Journal of Peptide Science
J. Pept. Sci. 2006; **12**: 780–789
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.813

JOURNAL OF PEPTIDE SCIENCE

Molecular dynamics study of amyloid formation of two Abl-SH3 domain peptides

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Received 31 July 2006; Revised 27 September 2006; Accepted 28 September 2006

Abstract: Molecular dynamics (MD) simulations were carried out for two-strand and ten-strand β-sheets constructed from two peptides corresponding to the diverging turn of two homologous Abl-SH3 domains, DLSFMKGE (MK; from Drosophila) and DLSFKKGE (KK; from man), in explicit water at the temperatures of 30, 170/190 and 300 K. It was found that the 2 × MK β-sheet is more stable than the 2 × KK β-sheet, and that the 10 × MK β-sheet is more stable than the 10 × KK β-sheet; this suggests that the MK systems are fibril-creating and the KK systems are not. These results might explain why most SH3 domains possess two conserved basic residues at the diverging turn, which may act as gatekeepers in order to avoid aggregation. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: amyloid; amyloid peptides; amyloid formation; molecular dynamics; β-sheet

INTRODUCTION

Amyloidosis is deposition of soluble proteins as insoluble fibrils or other supramolecular structures in living organisms. The mechanism of amyloid formation is still unclear and it has become one of the leading subjects of biomolecular research.

Amyloid formation and deposition is connected with conformation-related diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, Finnish familial amyloidosis, type II diabetes, and the prion-related diseases. No sequence or structural similarities are apparent among any of the proteins that display the ability to form amyloids. In spite of this diversity, all amyloid fibrils display similar features regardless of their source: (i) they are long, straight and unbranched fibrils; (ii) they bind to dyes, such as Congo Red and Thioflavin-T (Th-T), and (iii) X-ray fibril diffraction studies have indicated that they all exhibit a cross-β-structure. In the last few years, globular proteins unrelated to any known human disease have been found to be converted into amyloid fibrils *in vitro*. These nonpathogenic proteins have become paradigmatic models to study protein aggregation. One of the most extensively studied amyloidogenic proteins not related to any classical amyloid disease is the SH3 domain from PI3 kinase [1,2].

In globular proteins, amyloidogenesis starts necessarily with a total or partial unfolding [3–6]. After protein misfolding, short-peptide sequences may act as the 'hot spots' that provide the driving force for protein aggregation in amyloid fibrils [7–11]. These regions are usually in the inner hydrophobic core in native proteins, but in unfolded proteins they become exposed to solvent and ready to establish intermolecular contacts [7–11]. Previously, we identified and characterized in detail one of these 'hot spots' in the diverging turn of the PI3-SH3 domain. The diverging turn is a member of a particular subset of type IIβ turns and forms part of the folding nucleus of SH3 domains. The diverging turn of PI3-SH3 shows a number of unusual features compared to the rest of the proteins in the family. First, while most SH3 proteins have two consecutive basic residues at the diverging turn (usually two Lys), in PI3-SH3 one of these positions is occupied by Leu, which appears exposed to solvent in the native structure of the domain. This finding is extremely unusual, given that only 10% of the SH3 sequences shows a hydrophobic residue at this position. The presence of this unusual residue converts this sequence into an aggregation of 'hot spot'. In contrast, Spectrin SH3, a protein domain with a canonical diverging turn, does not form amyloid fibrils under any of the conditions we have so far explored. We have shown that these sequential differences account, at least partially, for the different amyloidogenicity of these two domains.

On the basis of a homology search, we have identified an aggregation-prone region in the same structural element of the related Abl-SH3 domain of Drosophila, with the sequence **DLSFMKGE** (hereafter referred to as [**MK**]), whereas the human homologous region with the canonical sequence **DLSFKKGE** (hereafter referred to as [**KK**]) is predicted to be less amyloidogenic, as is the case with the Spectrin SH3 domain. Preliminary

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experimental studies indicate that this assumption is correct (Ventura, unpublished results).

Molecular dynamics (MD) simulations has been widely used for the investigation of aggregation at the molecular level [12–20].

The present study is aimed at examining, by means of MD simulations, the molecular origin of the difference in the stability of the fibrils formed from the MK peptide and those formed from the KK peptide.

METHODS

In order to search for two SH3 domain diverging turn regions that exhibit similarities either to the same region in the PI3-SH3 domain or to the one in Spectrin-SH3, while keeping the number of differences between them to a minimum, the SMART (Simple Modular Architecture Research Tool, http://smart.embl-heidelberg.de) SH3 alignment was analyzed. The following two sequences were identified: DLSFKKGE from human Abl-SH3 with the canonical two basic consecutive residues and DLSFMKGE from the homologous protein domain from Drosophila, with a hydrophobic residue (Met) in position 5, analogous to the Ile of the amyloid forming PI3-SH3 domain. Although differing in only one residue, these two short amino acid stretches are predicted to have different aggregational properties.

For modeling of amyloid fibrils the antiparallel β-sheet was chosen because it is more stable than the parallel β-sheet. The antiparallel alanine flat β-sheets consisting of two and ten strands, respectively, were constructed, minimized, and mutated to the sequences DLSFMKGE and DLSFKKGE with the program SYBYL. We created the following four systems: (i) two strands of DLSFMKGE (**2 × MK**) (Figure 1(a)–(d)), (ii) two strands of DLSFKKGE (**2 × KK**) (Figure 1(e)–(h)), (iii) ten strands of DLSFMKGE (**10 × MK**) (Figure 2(a)–(d)), and (iv) ten strands of DLSFKKGE (**10 × KK**) (Figure 2(e)–(h)). Each of these β-sheet systems was surrounded by a 10 Å layer of water molecules over the solute and subjected to MD simulations with the Amber 8.0 force field in the NPT (constant number of molecules, pressure, and temperature) scheme. The MD runs were started at a temperature of 10 K and the temperature was increased stepwise by 10 degrees until 300 K. Longer MD runs were carried out at 30 K (78 ns), 170 K (140 ns), and 300 K for the two-strand systems, and at 30 K (47 ns), 190 K (55 ns), and 300 K for the ten-strand systems.

