J Comput Aided Mol Des (2008) 22:53–58
DOI 10.1007/s10822-007-9155-6

Molecular dynamics studies of α-helix stability in fibril-forming peptides

Erik Nordling · Yvonne Kallberg · Jan Johansson · Bengt Persson

Received: 21 January 2007 / Accepted: 17 November 2007 / Published online: 6 December 2007

Abstract Diseases associated with protein fibril-formation, such as the prion diseases and Alzheimer's disease, are gaining increased attention due to their medical importance and complex origins. Using molecular dynamics (MD) simulations in an aqueous environment, we have studied the stability of the α-helix covering positions 15–25 of the amyloid β-peptide (Aβ) involved in Alzheimer's disease. The effects of residue replacements, including the effects of Aβ disease related mutations, were also investigated. The MD simulations show a very early (2 ns) loss of α-helical structure for the Flemish (Aβ(A21G)), Italian (Aβ(E22K)), and Iowa (Aβ(D23N)) forms associated with hereditary Alzheimer's disease. Similarly, an early (5 ns) loss of α-helical structure was observed for the Dutch (Aβ(E22Q)) variant. MD here provides a possible explanation for the structural changes. Two variants of Aβ, Aβ(K16A,L17A,F20A) and Aβ(V18A,F19A,F20A), that do not produce fibrils in vitro were also investigated. The Aβ(V18A,F19A,F20A) initially loses its helical conformation but refolds into helix several times and spends most of the simulation time in helical conformation. However, the Aβ(K16A,L17A,F20A) loses the α-helical structure after 5 ns and does not refold. For the wildtype Aβ(1–40) and Aβ(1–42), the helical conformation is lost after 5 ns or after 40 ns, respectively, while for the "familial" (Aβ(A42T)) variant, the MD simulations suggest that a C-terminal β-strand is stabilised, which could explain the fibrillation. The simulations for the Arctic (Aβ(E22G)) variant indicate that the α-helix is kept for 2 ns, but reappears 2 ns later, whereafter it disappears after 10 ns. The MD results are in several cases compatible with known experimental data, but the correlation is not perfect, indicating that multimerisation tendency and other factors might also be important for fibril formation.

Keywords Amyloid β-peptide · Amyloid fibril · Molecular dynamics · Secondary structure

E. Nordling
Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm 171 77, Sweden

E. Nordling (✉)
Biovitrum AB, Stockholm 117 62, Sweden
e-mail: erik.nordling@biovitrum.com

Y. Kallberg · B. Persson
IFM Bioinformatics, Linköping University, Linkoping 581 83, Sweden

Y. Kallberg · B. Persson
Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm 171 77, Sweden

J. Johansson
Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, The Biomedical Centre, Uppsala 751 23, Sweden

Abbreviations

Aβ	Amyloid β-peptide
MD	Molecular dynamics

Introduction

Amyloid fibril-formation as a cause of disease has gained increased attention due to the prion diseases, bovine spongiform encephalopathy and the human counterpart Creutzfeld-Jakob's disease [1]. Another amyloidal disease is Alzheimer's disease, of importance in dementia among

an aging population. Presently, over 20 diseases, systemic or localised to various organs are associated with fibril formation [2]. These diseases have in common that endogenous proteins act as potential pathogenic agents. The fibril-forming proteins share no known patterns in sequence or structure, e.g. they range from all-helical to all-sheet proteins. However, they appear to have a common tendency to unfold and assume a conformation that can multimerise and form inter-molecular β-sheets that can grow into insoluble fibrils [3]. The neuronal toxicity in Alzheimeŕs disease is thought to originate from the extracellular neuronal plaques that are created by the fibril-forming amyloid β-peptide (Aβ) [4], but recent studies indicate that soluble Aβ oligomers, also known as ADDLs (Aβ-derived diffussible ligands) may also be toxic [5]. ADDLs appear spontaneously in human brain cells [6], and in transgenic mice Aβ accumulates intracellularly and cause neurodegeneration prior to amyloid plaque formation [7]. Recently, a 56 kDa oligomer of Aβ, dubbed Aβ * 56, was shown to associate with memory loss in transgenic mice [8]. Also oligomeric fibrillar structures, but not mature fibrils, formed from proteins that are not associated with disease are cytotoxic, suggesting that the toxicity is not protein-specific but mediated by the oligomeric fibrillar structure as such [9]. The toxicity of Aβ is coupled to the loss of the native structure [10]. Irrespective of the exact nature of the toxic form(s) of Aβ, they most likely require loss of the helical structure and formation of β-sheet structure.

