, and $\sigma \equiv 5$ Å is a typical value. For each pair of interacting amino acids, the two potentials have a minimum energy of $-\epsilon$ and are cut off at 20 Å. 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 5 Å. 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:

$$C_i = \frac{(\mathbf{v}_{i-1} \times \mathbf{v}_i) \cdot \mathbf{v}_{i+1}}{d_0^3}, \tag{4}$$

where $\mathbf{v}_i = \mathbf{r}_{i+1} - \mathbf{r}_i$. A positive C_i corresponds to right-handed chirality. Otherwise the chirality is left-handed. V^{CHIR} is given phenomenologically[14] by:

$$V^{\text{CHIR}} = \sum_{i=2}^{N-2} \frac{1}{2} \kappa C_i^2 \Theta(-C_i^{\text{NAT}}), \tag{5}$$

where Θ 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 κ is taken to be equal to ϵ. 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.

2.2. *The time evolution*

The time evolution of unfolded conformations to the native state is simulated by MD as described in Refs. 9 and 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:

$$m\ddot{\mathbf{r}} = -\gamma\dot{\mathbf{r}} + F_c + \Gamma, \tag{6}$$

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, γ is the damping constant, and Γ is a Gaussian noise term with dispersion $\sqrt{2\gamma k_B T}$. For both kinds of contact potentials, time is measured in units of $\tau \equiv \sqrt{m\sigma^2/\epsilon}$, where σ is 5 Å. 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 ϵ of order 4 kcal/mol, τ is of order $3ps$. We have found that the folding time, t_{fold}, depend on γ linearly so going to more realistic larger values of viscosity, as controlled by γ, involves simple rescaling. The equations of motion are solved by means of the fifth order Gear predictor–corrector algorithm[15] with a time step of 0.005τ.

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 versus T. For small values of N, the U-shaped dependence of t_{fold} on temperature may be very broad and 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 $1/2$. The calculation is based on at least five 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}.[16]

3. Dependence of Folding on R_c

In our studies of the effects of the cutoff 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 6 Å 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 stabilized 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 strongly on R_c. 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 Fig. 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 then be considered as an equivalent
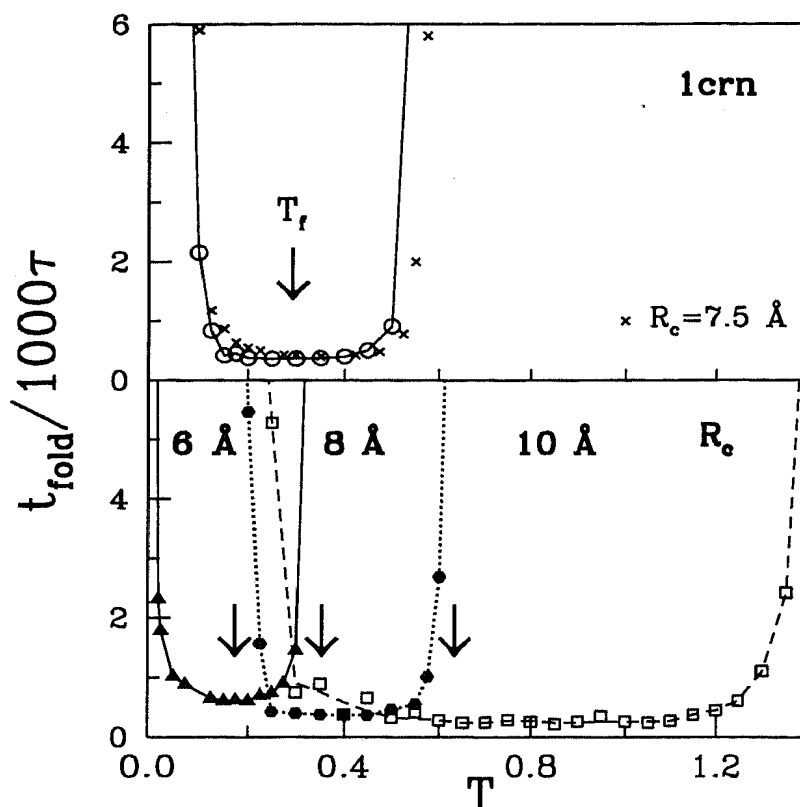
Fig. 2.   The dependence of the folding time on temperature for various Go models of crambin. The top panel is for contacts determined through the criterion that involves the van der Waals radii of the residual atoms. The bottom panel is for contacts determined by the cutoff based criterion. The corresponding 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.