H-bond analysis was applied for the total number of H-bonds of the system, including the side chain H-bonds and the backbone H-bonds. H-bond analysis included all hydrogen bonds involving side chains, including those from the salt bridges. As the MD simulations were started from the ideal β-sheets, the number of backbone H-bonds was used as the measure of the β-sheet structure of the system.

RESULTS AND DISCUSSION

Two-strand β-sheets of DLSFMKGE and DLSFKKGE Peptides

A few of the snapshots from the MD simulation of the two-strand systems are shown in Figure 1. These

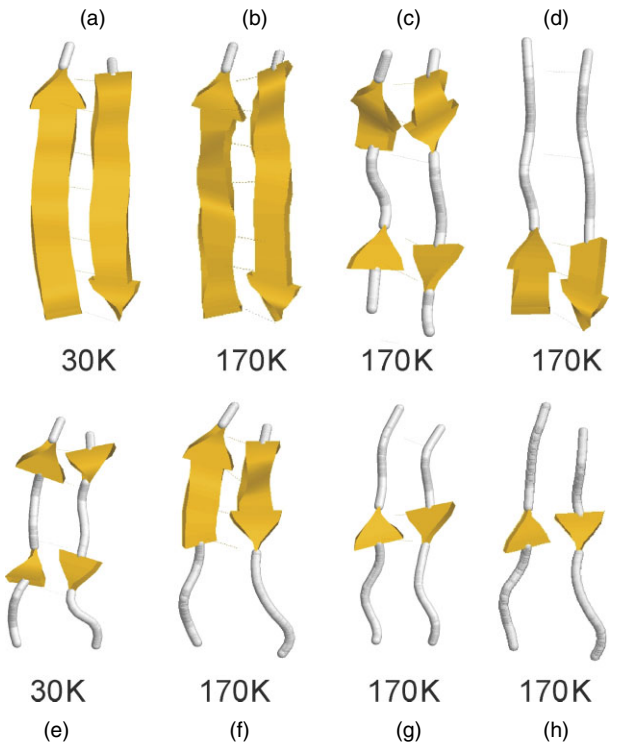

Figure 1 MD snapshots of **DLSFMKGE** two-strand system (a) at 30 K, 23 159 ps; (b) at 170 K, 42 359 ps; (c) at 170 K, 91 467 ps; (d) at 170 K, 99 126 ps. MD snapshots of **DLSFKKGE** two-strand system (e) at 30 K, 19 941 ps; (f) at 170 K, 42 880 ps; (g) at 170 K, 92 658 ps; (h) at 170 K, 98 969 ps.

structures are the representatives of the time evolution of the beta sheet oligomer.

Molecular dynamics simulations of the two-strand systems of the 'hot spot' peptides DLSFMKGE (**2 × MK**) and DLSFKKGE (**2 × KK**) have shown that at a temperature of 30 K the 2 × MK system forms a more regular β-sheet than does the 2 × KK system (Figure 1). At 170 K, the β-sheet formed by the 2 × MK peptide is not regular, but it remains stable for a longer time compared to the one formed from the 2 × KK peptide (Figure 1). At 170 K the distance between the mass centers of the strands of the 2 × MK system slowly increases, while the distance between the mass centers of the strands of the 2 × MK system at 30 K remains rather constant (Figure 3). Augmentation of the distance between the mass centers of strands of the 2 × MK system could be explained by the lack of stabilization from the neighboring strands; both strands of the system are the edge strands. At 170 K, the Cα distances between strands for the 2 × KK system are more scattered than those for the 2 × MK system (Figure 4), denoting that the 2 × MK β-sheet is more stable than the 2 × KK β-sheet.

The total number of hydrogen bonds for the 2 × MK system is similar to the one for the 2 × KK system (Figure 5), but the number of backbone hydrogen bonds for the 2 × MK system is bigger than that for the 2 × KK

DOI: 10.1002/psc

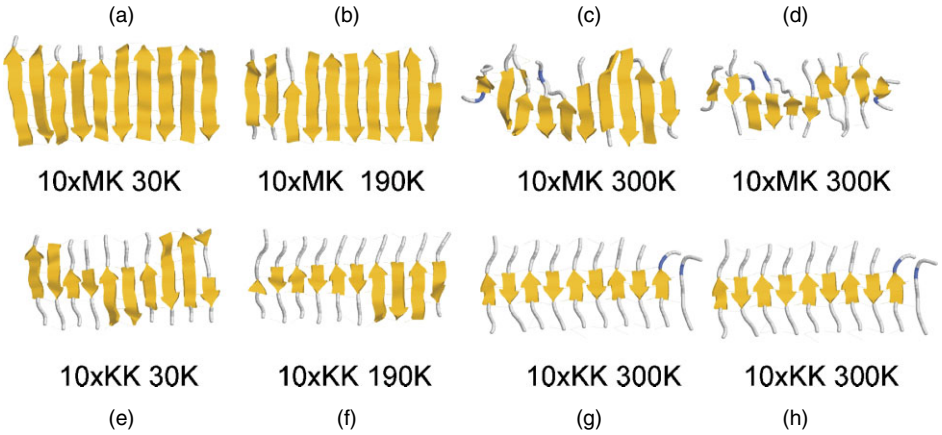

Figure 2 MD snapshots of **DLSFMKGE** ten-strand system (a) at 30 K, 15 343 ps; (b) at 190 K, 58 023 ps; (c) at 300 K, 58 489 ps; (d) at 300 K, 64 419 ps. MD snapshots of **DLSFKKGE** ten-strand system (e) at 30 K, 15 950 ps; (f) at 190 K, 58 588 ps; (g) at 300 K, 58 466 ps; (h) at 300 K, 64 038 ps.