Some fibril-forming proteins contain discordant α-helices, which are composed of a stretch of amino acid residues that statistically are predicted to be found in an extended conformation [11]. These proteins include the prion protein and Aβ. The occurrence of discordant helices in fibril-forming proteins indicates that destabilising factors are present in their amino acid sequences. Replacement of the β-preferring amino acid residues in some of the discordant helices has been shown to change the potential to form fibrils. In Aβ, replacing Val18 with Ala, or Lys16, Leu17 and Phe20 with Ala, or replacing Val18, Phe19 and Phe20 with Ala prevents fibril formation [12–14]. In this study, molecular dynamics (MD) simulations were used to study the influence of the amino acid sequences of the Aβ variants on the stabilities of their respective helices.

The Aβ mutations that are associated with hereditary cerebral haemorrhage with amyloidosis of the Dutch type (Aβ(E22Q); [15]) and the Italian type (Aβ(E22K); [16]), like Aβ(E22Q) have been shown to fibrillate fast in vitro while Aβ(E22K) is similar to wild-type Aβ in fibrillation velocity [16]. Hereditary cerebroarterial amyloidosis of the Iowa type (Aβ(D23N)) has an onset in the 6th or 7th decade of life [17] and the peptide displays fast fibrillation in vitro [18]. Hereditary cerebral amyloidosis of the Flemish (Aβ(A21G); [19]) and the Arctic (Aβ(E22G); [20]) types both have an onset in the 5th decade of life [19, 20]. Aβ(E22G) shows fast fibril formation in model systems [21], while Aβ(A21G) mainly increases the rate of secretion of Aβ-peptide [18]. Finally a mutation adjacent to the γ-secretase cleavage site associated with familial Alzheimer disease (Aβ(A42T); [22]) was included in the study. This mutation has not been reported to increase the fibrillation velocity but rather to alter the cleavage of the Aβ precursor and influence the ratio of Aβ1–40 to Aβ1–42 [23].

Two Aβ variants that do not fibrillate under the same conditions as wild-type Aβ, Aβ(K16A,L17A,F20A) [13] and Aβ(V18A,F19A,F20A) [14] were also included in the simulations.

Materials and methods

The conformation of Aβ was taken from the NMR structure (PDB-code 1ba6, [24]). The mutated forms were constructed from the Aβ(1–42) peptide by initially giving the replaced residues the same rotamer as the original counterpart. Unfavourable contacts introduced by the substitution were relieved by a 1,000 steps conjugate gradient minimisation of the side-chains. The replacement and the following minimisations were carried out using ICM (Molsoft LLC, San Diego, Calif.).

MD simulations were performed using the GROMACS molecular dynamics program, version 3.31 [25] with the OPLS-AA force field [26]. The simulations were executed with explicit water molecules, using the simple point charge model for the water molecules [27]. The temperature was kept constant during the simulations using a Berendsen thermostat [28] with a coupling time of 0.1 ps. The pressure was maintained by coupling to a reference pressure of 1 bar with a coupling time of 1 ps and an isothermal compressibility of 4.5×10^5 bar^{-1}. A twin range cut-off of 0.8 and 1.4 nm was used for the evaluation of the non-bonded interactions. Interactions within the larger cut-off were updated every 10 steps. The time step used was 2 fs. The simulations lasted for 100 ns and were carried out at 310 K.

