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Calculation of the free energy barriers in the oligomerisation of Aβ peptide fragments

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1. ABSTRACT

Protein misfolding and aggregation are associated with a range of severe human neurodegenerative conditions. We use all-atom simulations to describe the process of assembly of the $A\beta_{16-22}$ and $A\beta_{25-35}$ fragments of Aβ, a peptide associated with Alzheimer's disease. Our results indicate that the pathways of aggregation of these two peptides depend predominantly on the relative strength of hydrophobic interactions and hydrogen bonding. In the $A\beta_{25-35}$ peptide, which is weakly hydrophobic, the tendency to form hydrogen bonds drives the crossing of a single major free energy barrier for the formation of a cross-β structure. By contrast, in the more hydrophobic $A\beta_{16-22}$ peptide, the process of ordered assembly is preceded by an initial collapse into disordered oligomers. These results provide support for a recently proposed two-step mechanism of amyloid formation. We have also found that the barriers for reordering are lower for large oligomers than for small oligomers, a result that provides an explanation of the recent experimental observation that the efficiency of the seeding reaction depends on the size of the seeds themselves.

2. INTRODUCTION

Misfolding and aggregation of proteins are intensely studied phenomena because of their links to a variety of human diseases (1-4). Although under normal circumstances the quality control mechanisms of the cell are able to refold or, if needed, degrade the pathological species resulting from the abnormal assembly of peptides and proteins, a range of factors like aging, specific pathological conditions, or even therapeutic treatments such as kidney dialysis can alter the balance between the processing of misfolded species and their intra or extracellular accumulation (2). As a result, many human disorders, including Alzheimer's and Parkinson's diseases, and type II diabetes have been recently related to the deposition of protein aggregates in various tissues (1,5-7).

Although the identification of the ensemble of molecular species that give rise to neurodegeneration is one of the most controversial topics in current studies in amyloid-related diseases, evidence is accumulating concerning the ability of the low molecular weight

oligomers of specifically disrupt cognitive functions (8-14). Interest in these species has increased since their initial detection in the brain of patients suffering from Alzheimer's disease (15,16). Despite much recent progress (8,17-21), however, a detailed description of the oligomerisation process at the molecular level remains in large part elusive because it is challenging to describe the early stages of aggregation of polypeptide chains by experiment, primarily because of the difficulties in characterising the small structurally heterogeneous transient species that are involved.

In order to obtain insight into the process of amyloid formation one strategy is to use computational approaches. The use of molecular simulations in explicit water has increased our understanding of the early molecular events that lead to the aggregation of peptides and proteins (22-32). Powerful intermediate-resolution models have also provided the opportunity of studying larger systems and longer timescales (33-40). These models have enabled the fundamental properties of polypeptide chains responsible for the process of amyloid formation to be identified. Of course, simple models are unable to describe in detail the complex phenomenology associated with the different behaviors of specific polypeptide chains, such as for example the different propensities of mutant forms of peptides and proteins to form amyloid fibrils or oligomeric intermediates (41), or their different toxicities in neurological disorders such as Alzheimer's, Parkinson's and Creutzfeldt-Jakob diseases (9). However, such models are able to play a crucial role in obtaining fundamental insights into the origin of the experimental observations of those aspects of the phenomenon of protein aggregation that are common to most of the peptides and proteins that have been analyzed, such as the existence of lag phases (5) and of a series of disordered oligomeric assemblies that appear prior to the formation of amyloid fibrils (19,42).

In this paper we investigate the early stages in the oligomerization process of two fragments of the Aβ peptide, $A\beta_{16-22}$ and $A\beta_{25-35}$. We have recently discussed how the mechanism of oligomerization of these two peptides creates transient oligomers whose structural properties are potentially related to their toxicity (32). Such a mechanism involves two steps – disordered coalescence, driven by hydrophobic interactions and reorganisation into cross-β structures, driven by the formation of intra-chain hydrogen bonds (32,39). The competition between these two fundamental interactions, which are common to all polypeptide chains, leads, for the more hydrophobic $A\beta_{16-22}$ fragment, to the formation of disordered oligomers, which subsequently undergo a process of conformational conversion and become rich in β-sheet structure. By contrast, in $A\beta_{25-35}$, which is less rich than $A\beta_{16-22}$ in hydrophobic residues, the initial coalescence phase is nearly suppressed, and the oligomerization proceeds by the direct formation of ordered β-rich oligomers (Figure 1).