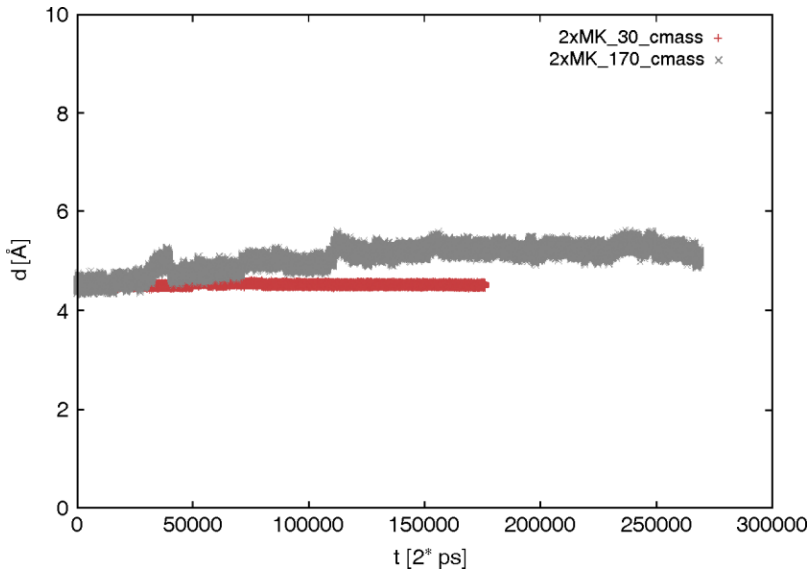

Figure 3 The distance between the centers of mass of neighboring strands for 2 × DLSFMKGE system at 30 K (black) and 170 K (gray).

system. Because the number of backbone hydrogen bonds could be regarded as the measure of the strength or regularity of a β-sheet, we can conclude that the 2 × MK β-sheet is stronger than the one formed from the 2 × KK system. The number of side chain hydrogen bonds for the 2 × KK system is greater than that for the 2 × MK system. At 300 K, both the two-strand systems lose their β-sheet structure, suggesting that the lack of stabilization effect from the nearby strands could be responsible for dissolving the systems.

Ten-strand β-sheets of DLSFMKGE and DLSFKKGE Peptides

A few snapshots from the MD simulation of the ten-strand systems are shown in Figure 2. These structures are the representatives of the time evolution of the beta sheet oligomer.

Molecular dynamics simulations of the ten-strand systems of the 'hot spot' peptides DLSFMKGE (**10** × **MK**) and DLSFKKGE (**10** × **KK**) have shown that at the temperature of 30 K the 10 × MK peptide forms a more regular β-sheet than the 10 × KK peptide (Figure 2). Also, at 190 K the 10 × MK peptide forms a more perfect β-sheet than the 10 × KK peptide. Two-strand β-sheet systems were stable until 170 K (Figure 1). The ten-strand β-sheets appear to be clearly more stable (Figure 2). In comparison with the two-strand systems, in the case of ten-strand β-sheets we can see the importance of the side-to-side interactions of the neighboring strands in the β-sheet. At 300 K, both systems are seen to dissociate, suggesting that a single sheet β-sheet is not stable and other face-to-face