To judge whether the simulations had reached a level of equilibrium, the potential energy and the root mean squared distance (RMSD) were plotted versus the simulation time. The RMSD is calculated based on all atoms in the peptide after it has been superimposed upon the original structure as implemented in the GROMACS package [25]. The individual simulations were then evaluated by calculating secondary structure content for the peptides for each 500:th ps of the entire simulation with the DSSP algorithm [29].

The statistical preference for each peptide to form α-helix or β-strand was calculated based upon the propensity values calculated from the fractional occurrences of residues in different secondary structures in 1,091 proteins [11]. For each residue, the value was calculated by averaging the propensities for all residues within a five-residue window centred on the residue in question.

Results

MD simulations of Aβ and analogues

The potential energy for the system is plotted for the entire calculation in Fig. 1a, worth noting is that Aβ(1–40) has a higher energy even though the total size of that system is smaller. The potential energy of all simulations is stable during the entire calculation. In Fig. 1b the RMSD versus the starting conformation is plotted against the simulation time. After 20 ns all simulations are within a span of 0.9–1.4 nm in RMSD from the original structure. The variant that diverts fastest from the original structure is Aβ(D23N) which has diverted to its maximum RMSD compared to the native structure during the initial 5–10 ns of the simulation.

In Fig. 2, the secondary structure states every 500:th ps for all peptides are plotted against the simulation time. The wild-type Aβ(1–40) (Fig. 2a) and Aβ(1–42) (Fig. 2b) differ by that the longer form maintains a helical region for about 40 ns of the simulation, while the shorter form loses the central helical region after approximately 5 ns. Aβ(1–40) also displays more β-sheet regions than Aβ(1–42).

For Aβ(A21G) (Fig. 2c) a disruption of the helical region is observed already during the first 5 ns. An unordered region is also clearly centered upon the mutation-site for most of the simulation. The initially α-helical region refolds into a 3_{10} helix, disrupted by the mutant glycine residue. A few ns long β-sheets are also visible.

Aβ(E22G) (Fig. 2d) shows an unordered region originating around the mutation-site. The first part of the helix is kept intact for the first 2 ns of the simulation, but reappears 2 ns later. However, after 10 ns there is no helical content. Aβ(E22Q) (Fig. 2e) maintains the original extent of the helical central region for approximately 5 ns. For Aβ(E22K) (Fig. 2f) the substitution seems to affect the local helical content rapidly, as the C-terminal part of the helix unwinds after 2 ns, but the N-terminal part of the helix is conserved and extended somewhat over time.

The Aβ(D23N) (Fig. 2g) assumes a more or less random conformation after 1 ns of the simulation.

For Aβ(A42T) (Fig. 2h) the central helical region is maintained for 11 ns and parts of it are restored during the simulation, but the substitution of the last amino acid residue appears the stabilise an intra-molecular β-sheet in the C-terminal part, which appear after 40 ns of the simulation and reoccurs multiple times during the simulation, lasting for periods of up to 7 ns.

The two designed non fibril forming variants, Aβ(K16A, L17A,F20A) (Fig. 2i) and Aβ(V18A,F19A,F20A) (Fig. 2j) behave in different manners. The first one loses its helical region after 5 ns and does not refold, while the latter fast loses its helical conformation but refolds into a helix several times and spends most of the simulation time including the last 30 ns in a helical conformation.

Secondary structure propensities

Secondary structure preferences, expressed as the quotient of α-helix propensity over β-strand propensity, for the Aβ variants studied herein are shown in Fig. 3. As expected, the analogues Aβ(K16A,L17A,F20A) and Aβ(V18A,F19A, F20A) both show substantially increased preferences for helical structure compared to wild-type Aβ. The disease related mutant forms of Aβ show a decreased helix/strand quotient compared to Aβ(1–42), in particular Aβ(A21G) and Aβ(E22G).