3. CALCULATION OF THE RATES OF ASSOCIATION AND DISASSOCIATION

The use of molecular simulations grants access, at least in principle, to a complete knowledge of the behaviour of polypeptide chains during their aggregation. We exploit here this opportunity to estimate the kinetic parameters that describe the growth of the oligomeric aggregates that appear prior to the formation of the amyloid assemblies.

All-atom simulations were performed with the ProFASi (PROtein Folding and Aggregation SImulator) program (43-47). The ProFASi interaction potential is composed of four terms that describe excluded volume repulsion, electrostatics interactions, hydrogen bonding and hydrophobic effects. This force field was shown to reproduce accurately the folded states and the melting temperatures of a range of polypeptide chains of both α and β structures, including Betanova, GB1p, LLM and F_S, with excellent agreement with both CD and NMR data (43-47). In addition, folding properties such as the α-helix content and the relative population of folded species was also found to be in excellent agreement with experimental data. ProFASi has also already been applied to study the aggregation of a series of short peptides, including the $A\beta_{16-22}$ and $A\beta_{25-35}$ peptides (32,47).

For both the $A\beta_{16-22}$ and $A\beta_{25-35}$ peptides we carried out a series of 100 independent simulations each of 10^9 Monte Carlo steps in a cubic box of 60 Å with periodic boundary conditions at constant temperature. In order to explore the effects created by the finite size of the system we also performed additional series of 50 simulations with 30 peptides. The initial configurations consisted in all cases in random distributions of monomeric peptides in the absence of any seed of the ordered phase since we were interested in the mechanisms of spontaneous oligomerization.

In order to characterise the structure of the oligomers we calculated the population $P(N_\beta)$ of oligomers containing a number N_β of β-sheets (Figure 2). We also calculated the population $P(N_{c\beta})$ of oligomers with a number $N_{c\beta}$ of peptides in a β strand conformation, and the population $P(N_c)$ of oligomers containing N_c peptides in any conformation (Figure 3). These simulations enable us to evaluate the rates of growth (R_+) and depletion (R_-) of the ordered component of the oligomer (Figure 4). According to transition state theory these rates can be expressed as

$$R_+(n) = Ae^{-(F^T_{n,n+1} - F^\beta_n)/k_BT} \qquad (1)$$

$$R_-(n+1) = Ae^{-(F^T_{n,n+1} - F^\beta_{n+1})/k_BT} \qquad (2)$$

where $F^T_{n\,n+1}$ is the free energy of the transition state between β-sheets of sizes n and $n+1$, F^β_n is the free

Calculation of the free energy barriers

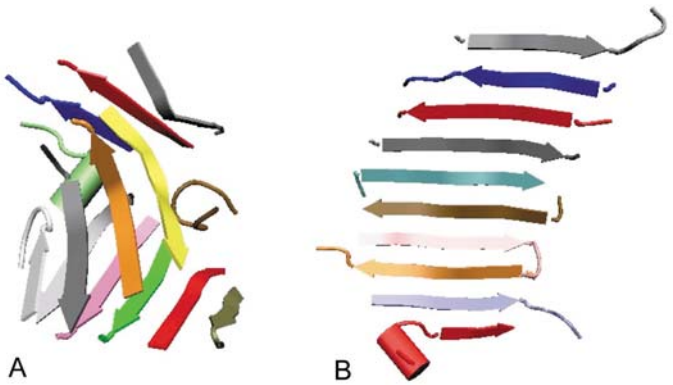

Figure 1. Examples of the oligomeric structures generated through the simulations that we have presented in this work for: (a) a disordered oligomer formed by $A\beta_{16\text{-}22}$ and (b) an ordered β-sheet structure formed by $A\beta_{25\text{-}35}$.