DOI: 10.1002/psc

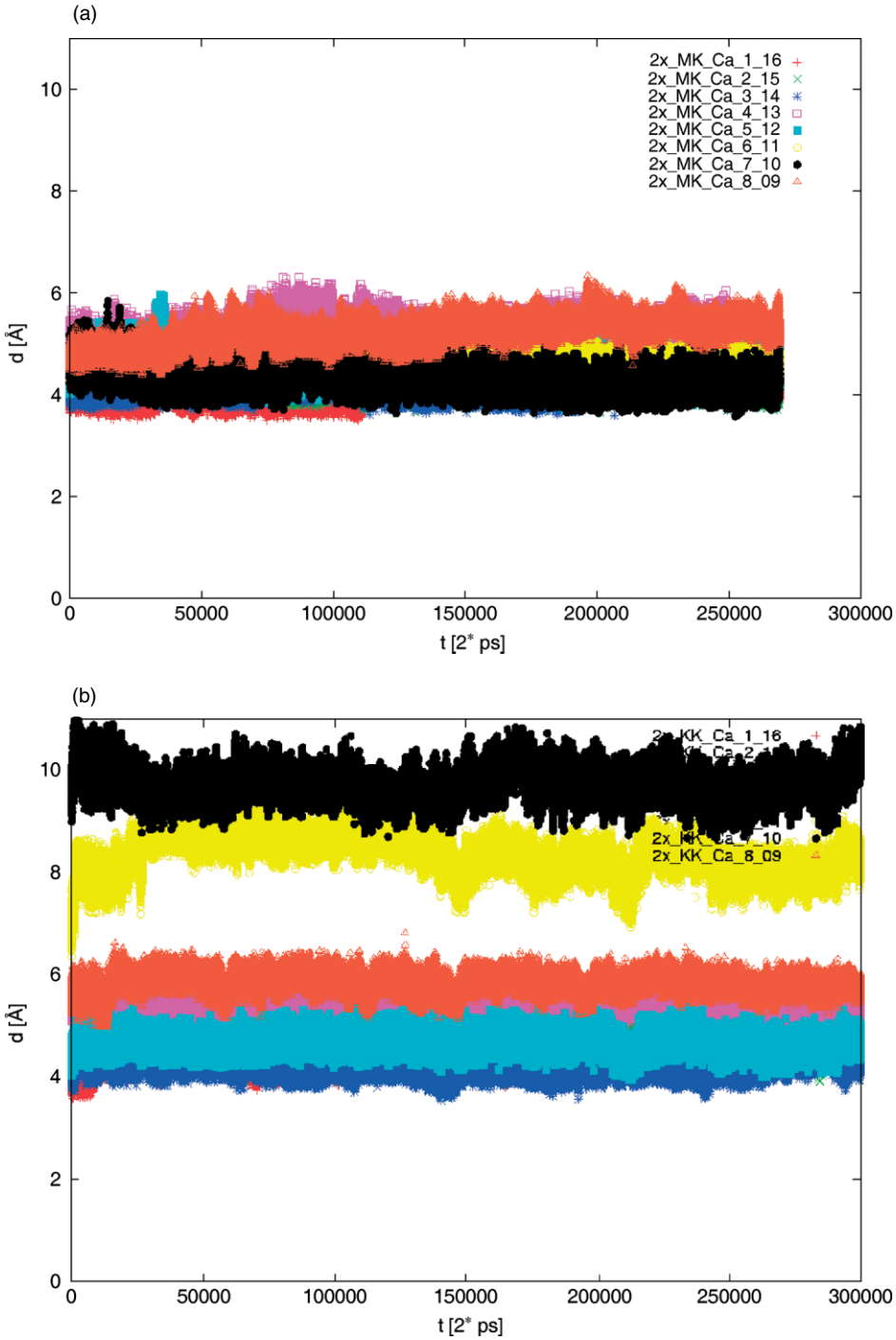

Figure 4 Cα distances between strands of 2 × DLSFMKGE (a) and 2 × DLSFKKGE (b) systems at 170 K.

β-sheets are needed for the stabilization of the systems.

The total number of hydrogen bonds for the 10 × MK system decreases with time (Figure 6), but the total number of hydrogen bonds for the 10 × KK system slightly increases with time because of the increase in the side chain hydrogen bonds of the system. The number of backbone hydrogen bonds for the 10 × MK system is clearly greater than that for the 10 × KK system, suggesting that the 10 × MK system forms a stronger and more regular β-sheet than does the 10 × KK system. The results (Figure 6) show considerably higher backbone hydrogen bond percentage for DLSFMKGE than for DLSFKKGE during the course of the simulation, thus suggesting that DLSFMKGE is a potential fibril maker whereas DLSFKKGE is not.

At 190 K, the distances between the mass centers of the neighboring strands of the 10 × KK system are more scattered than those of the 10 × MK system (Figure 7).

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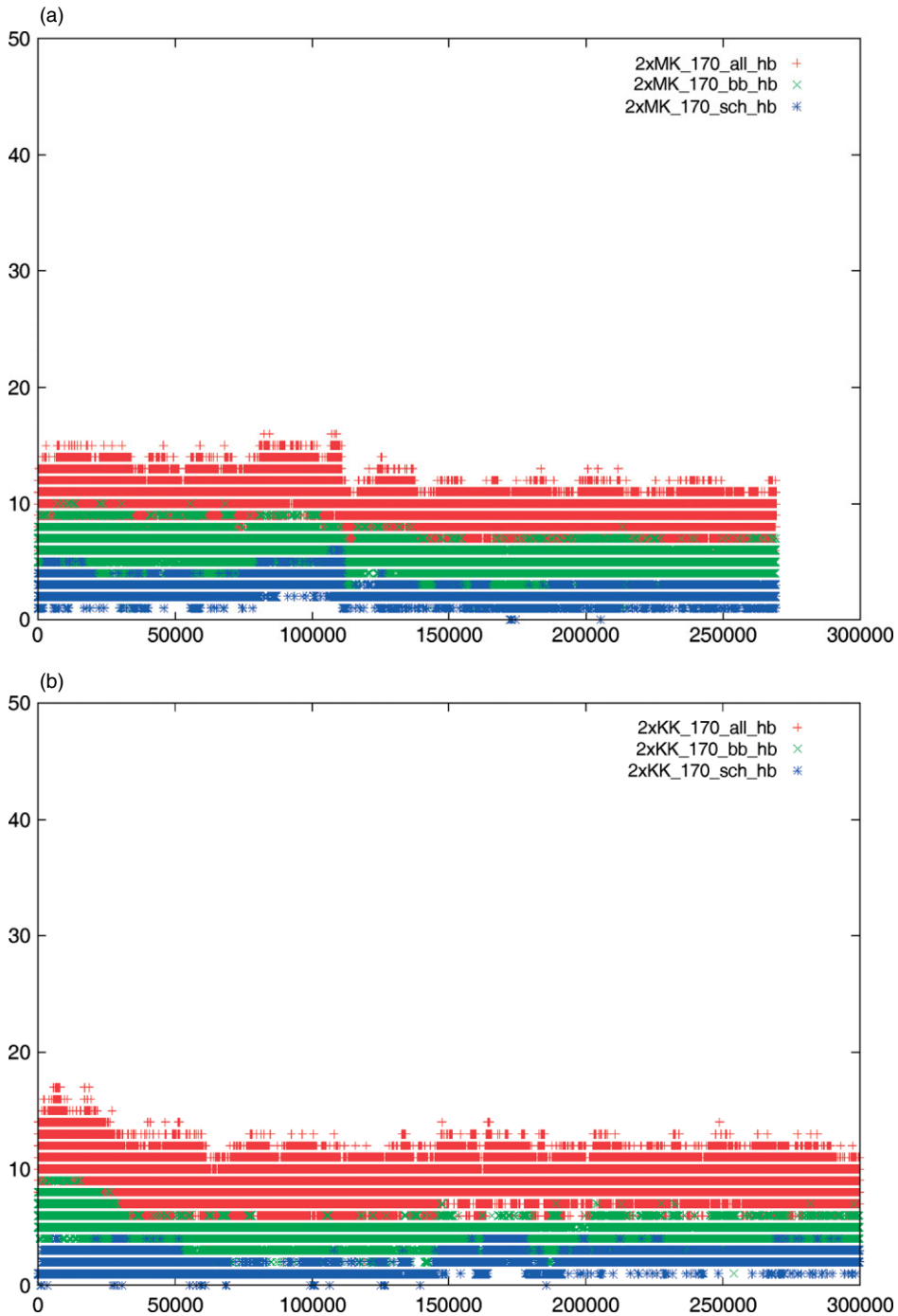