value, but this quantity is protein-dependent (7.5 Å appears too big for the domain of titin[14]).

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 nonmonotonic around R_c of 8.75 Å even though the number of contacts, n_{nat} remains monotonic throughout.

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 it. This is seen in Fig. 2 and also in Fig. 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,
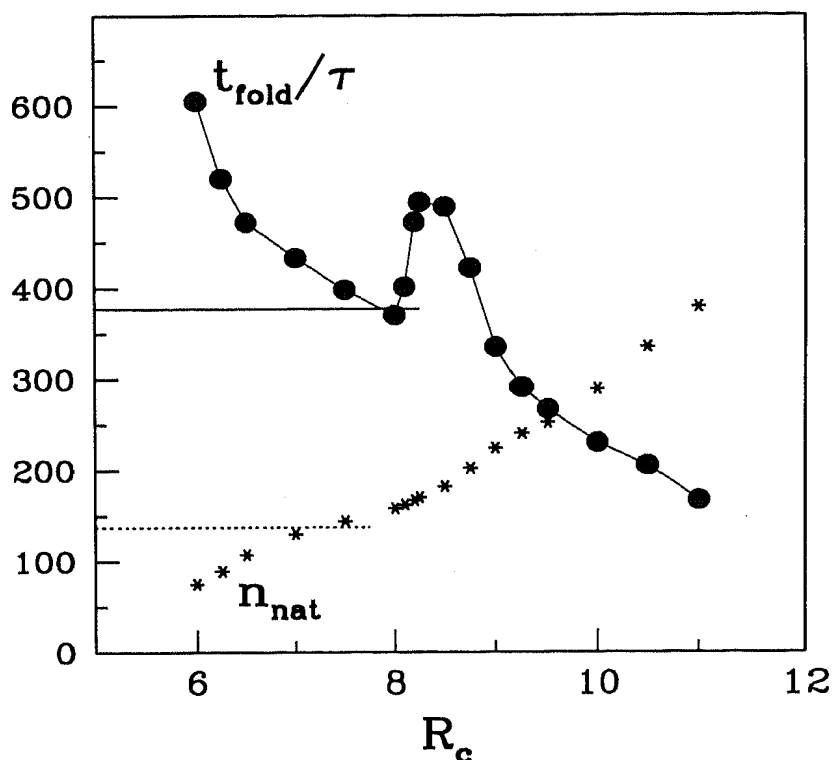
Fig. 3. The folding time at T_{min} as a function of the cutoff distance R_c. The error bars are less than double the circular symbol size. The stars represent the numbers of the native contacts corresponding to a given value of R_c.
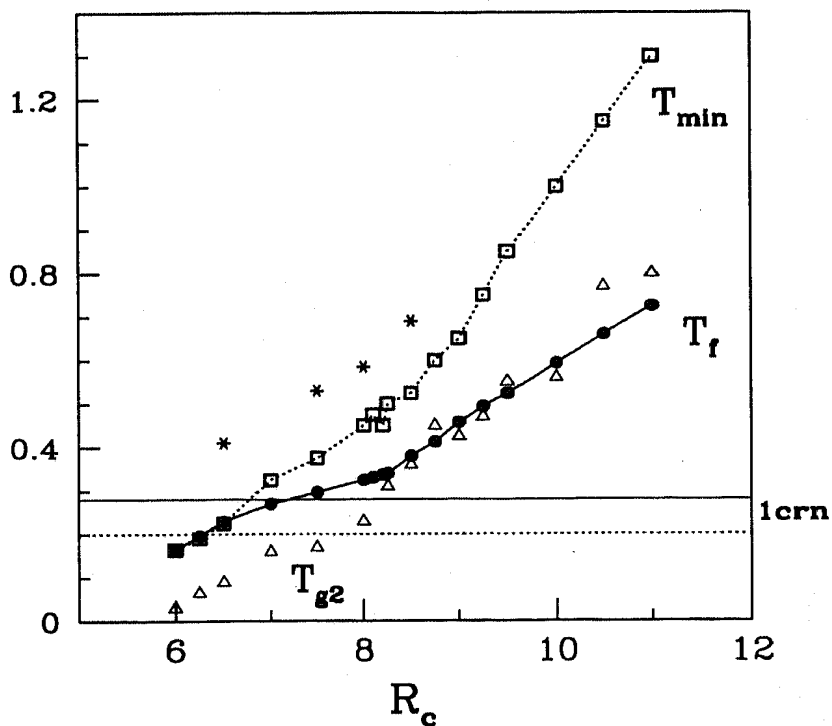