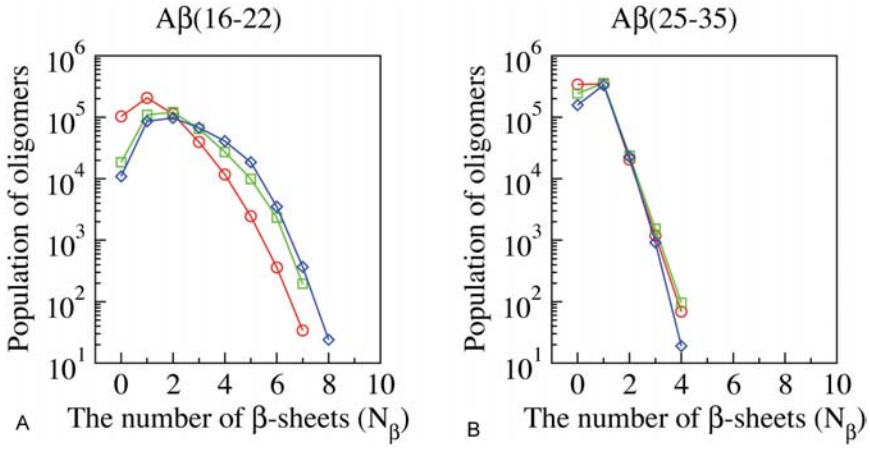

Figure 2. Populations $P(N_\beta)$ of oligomers containing a number N_β of β-sheets for: (a) $A\beta_{16\text{-}22}$ and (b) $A\beta_{25\text{-}35}$. Different colours indicate the three time windows that we considered: $0-2\cdot10^8$ (red), $4-6\cdot10^8$ (green), and $8-10\cdot10^8$ Monte Carlo steps (blue line).

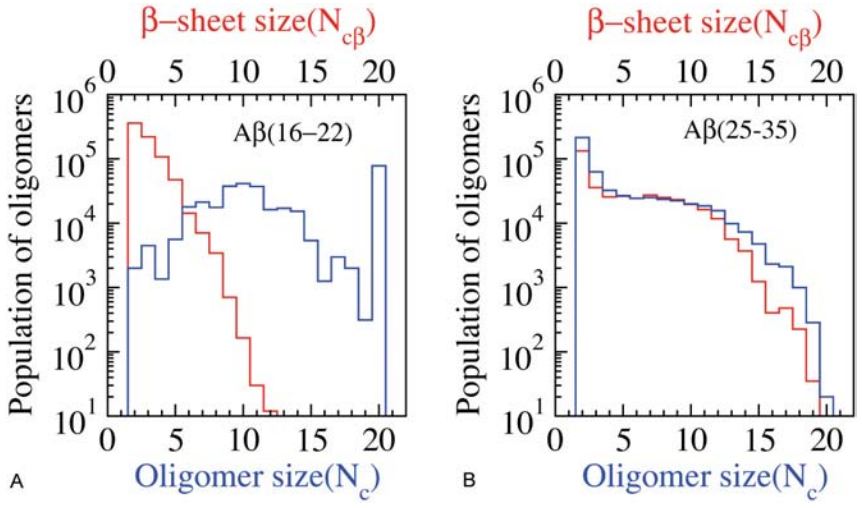

Figure 3. Populations in the final time window ($8-10\cdot10^8$ Monte Carlo steps) for: (a) the $A\beta_{16\text{-}22}$ and (b) the $A\beta_{25\text{-}35}$ peptides. The blue line represents the population $P(N_c)$ of oligomers of N_c peptides; the red line represents the population $P(N_{c\beta})$ of oligomers with $N_{c\beta}$ peptides in a β strand conformation.