Figure 5 The number of hydrogen bonds of 2 × DLSFMKGE system at 170 K (a) and 2 × DLSFKKGE system at 170 K (b): all-red, backbone-green, side chain-blue, H-bond (2.5Å, 120°).

The Cα distances between neighboring strands of the 10 × MK system at 190 K show the stabilization effect of the neighboring strands; the Cα distances are more scattered between the 1 and 2, 4 and 5, 7 and 8, and 9 and 10 strand pairs than those between the 2 and 3, 5 and 6, 6 and 7, 3 and 4, and 8 and 9 strand pairs (Figure 8). Also the Cα distances between strands of the 10 × KK system at 190 K show the stabilization effect of the neighboring strands: the Cα distances are more scattered between the 1 and 2, 6 and 7, and 9 and 10 strand pairs than the ones between the 2 and 3, 3 and 4, 4 and 5, 5 and 6, 7 and 8, and 8 and 9 strand pairs (Figure 9). The fact that, in the course of the simulation, a β-sheet partly or completely melts at higher temperatures suggests the need for a complex with several face-to-face oriented β-sheets in order to provide stability to the amyloid fibril.

At 190 K, the lipophylic surface of 10 × MK β-sheet has a hydrophobic patch. In contrast, the

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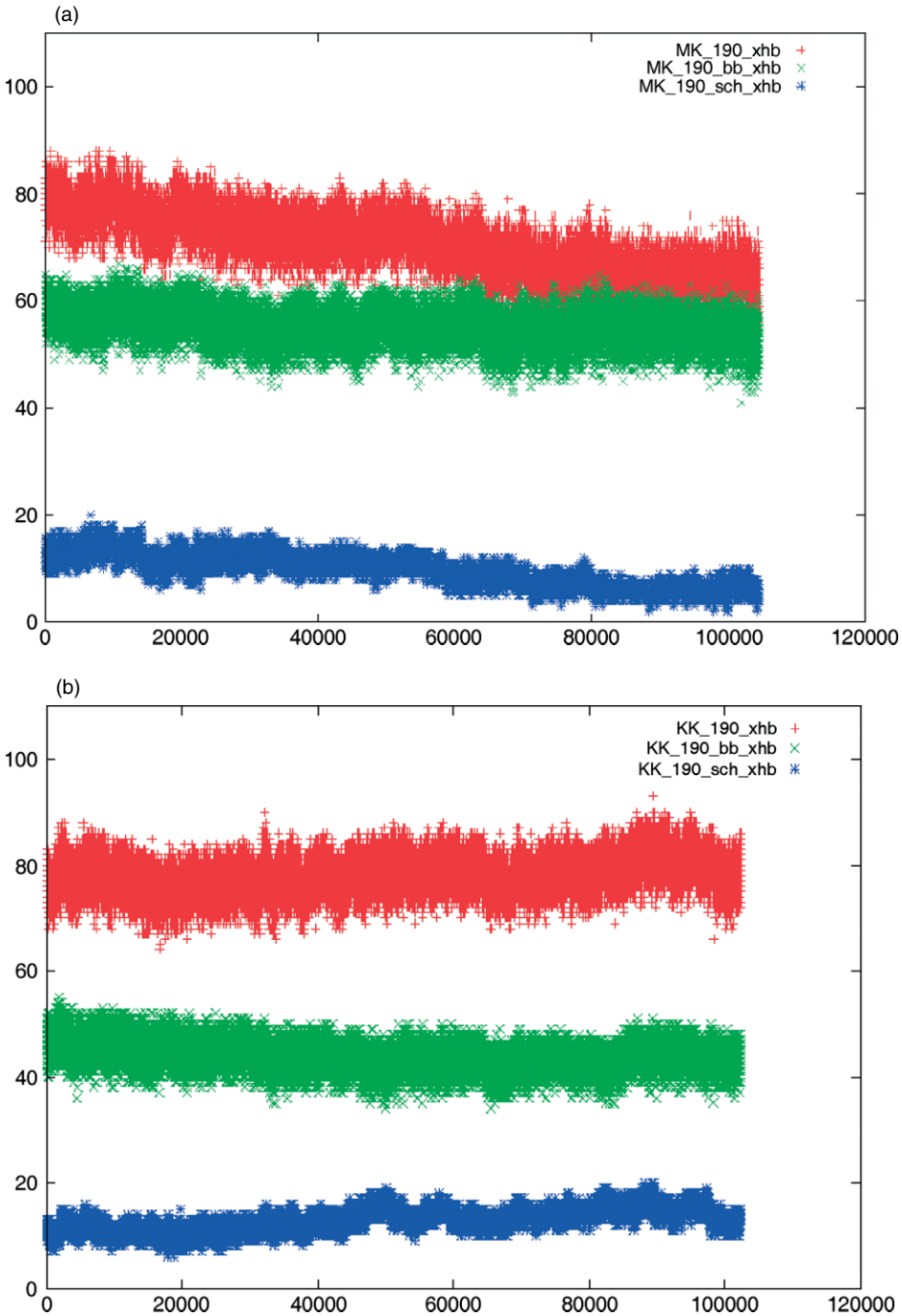

Figure 6 Comparison of the number of hydrogen bonds of 10 × DLSFMKGE (a) and 10 × DLSFKKGE (b) systems at 190 K: all-red, backbone-green, side chain-blue hydrogen bonds.