Discussion

In this study MD simulations were used to investigate the stability of the helical form of Aβ and analogues of it with altered potential to form fibrils. Aβ is a cleavage product of the transmembrane amyloid precursor protein (APP), and the region containing the helix now investigated (residues 15–25) is located just N-terminally to the predicted membrane-spanning region starting at residue 29 [30]. Aβ is structurally flexible in aqueous solution [31] but the central helix is stabilised in the presence of trifluoroethanol and detergent micelles [32]. The simulations of Aβ(1–40) and Aβ(1–42) hint that Aβ(1–42) has a more stable central helical region during the time of the simulation. This is surprising as Aβ(1–42) is more prone to form fibrils compared to Aβ(1–40). NMR experiments have previously reported that Aβ(1–40) and Aβ(1–42) have similar helical stability [31], which could indicate that the results from the MD simulations reflect that neither of the peptides is particularly stable. It was recently proposed that a major difference between Aβ(1–40) and Aβ(1–42) is that Aβ(1–42) forms trimers and tetramers more favourably than Aβ(1–40) [33]. A more favourable oligomerisation of Aβ1–42 may drive fibril formation.

The non-fibrillating variants Aβ(K16A,L17A,F20A) and Aβ(V18A,F19A,F20A) appear to resist fibrillation via different mechanisms. Aβ(K16A,L17A,F20A)