Calculation of the free energy barriers

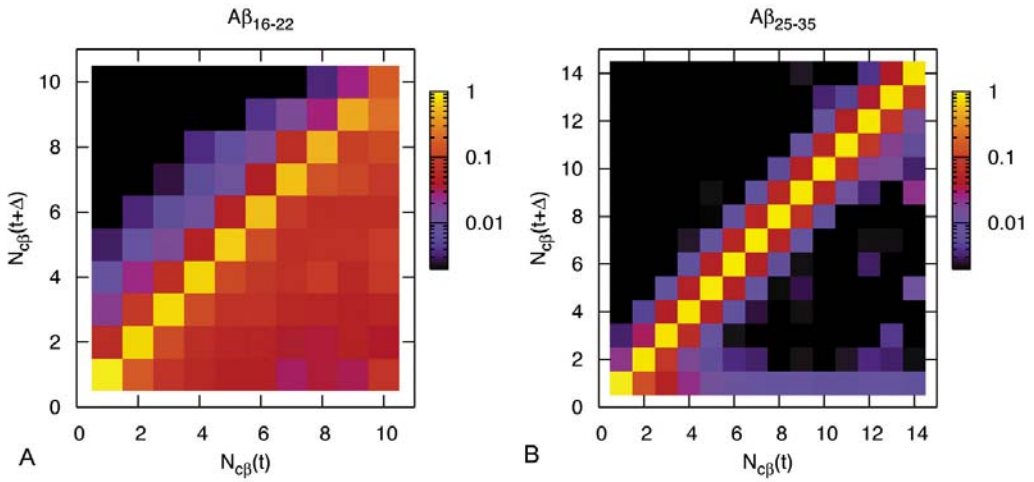

Figure 4. Transition probabilities from $N_{c\beta}(t)$ to $N_{c\beta}(t+\Delta)$ for (a) $A\beta_{16\text{-}22}$ and (b) $A\beta_{25\text{-}35}$.

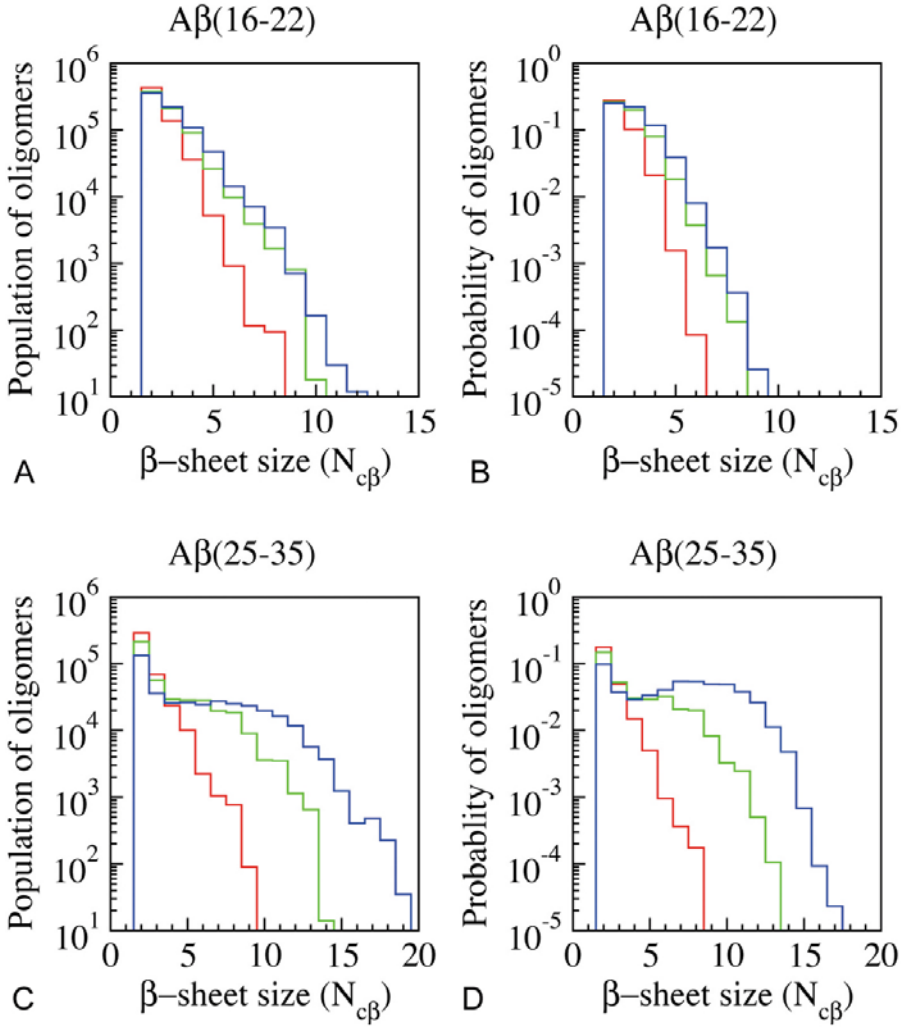

Figure 5. Populations of oligomers for the $A\beta_{16\text{-}22}$ (a) and $A\beta_{25\text{-}35}$ (c) peptides for three different time windows $0-2\cdot10^8$ (red), $4-6\cdot10^8$ (green), and $8-10\cdot10^8$ (blue) Monte Carlo steps. For comparison, the populations of oligomers obtained from the free energies are also shown for the $A\beta_{16\text{-}22}$ (b) and $A\beta_{25\text{-}35}$ (d) peptides.