Fig. 4. The values of the characteristic temperatures T_{min} ($\square$), T_f ($\bullet$), and T_{g2} ($\triangle$) and positions of the maximum in the specific heat ($*$) for the Go models of crambin for various values of R_c.
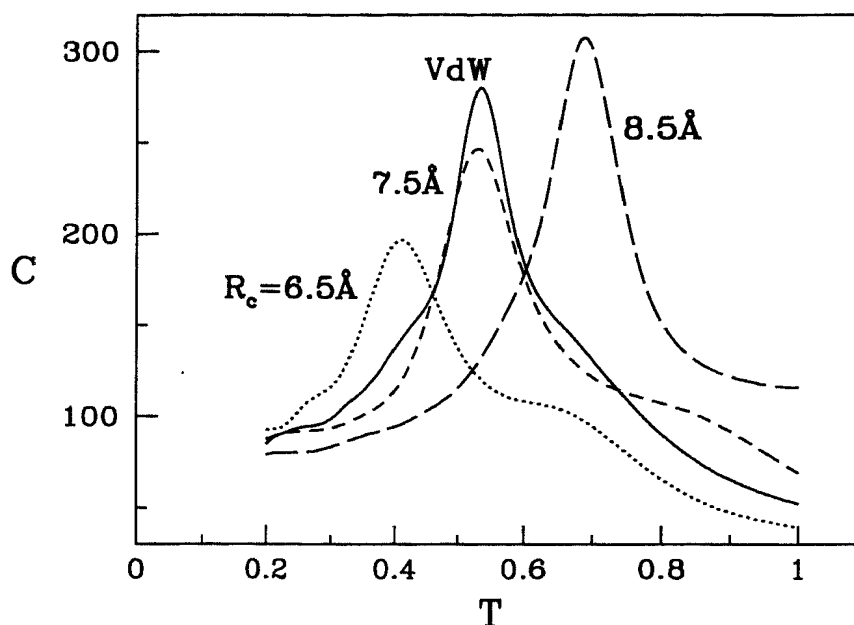
Fig. 5. The plots of specific heat as a function of T for several values of R_c, as indicated. The solid line (denoted by VdW) corresponds to the reference model in which the native contacts are determined based on the van der Waals radii of the atoms.

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 versus 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_{min} of the reference system (with the microscopically calculated contact map) 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 versus 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_{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.

4. 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 versus 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 ~ 60, the systems are bad folders.