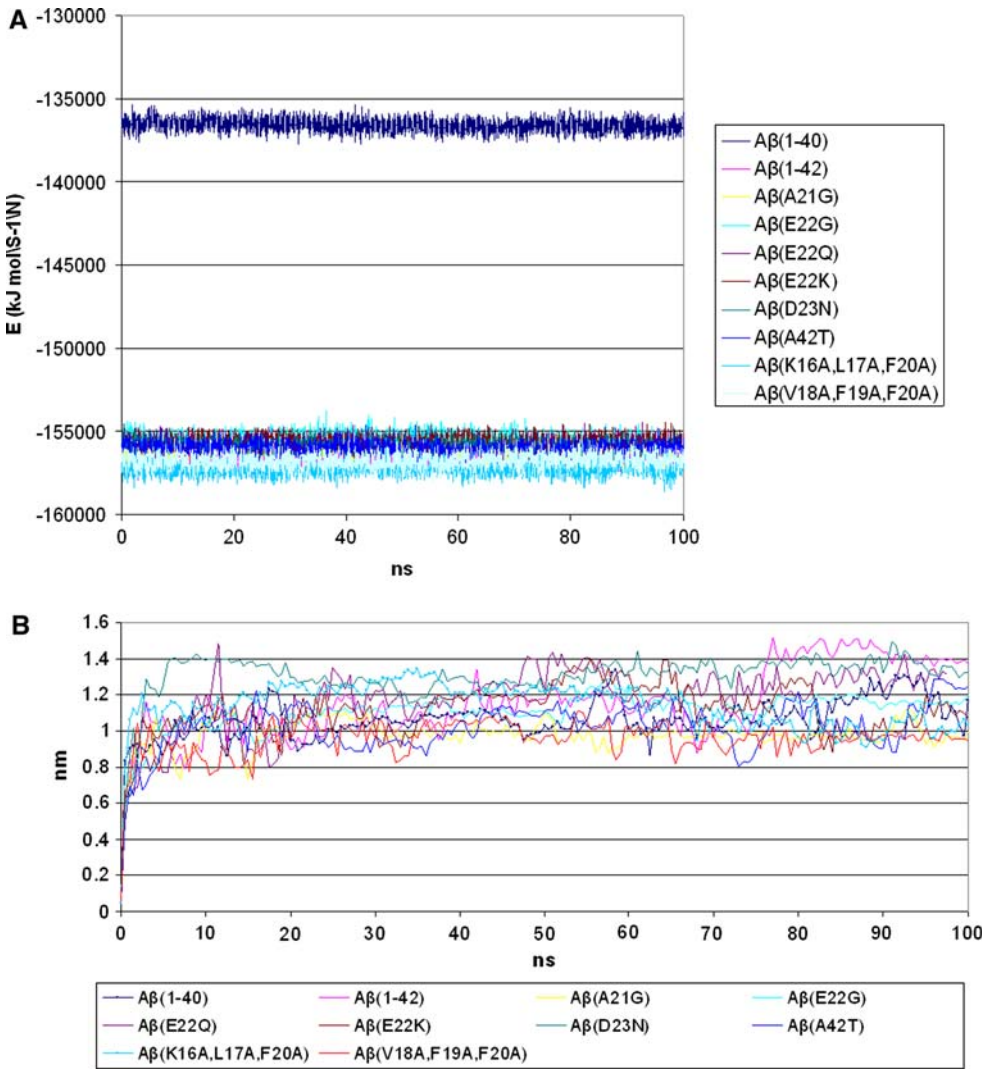

Fig. 1 (**a**) Potential energy of the system during the 100 ns simulation. (**b**) RMSD in nm of all atoms during the 100 ns simulation compared to the original conformation

remains in a helical conformation for 5 ns after which it adopts a more random conformation centrally. Its resistance to fibril formation can be linked to a reduced ability to form intermolecular hydrogen bonds, as was the rationale behind its design [13]. Replacing large amino acid residues like Lys, Leu and Phe with the conformationally less strained Ala could add flexibility to the helix, which may result in a less stable helix in the simulations. This could also make the coil to β-sheet transformation in the fibrillation process more unfavourable, as more entropy is lost. In Aβ(V18A,F19A,F20A), the replacement of the β-sheet preferring residues with the α-helical preferring alanine appears to allow the peptide to re-enter an α-helical conformation during the simulation.

The apparent stability of Aβ(E22Q) in the simulations is unexpected since it has been reported to fibrillate fast in vitro [16]. One possibility is that removal of a negative charge close to Asp23, which has been shown to form a salt-bridge in Aβ fibrils, may ease the correct matching of the salt-bridge that stabilises the bend in the fibrils [34], which in turn could speed up the fibrillation process. Another possibility is that the mutation leads to a lower solubility of the peptide compared to wild-type Aβ, which also should increase the velocity of the fibrillation process [35]. Aβ(E22G) (Fig. 2d) and Aβ(D23N) (Fig. 2g) both show high fibrillation rates and aggressive fibrillation [18, 20, 21], which seems to match their low helical stability in the simulations.

Aβ(E22K) forms fibrils with similar kinetics as wild-type Aβ [16] and the simulation show a more stable helical region compared to the wild-type (Fig. 2f). The simulation of Aβ(A42T) shows that the mutation appears to stabilise the formation of a β-sheet in the C-terminal region, but it also shows a recurring helical segment in the central region (Fig. 2h), which may act as a counterweight to fibrillation for this peptide. Previously it has been suggested that Aβ(A42T) primarily induce amyloidosis by influencing the

