Food & Function

RSC Publishing

PAPER

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Cite this: *Food Funct.*, 2013, **4**, 1474

How curcumin affords effective protection against amyloid fibrillation in insulin†

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Received 12th January 2013
Accepted 5th August 2013

DOI: 10.1039/c3fo00019b

www.rsc.org/foodfunction

Since the formation of amyloid structures from proteins was recognized in numerous diseases, many efforts have been devoted to the task of finding effective anti-amyloidogenic compounds. In a number of these investigations, the existence of "generic" compounds is implicitly acknowledged. Curcumin seems to be one of these compounds, possessing key structural components effective toward fibrillation prevention, and its anti-amyloidogenic property has been reported for a number of model and disease-related proteins such as lysozyme and alpha-synuclein. In this study, insulin amyloid formation has been shown to be effectively influenced by micromolar concentrations of curcumin. Under amyloidogenic conditions (pH 2.5 and 37 °C), the compound was observed to inhibit fibril formation of insulin in a dose-dependent manner. Moreover, addition of curcumin to the protein incubated under such conditions at different time points resulted in reduced amounts of final fibrils. Disaggregation of pre-formed fibrils was also observed upon addition of curcumin, as well as reduction in final fibril amounts after seeding. Overall, this compound appears to be able to interact with native, intermediate and fibrillar forms. Docking experiments suggest a potential interacting site with the B-chain of insulin, as well as the possibility for beta-sheet breaker activity.

1 Introduction

Amyloid fibrils are protein nanostructures composed of overlapping beta-pleated sheet domains.[1,2] These structures are generally formed from partially unfolded (or misfolded) segments of the starting proteins.[3] Formation of amyloid fibrils is accompanied by deleterious effects on cells, as observed in a large number of diseases,[4–7] prompting the search for suitable ligands that could control the process.[8,9]

In general, amyloid formation occurs through a nucleation process and further growth of the nuclei formed. If the nucleation process is present, a "lag phase" is observed which may vary upon the use of different experimental conditions *in vitro*. The lag phase corresponds to the formation of oligomers, which then assemble and grow to mature fibrils. The structural variability observed in nanofibrillar architecture may be related to the interactions between protein side chains and solvent, while definition of the main frame of amyloid fibrils depends on interactions between protein main chains.[10] The final structure of amyloid fibrils has been investigated for a number of years employing various techniques including electron microscopy and X-ray fiber diffraction; more recently, solid-state NMR and cryoelectron microscopy have also been used in this regard.[10–12] Amyloid structures' deposition has been associated with diseases such as Alzheimer's and Parkinson,[10] and much effort has been devoted to understanding the nature of the pathogenic amyloid species. Several studies have shown the higher cytotoxicity of prefibrillar intermediates/oligomers compared to mature fibrils in various proteins such as Abeta,[13] PI3-SH3 and HypF-N,[14] and W7FW14F apomyoglobin.[15,16]

As a potential therapeutic strategy, prevention of formation of amyloid structures (preferably in early stages) may be achieved with the use of small molecules. So far, some of the tested ligands have been shown to be effective against amyloid structure formation of multiple proteins, suggesting existence of "generic" anti-amyloidogenic compounds.[17] The efficient features of this type of ligand may be related to common interactions that are found between these molecules and their targets. Aromatic interactions are believed to have an important role in protein–protein interactions in general[18] which is also the case related to the formation of beta segments in amyloid structures and their stabilization.[19] In fact, various "atomic zippers" (*i.e.* the complementing interactions of beta-sheets side chains) have been detected in amyloid structures, in which aromatic residues seem to have a greater stabilizing effect,

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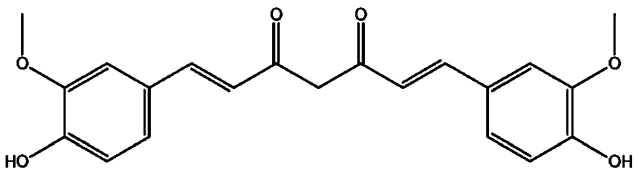
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3fo00019b



Scheme 1 Curcumin structure.