Calculation of the free energy barriers

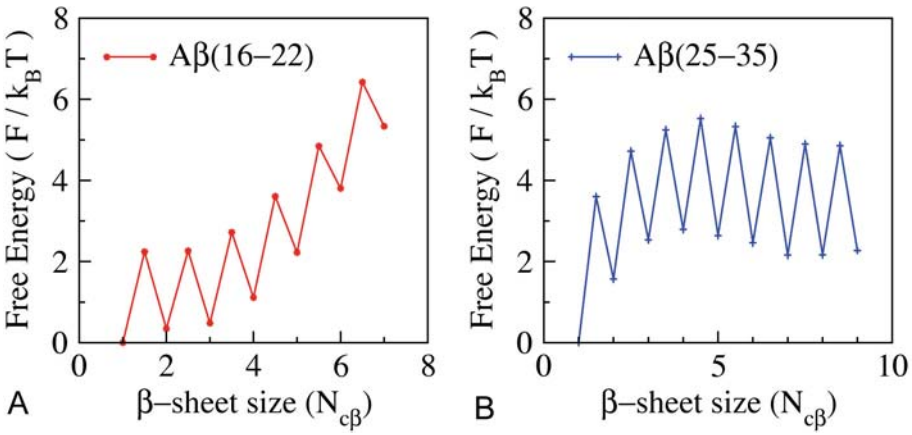

Figure 6. Free energy as a function of the number $N_{c\beta}$ of peptides in a β strand conformation. The free energy is estimated from the association (R_+) and dissociation (R_-) rates for (a) $A\beta_{16\text{-}22}$ and (b) $A\beta_{25\text{-}35}$. The free energy of $A\beta_{16\text{-}22}$ illustrates the uphill process in the formation of β-sheets by reorganisation. By contrast the free energy of $A\beta_{25\text{-}35}$ shows that a nucleation process without coalescence and with a critical nucleus of $N_{c\beta} = 4$ is involved in the formation of oligomers in this case.

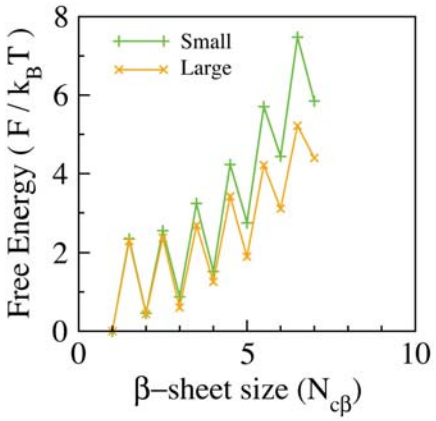

Figure 7. Comparison of the free energy of reorganisation in the case of the $A\beta_{16\text{-}22}$ peptide for small oligomers (less than 15 peptides, green) and for large oligomers (greater than 15 peptides, orange). The lower barriers for the large oligomers reflect their greater ease of reorganisation comparer with small oligomers.

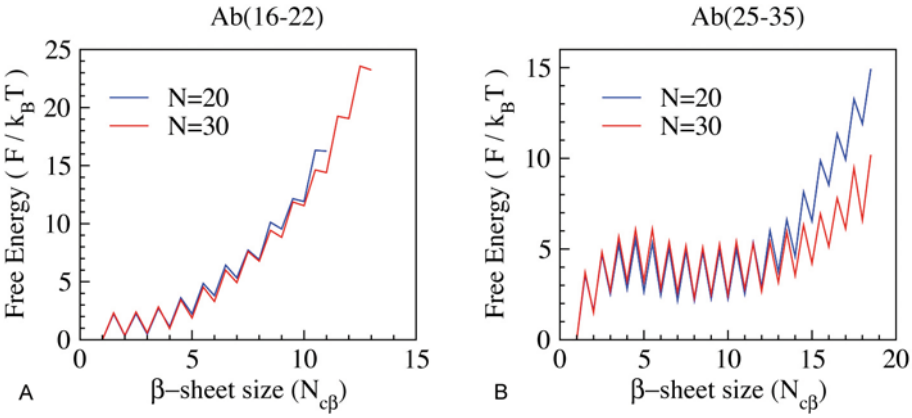

Figure 8. Comparison of the free energy barriers of systems of N=20 and 30 peptides, respectively. Finite size effects are significant for large β-sheets, but much less so in small β-sheets.