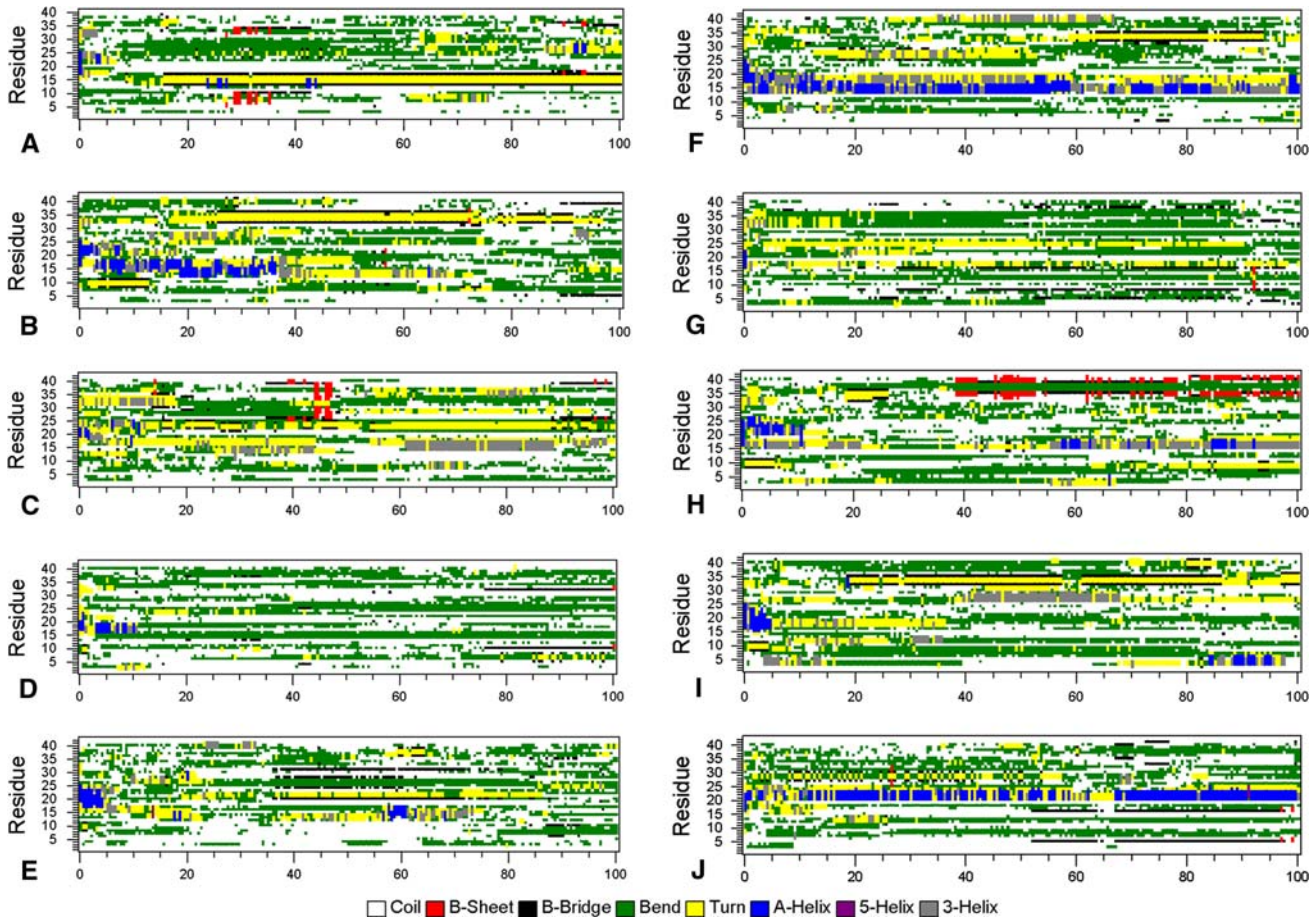

Fig. 2 A visualisation of the secondary structure of the simulations during the 100 ns simulations for (**a**) Aβ(1–40), (**b**) Aβ(1–42), (**c**) Aβ(A21G), (**d**) Aβ(E22G), (**e**) Aβ(E22Q), (**f**) Aβ(E22K), (**g**) Aβ(D23N), (**h**) AB(A42T), (**i**) Aβ(K16A,L17A,F20A) and (**j**) Aβ(V18A,F19A, F20A)