10 × KK β-sheet has a hydrophobic region with fewer hydrophobic islands (Figure 10). It is possible that the hydrophobic patch of 10 × MK β-sheet is responsible for the better amyloid formation of MK peptide in comparison with KK peptide. It is worth mentioning that preliminary results obtained with octapeptides by expanding the two respective regions support this conclusion (Ventura, unpublished results).

CONCLUSIONS

The results reported in this paper allow us to conclude that the 2 × MK β-sheet is more stable than the 2 × KK β-sheet, and that the 10 × MK β-sheet is more stable than the 10 × KK β-sheet; this suggests that the MK peptide is more prone to fibril formation than the KK peptide. The increased stability of the β-sheet formed from the 10 × MK system arises because of the

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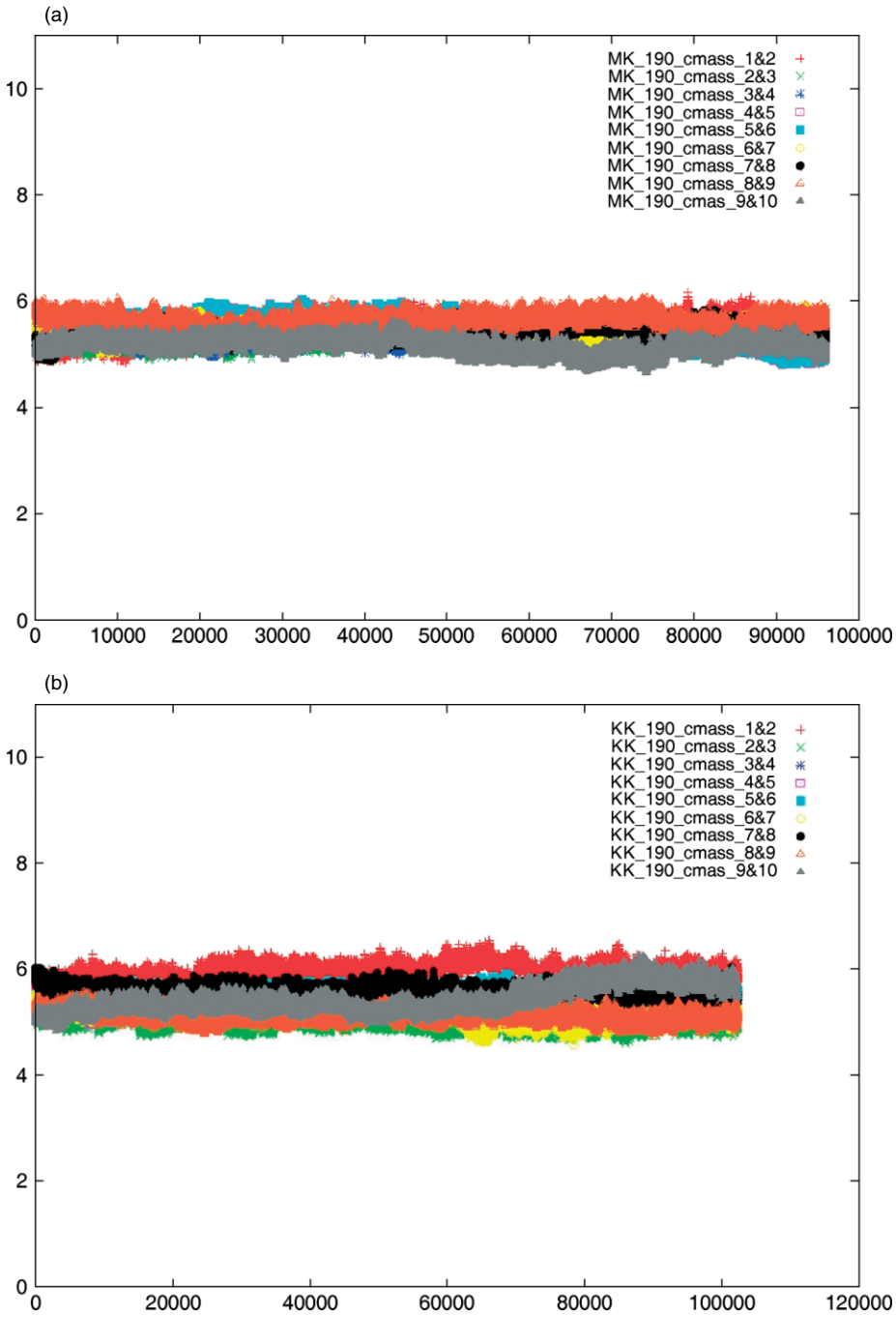

Figure 7 Mass center distances of neighboring strands of 10 × DLSFMKGE (a) and 10 × DLSFKKGE (b) systems at 190 K.

interactions between the methionine and the phenylalanine residues of the neighboring strands. Replacement of Met by Lys removes this stability factor. Single β-sheet systems, both two-strand and ten-strand, are not sufficiently stable at 300 K, which means that they should be stabilized by other β-sheets that are parallely placed in order to form fibrils. It appears that the presence of the canonical Lys–Lys sequence in the KK peptide significantly reduces its propensity to aggregate, suggesting that a significant degree of protection could occur from these two residues in SH3 domains. It is likely that these residues are strongly favored at these positions because they function as gatekeepers with a protective role against aggregation.