energy of a β-sheet of size n, and k_B is the Boltzmann constant. By neglecting a possible dependence on n in the pre-factor A, we obtain

$$F^T_{n,n+1} - F^\beta_{n,} = -k_B T \log(R_+(n)) \qquad (3)$$

$$F^T_{n,n+1} - F^\beta_{n+1,} = -k_B T \log(R_-(n+1)) \qquad (4)$$

We evaluate these free energy barriers from the last two hundred million Monte Carlo steps of each simulation. In order perform a consistency test on the free energy barriers estimated through these rates, we back-calculate the populations of β-sheets of each size as $P(N_{c\beta}) \approx e^{-F^\beta_n / k_B T}$ and compare them to the populations obtained from the simulations. In Figure 5 we compare the two sets of populations for the $A\beta_{16\text{-}22}$ and $A\beta_{25\text{-}35}$ peptides. The small differences between these distributions are likely to be caused by the assumption of considering that only one peptide at the time can associate or dissociate from an oligomer, and to the finite size effects that we are going to discuss below.

The mechanism of aggregation of the $A\beta_{16\text{-}22}$ peptide involves two distinct phases - coalescence and reorganisation (32). Under the conditions used in the present work, the coalescence phase is downhill in free energy and is characterised by the presence of large disordered oligomers already at the very early stages of the simulation. By contrast, the reorganisation phase in which β-sheet structures are formed within the oligomers is uphill in free energy. In our simulations, we observed only the free energy increase prior to the formation of a critical nucleus (Figure 6a). This coalescence phase is suppressed in the $A\beta_{25\text{-}35}$ peptide as a consequence of its low hydrophobicity. The difference in the mechanism of aggregation of the two peptide fragments is evident form the comparison of their respective populations of β-rich oligomers (32) (Figure 3). The free energy profile of the β-sheet formation for $A\beta_{25\text{-}35}$ shows a maximum for β-sheets composed of $N_{c\beta} = 4$ peptides. For large $N_{c\beta}$ values the free energy of reorganisation increases again due to finite size effects, which will be discussed later in more detail.

4. DEPENDENCE OF THE FREE ENERGY BARRIERS ON THE SIZE OF THE OLIGOMERS

In the $A\beta_{16\text{-}22}$ fragment the coalescence phase is separated from the reorganisation phase. Under the conditions used in the simulations that we present here, we are able to investigate whether the rate of β-sheet formation is dependent on the size of the disordered oligomer. Our results indicate that the free energy barriers for β-sheet formation are different for the small and the large oligomers. These barriers are shown in Figure 7 for oligomers formed by less than 15 peptides and more than 15 peptides, respectively, for a system containing 30 peptides. These results suggest that the barriers for reordering are lower in large oligomers than in small oligomers. These results provide a possible explanation of recent experimental findings (48) in which the efficiency of the seeding reaction was found to be dependent on the size of the seed.

These results can be rationalised by the following argument. The process of aggregation is governed by the two distinct rates of coalescence and reorganisation, which depend on the thermodynamic conditions of the system such as temperature and concentration and on the competition between hydrophobic, hydrogen bonding and electrostatic interactions. If the rate of coalescence is larger than the rate of reorganisation, as it is the case of the $A\beta_{16\text{-}22}$ peptides under the conditions studied here, the reaction proceeds with the formation of large disordered oligomers, as the rate of reorganisation is dependent on the presence of β-rich structural templates. The formation of these ordered nuclei on the surface of the oligomers increases the rate of reorganisation into β-sheet structures of the disordered peptides within the oligomers. This reaction continues until the two rates (coalescence and reorganisation) become equal after which the oligomesr grow only in an ordered fashion. Importantly, large oligomers exhibit larger numbers of ordered templates on their surface with respect to small oligomers, hence resulting in more efficient ordering mechanism for large oligomers. By contrast, in the oligomerization of the $A\beta_{25\text{-}35}$ peptide, for which the rate of collapse is very slow, the two rates appear to be similar at all times, and the oligomers grow directly as β-sheets.