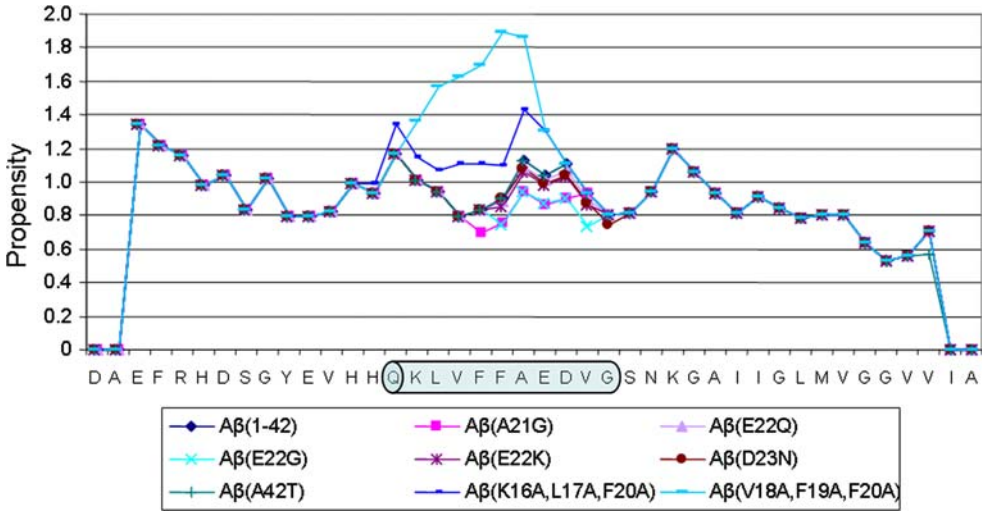

Fig. 3 Quotient of α-helix propensity and β-strand propensity for Aβ and analogues thereof. The helix in the PDB-structure of Aβ is marked with a cylinder. Aβ1–40 is not shown as the propensity is equal to Aβ1–42 for the entire length of the sequence

cleavage of APP and the ratio between Aβ(1–40) and Aβ(1–42) [23]. The Aβ(A21G) mutant is one of the most unstable peptides during the simulations, with a break-up of the central helical region after just 1 ns and the appearance of a C-terminal β-sheet that lasts 2 ns at a time. However, the Aβ(A21G) mutation has been reported not to increase the fibrillation rate [18, 36] but instead increase the rate of release of Aβ [18]. Van Nostrand et al. [18] did

note a pronounced neurotoxicity of Aβ(A21G), which suggests that the observed low helical stability of Aβ(A21G) could promote formation of toxic oligomers.

Whether helical instability is important for Aβ fibril formation in vivo needs to be investigated further. Additional effects of the mutations, like increased potential to form β-sheets and reduced solubility, are not addressed by the current simulations. Also, possible interactions between multiple Aβ molecules have not been considered in this study, due to computational time restraints. In spite of these caveats, the MD results suggest that helix destabilisation should be considered as one potential mechanism associated with mutations in the central part of Aβ.

Overall, the MD simulations are compatible with the idea that stabilisation of helices in peptides/proteins involved in amyloid diseases affect the potential to form fibrils. Stabilising a helical monomeric conformation may be more attractive than to inhibit fibril growth by preventing β-sheet formation [37, 38], since the latter may result in an increased amount of potentially toxic Aβ oligomers.

Acknowledgements Financial support from the Swedish Research Council, the Swedish Foundation for Strategic Research, Linköping University and Karolinska Institutet Funds is gratefully acknowledged.