Acknowledgements

This work was supported by NATO Collaborative Linkage Grant LST.CLG.979807, by a stipend for I.L. from the 'Kasa im. Jozefa Mianowskiego' Fund, Poland, by the Latvian Science Council Grant 05.1768, and by Gdansk Academic Computer Center TASK.

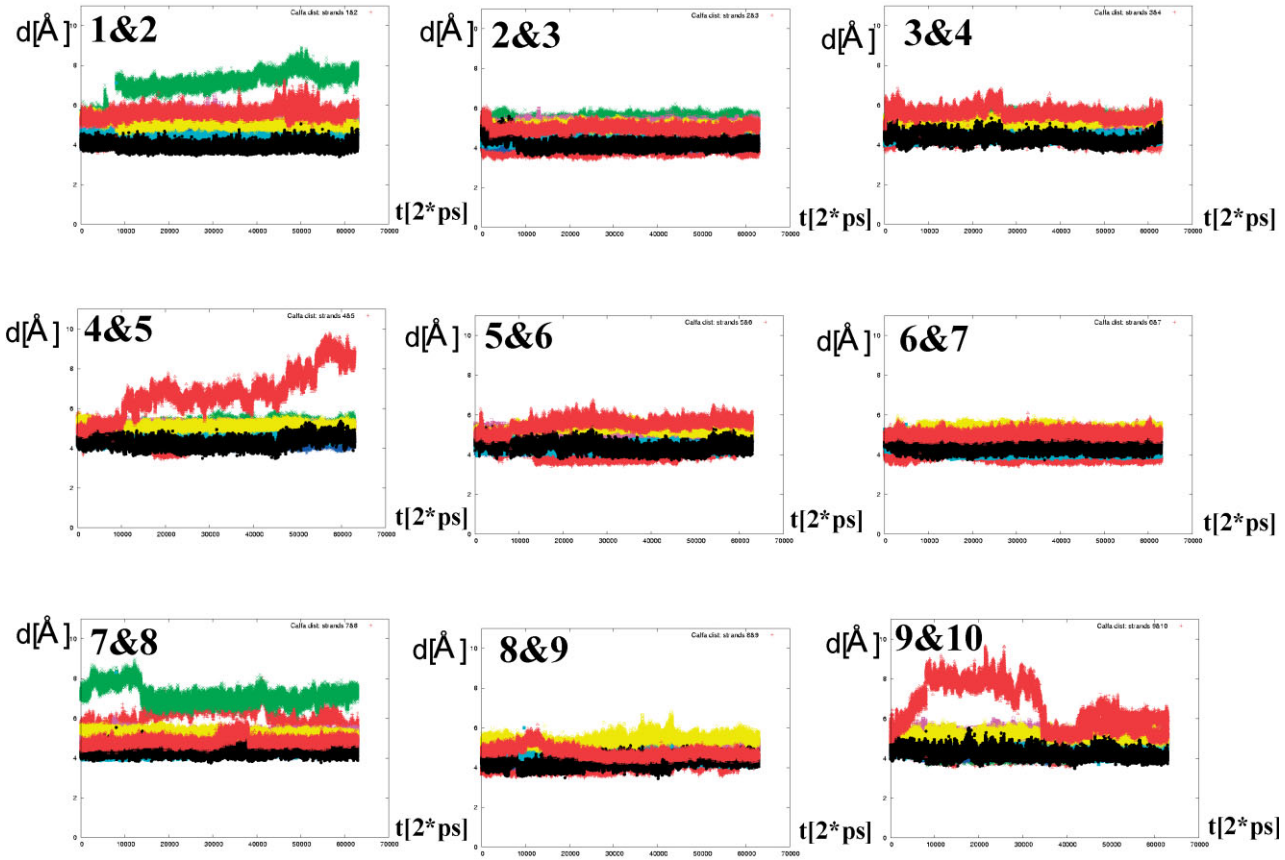

Figure 8 Cα distances between strands of 10 × DLSFMKGE system at 190 K.