The fastest folding time is shown in Fig. 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 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.
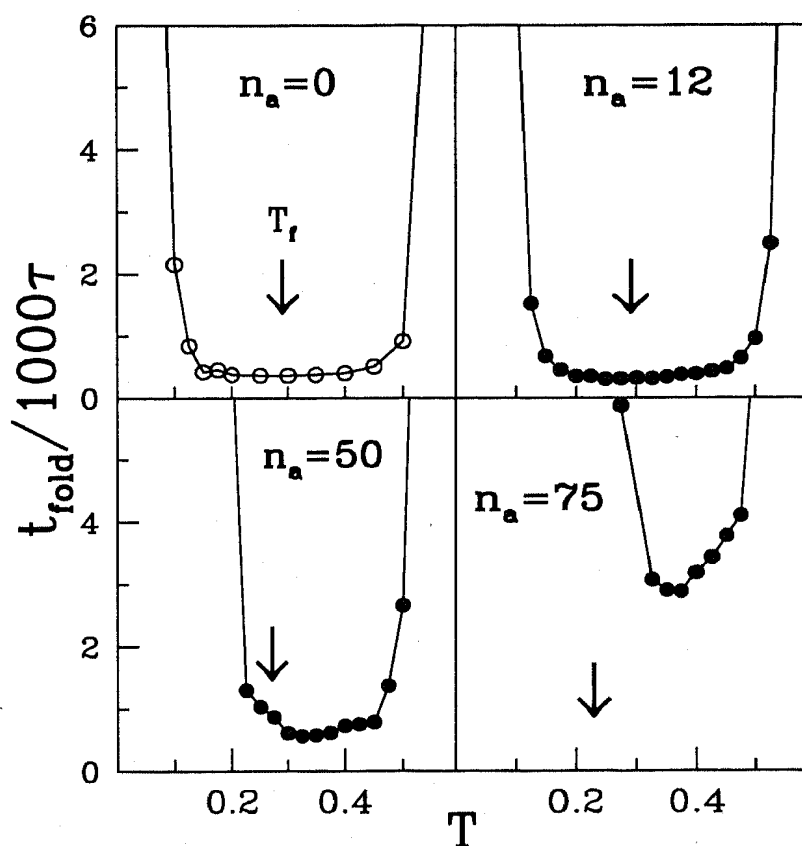

Fig. 6. The dependence of the folding time on temperature for Go models of crambin in which n_a extra non-native attractive contacts are added. The values of n_a are indicated. The arrows show values of T_f.
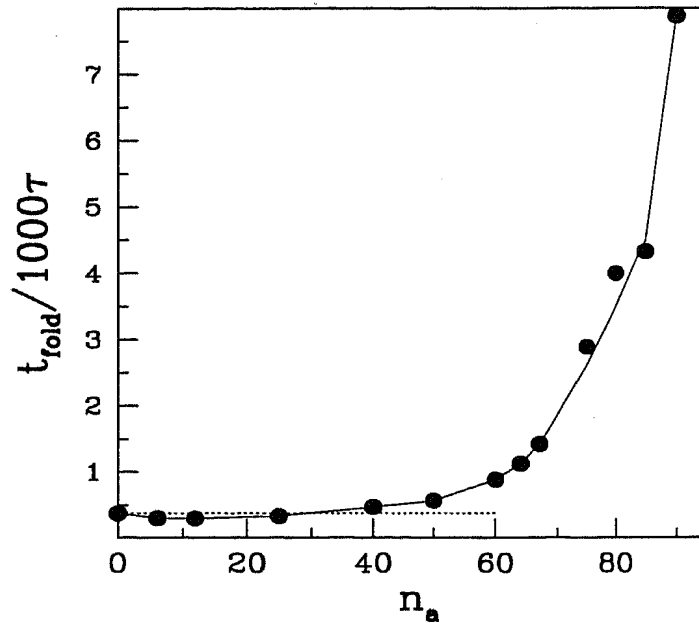
Fig. 7.   The folding time as a function of n_a. The dotted line indicates the value corresponding to $n_a = 0$.
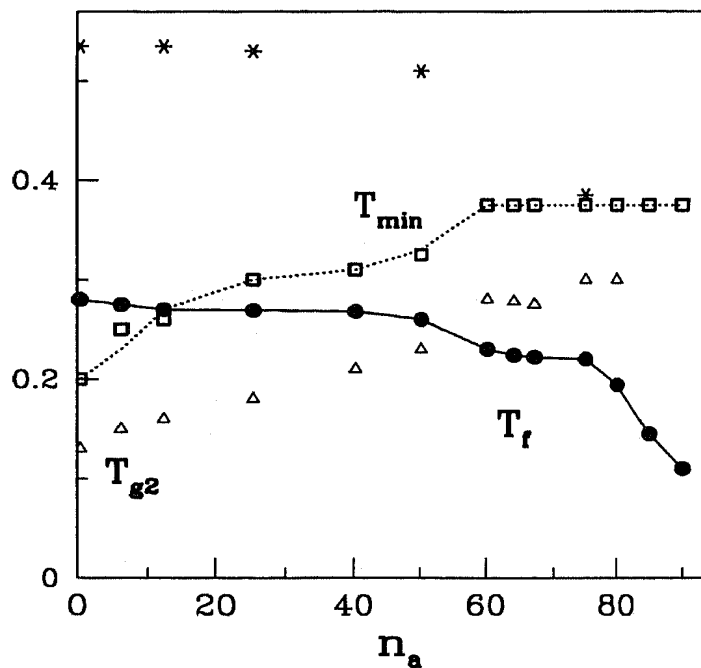