5. FINITE SIZE EFFECTS ON THE FREE ENERGY

The simulations that we discuss in this work are carried out in systems containing 20 or 30 peptides. Since these numbers are comparable to the sizes of the oligomers that we are studying, finite size effects can be expected on the free energy of these systems. Such effects appear because the formation of oligomers leads to a depletion of the number of free monomers in the system and thus to a decrease in their effective concentration.

These effects have an influence on the estimates of the rates of aggregation that we presented. In order to estimate such influence, we compare the free energies of a system of 20 peptides with the free energy of a system of 30 peptides (Figure 8). In the case of the $A\beta_{25\text{-}35}$ peptide we found that the increase in free energy at large values of $N_{c\beta}$ is indeed a finite size effect that tends to be reduced by increasing the number of peptides in the simulations. Therefore we conclude that the increase in free energy for $N_{c\beta} > 10$ in Figure 8b does not indicate the presence of a further transition after the formation of ordered oligomers. By contrast the increase with $N_{c\beta}$ of the free energy for the $A\beta_{16\text{-}22}$ peptide shown in Figure 8a does not change significantly with the number of peptides in the simulations, as expected since such an increase correspond to the transition between disordered and ordered oligomers.

6. DISCUSSION

The mechanism of ordered aggregation of peptides and proteins has been recently described in terms of a two-step model in which a disordered collapse takes places prior to the growth of ordered filaments (19,32,33,37,39). As we have shown in the case of the $A\beta_{16-22}$ and $A\beta_{25-35}$ peptides, the lifetime of the disordered intermediate oligomeric species depends on the relative strengths of the hydrophobic and hydrogen bonding interactions (32). These peptides exhibit different behaviours since they have different contents of hydrophobic amino acids. In the present work, from the relative population of each oligomeric species, we have calculated the rates of growth R_+ and depletion R_- for each such species and from these rates we estimate the relative free energy between them.

In the case of $A\beta_{25-35}$, for which the growth of oligomers coincides with the growth of β-sheets, we found that the free energy as a function of the number of peptides in an oligomer grows for β-sheets containing up to four peptides, thus suggesting that the critical nucleus for ordered aggregation should be made up by four peptides, at least under the conditions that we investigated here. In the case of the $A\beta_{16-22}$ peptide, the coalescence of peptides into disordered oligomers appears to be downhill in free energy while their subsequent reorganisation into β-sheet structures is uphill. We do observe, in addition, a size dependence for the β-sheet growth. Large disordered oligomers, containing more ordered nuclei, experience a lower free energy barrier in order to increase their β-sheet size.

We have suggested an explanation for these results by considering the growth of large protofibrillar structures as driven by two distinct rates - of coalescence and of reorganisation. When the rate of coalescence is larger than the rate of reorganisation, the oligomers grow at first in a disordered fashion, and then the peptides reorganise slowly leading to the formation of a series of ordered nuclei that have in turn the effect of increasing the rate of reorganisation. Once the two rates become equal the oligomers grow in an orderly fashion resulting in cross-β filaments (Figure 9). If a disordered oligomer fails to reorganise and produce ordered nuclei it will continue to grow resulting in a large amorphous aggregate. When the amino acid sequence of the polypeptide chain and the conditions of the experiment are such that the rate of coalescence is much larger than the rate of reorganisation, the two rates will never become comparable and the polypeptide chains will coalesce into amorphous aggregates.

These considerations provide an explanation for the simultaneous presence of amyloid fibrils and amorphous aggregates in protein samples and also for the coexistence of amyloid fibrils and small oligomeric protofibrillar species (7). Furthermore, the formation of ordered structures stabilised by hydrogen bonding interactions results in the solvent exposure of the hydrophobic residues, a phenomenon that may be linked to toxic properties of these oligomers (32). These results provide further support to the hypothesis that the ability to form fibrillar and protofibrillar species is a generic property of polypeptide sequences, while the specificity of the amino acid sequence regulates the propensity of proteins and peptides to form ordered aggregates (49,50). Such propensity, as we have shown here, depends on the competition between hydrophobic and hydrogen bonding interactions and therefore on the particular amino acid sequence of the protein. In order to form protofibrillar and fibrillar species, protein sequences need to be composed by specific combinations of hydrophobic and charged residues that result in rates of reorganisation not much lower than the rate of coalescence of the system.

7. ACKNOWLEDGEMENTS

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Abbreviations: Protein misfolding, Protein aggregation, Amyloid fibrils, Oligomers, Alzheimer's disease.

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