References

1. Jackson GS, Collinge J (2001) Mol Pathol 54:393
2. Westermark P (2005) FEBS J 272:5942
3. Nelson R, Eisenberg D (2006) Curr Opin Struct Biol 16:260
4. Lorenzo A, Yankner BA (1996) Ann NY Acad Sci 777:89
5. Lambert MP, Barlow AK, Chromy BA, Edwards C, Freed R, Liosatos M, Morgan TE, Rozovsky I, Trommer B, Viola KL, Wals P, Zhang C, Finch CE, Krafft GA, Klein WL (1998) Proc Natl Acad Sci USA 95:6448
6. Walsh DM, Tseng BP, Rydel RE, Podlisny MB, Selkoe DJ (2000) Biochemistry 39:10831
7. Chui DH, Tanahashi H, Ozawa K, Ikeda S, Checler F, Ueda O, Suzuki H, Araki W, Inoue H, Shirotani K, Takahashi K, Gallyas F, Tabira T (1999) Nat Med 5:560
8. Lesne S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) Nature 440:352
9. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M (2002) Nature 416:507
10. Monji A, Utsumi H, Ueda T, Imoto T, Yoshida I, Hashioka S, Tashiro K, Tashiro N (2001) J Neurochem 77:1425
11. Kallberg Y, Gustafsson M, Persson B, Thyberg J, Johansson J (2001) J Biol Chem 276:12945
12. Soto C, Castano EM, Frangione B, Inestrosa NC (1995) J Biol Chem 270:3063
13. Tjernberg LO, Naslund J, Lindqvist F, Johansson J, Karlstrom AR, Thyberg J, Terenius L, Nordstedt C (1996) J Biol Chem 271:8545
14. Paivio A, Nordling E, Kallberg Y, Thyberg J, Johansson J (2004) Protein Sci 13:1251
15. Levy E, Carman MD, Fernandez-Madrid IJ, Power MD, Lieberburg I, van Duinen SG, Bots GTAM, Luyendijk W, Frangione B (1990) Science 248:1124
16. Miravalle L, Tokuda T, Chiarle R, Giaccone G, Bugiani O, Tagliavini F, Frangione B, Ghiso J (2000) J Biol Chem 275:27110
17. Grabowski TJ, Cho HS, Vonsattel JPG, Rebeck GW, Greenberg SM (2001) Ann Neurol 49:697
18. van Nostrand WE, Melchor JP, Cho HS, Greenberg SM, Rebeck GW (2001) J Biol Chem 276:32860
19. Hendriks L, van Duijn CM, Cras P, Cruts M, Van Hul W, van Harskamp F, Warren A, McInnis MG, Antonarakis SE, Martin J-J, Hofman A, Van Broeckhoven C (1992) Nat Genet 1:218
20. Kamino K, Orr HT, Payami H, Wijsman EM, Alonso ME, Pulst SM, Anderson L, O'dahl S, Nemens E, White JA, Sadovnick AD, Ball MJ, Kaye J, Warren A, McInnis M, Antonarakis SE, Korenberg JR, Sharma V, Kukull W, Larson E, Heston LL, Martin GM, Bird TD, Schellenberg GD (1992) Am J Hum Genet 51:998
21. Cheng IH, Palop JJ, Esposito LA, Bien-Ly N, Yan F, Mucke L (2004) Nat Med 10:1190
22. Carter DA, Desmarais E, Bellis M, Campion D, Clerget-Darpoux F, Brice A, Agid Y, Jaillard-Serradt A, Mallet J (1992) Nat Genet 2:255
23. Rossi G, Giaccone G, Maletta R, Morbin M, Capobianco R, Mangieri M, Giovagnoli AR, Bizzi A, Tomaino C, Perri M, Di Natale M, Tagliavini F, Bugiani O, Bruni AC (2004) Neurology 63:910
24. Watson AA, Fairlie DP, Craik DJ (1998) Biochemistry 37:12700
25. Lindahl E, Hess B, van der Spoel D (2001) J Mol Model 7:306
26. Kaminski GA, Friesner RA, Tirado-Rives J, Jorgensen WL (2001) J Chem Phys B 105:6474
27. Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J (1981) In: Pullman B (ed) Intermolecular forces. Reidel, Dordrecht, pp 331–342
28. Berendsen HJC, Postma JPM, van Gunsteren WF, DiNola A, Haak JR (1984) J Chem Phys 81:3684
29. Kabsch W, Sander C (1983) Biopolymers 22:2577
30. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A (2003) Nucleic Acids Res 31:3784
31. Riek R, Guntert P, Dobeli H, Wipf B, Wuthrich K (2001) Eur J Biochem 268:5930
32. Serpell LC (2000) Biochim Biophys Acta 1502:16
33. Chen YR, Glabe CG (2006) J Biol Chem 281:24414
34. Lührs T, Ritter C, Adrian M, Riek-Loher D, Bohrmann B, Dobeli H, Schubert D, Riek R (2005) Proc Natl Acad Sci USA 102:17342
35. Chiti F, Stefani M, Taddei N, Ramponi G, Dobson CM (2003) Nature 424:805
36. Murakami K, Irie K, Morimoto A, Ohigashi H, Shindo M, Nagao M, Shimizu T, Shirasawa T (2003) J Biol Chem 278:46179
37. Gordon DJ, Sciarretta KL, Meredith SC (2001) Biochemistry 40:8237
38. Watanabe K, Nakamura K, Akikusa S, Okada T, Kodaka M, Konakahara T, Okuno H (2002) Biochem Biophys Res Commun 290:121