Fig. 8.   Similar to Fig. 4 but for the model with the added non-native attractive contacts.

Figure 8 shows T_f, T_{min}, and T_{g2} as 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 Fig. 9. In contrast to the picture shown in Fig. 5, which also deals with a growing number of contacts, an
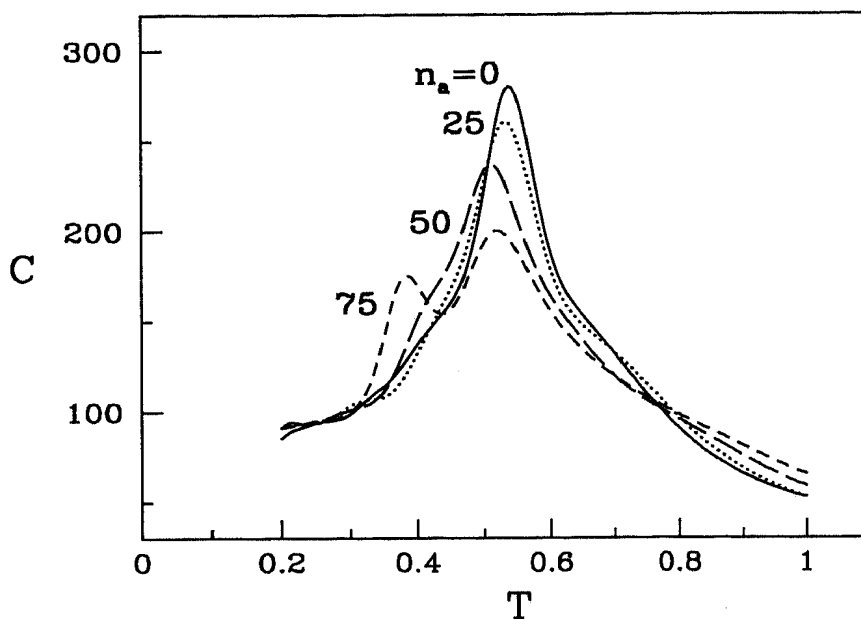
Fig. 9.   Specific heat as a function of T for the indicated values of n_a.

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 compactification with the former appearing at the lower temperature. The emergence of these two transitions indicates conditions of bad foldablity. Notice also that in accordance with what 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).

5. Conclusions

In this paper, we demonstrate 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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