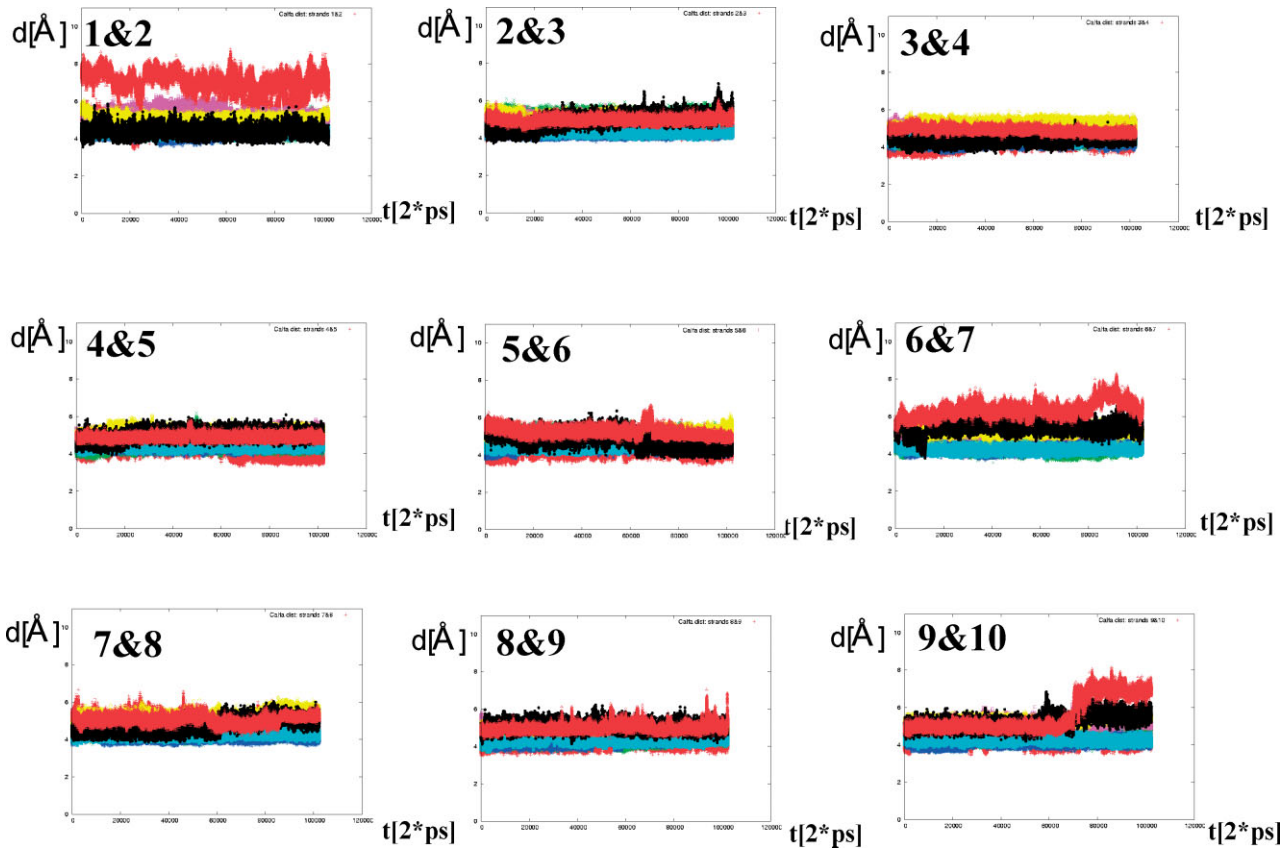

Figure 9 Cα distances between strands of 10 × DLSFKKGE system at 190 K.

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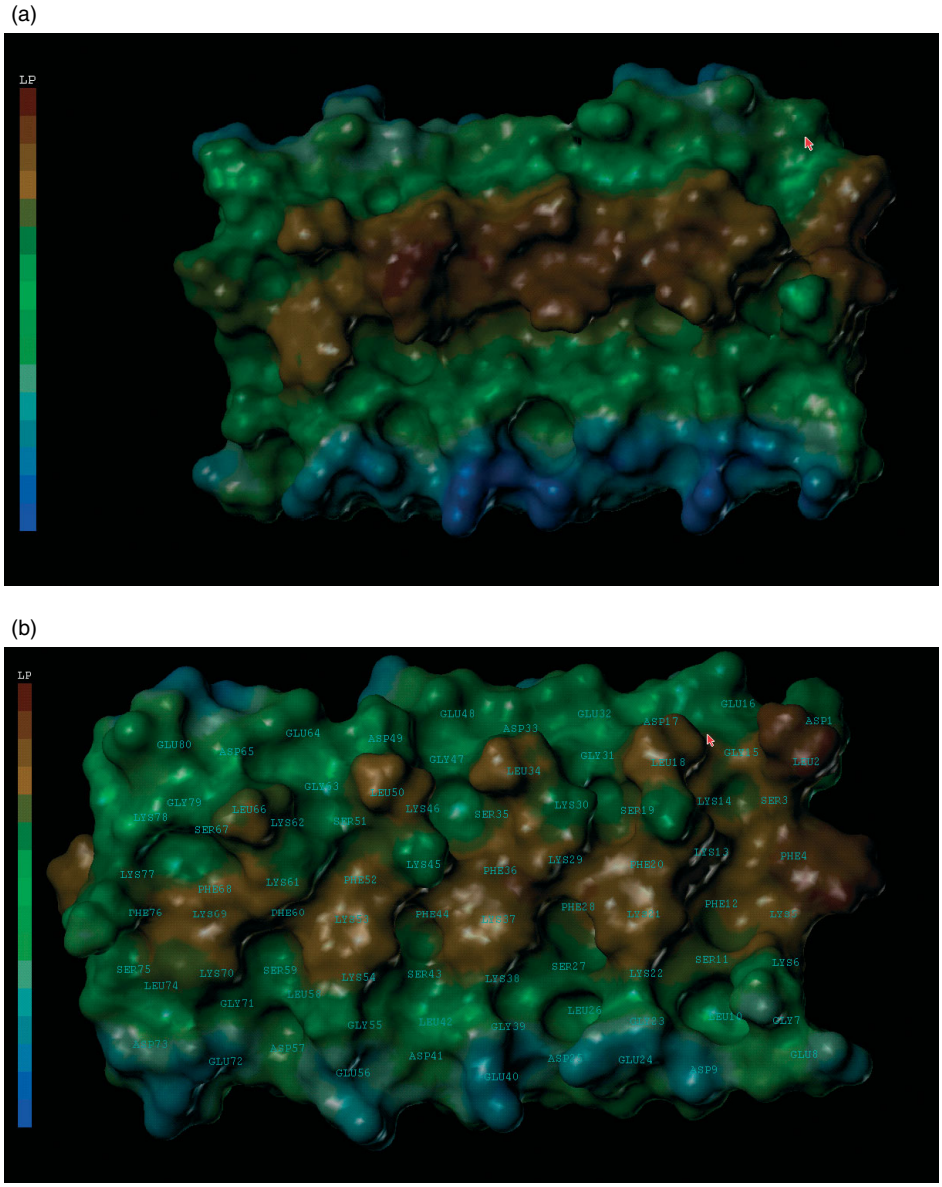

Figure 10 Lipophylic surfaces of 10 × MK (a) and 10 × KK (b) β-sheets.

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