compared with their aliphatic counterparts.[20] On the other hand, main-chain interactions between adjacent sheets occur *via* hydrogen bonds,[10] leading to the concept of "beta-sheet breakers" as potential anti-amyloidogenic small molecules.[21] A ligand that could make both pi–pi and hydrogen bonding interactions could thus be of special interest as a potential generic anti-amyloidogenic compound. Curcumin (diferuloylmethane) appears to possess these structural characteristics (Scheme 1), and has been previously shown to be an effective inhibitor of amyloid formation in Aβ-amyloid,[22] α-synuclein,[23] hen egg-white lysozyme,[24] and prion protein.[25] This compound has numerous other potential therapeutic uses (*e.g.* anti-inflammatory and anticancer),[26] which could be realized if its bioavailability is improved.[27]

In this study, the effect of curcumin on insulin fibrillation was investigated. Amyloid formation of this polypeptide may be problematic with regard to its production process and storage as a therapeutic protein,[28,29] but insulin could also form amyloid deposits *in vivo*, when frequently injected at the same site, in diabetic patients.[30,31] Insulin fibril formation needs the native, all-alpha polypeptide to partially unfold and subsequently refold to form amyloid beta structures.[32,33] The observed anti-amyloidogenic effect of curcumin on insulin is discussed here with regard to the different stages of the fibrillation process.

2 Materials and methods

2.1 Protein and reagents

Bovine insulin, curcumin, Thioflavin T (ThT), and Congo red were purchased from Sigma (St Louis, MO, USA) and all salts and organic solvents were from Merck (Darmstadt, Germany). Protein concentration was determined by measuring absorbance at 280 nm, using an extinction coefficient of 1.0.[34] All experiments were done at least in duplicates and typical results are reported. The acceptable reproducibility error margin was set at 10%.

2.2 Amyloid induction

Bovine insulin was dissolved at 1 mg ml^{-1} in 50 mM glycine (pH 2.5 or pH 7.5) containing 1 mM EDTA, and dialyzed against the same buffer (excluding EDTA) for 24 hours. The protein solution was then incubated at 37 °C for the specified durations while being stirred gently by Teflon magnetic bars. To investigate the effect of curcumin, the compound was diluted into DMSO (1 mg ml^{-1}) and added to insulin solution at the specified times. Final concentrations of 1, 2, 3, 4, 6, and 8 μM of curcumin were used. For the seeding experiment, pre-formed aggregates that were obtained after 2 hours of insulin incubation under amyloidogenic conditions were used.

2.3 Thioflavin T binding assay

All fluorescence experiments were carried out on a Cary Eclipse VARIAN fluorescence spectrophotometer (Mulgrare, Australia) at room temperature. To investigate amyloid formation, 10 μl of insulin samples (1 mg ml^{-1}) were added to 590 μl of 13 μM Thioflavin T (ThT) solution (from 2.5 mM ThT stock solution in 10 mM sodium phosphate, 150 mM NaCl, pH 7, passed through a 0.45 μM filter paper), mixed completely, and incubated for 5 minutes. Fluorescence emission spectra were then taken using excitation at 440 nm. The excitation and emission slit widths were set at 5 nm (ref. 35) and the cuvette pathlength was 1 cm.

2.4 Congo red binding assay

A stock solution of 7 mg ml^{-1} Congo red (CR) was prepared in 5 mM potassium phosphate containing 150 mM NaCl. Insoluble particles of this solution were removed by filtering twice through a center-glass N4 filter. The filtered solution was diluted 2000 times with the same buffer.10 μl of 1 mg ml^{-1} protein solution was taken at 24 hours after incubation for amyloid formation and was diluted into CR solution with a final volume of 235 μl followed by incubation at 25 °C without further stirring at least for 5 minutes in the dark. The absorbance spectrum was acquired on the Shimadzu UV-visible spectrophotometer (Kyoto, Japan) in the 400–600 nm region.[35]

2.5 Circular dichroism

Circular dichroism (CD) spectra in the far-UV region (190–260 nm) were obtained on an AVIV 215 spectropolarimeter (Aviv Associates, Lakewood, NJ, USA), using a 1 mm path cell at room temperature. The protein concentration was 0.2 mg ml^{-1}.

2.6 Transmission electron microscopy (TEM)

10 μl of incubated samples were placed on a copper 400 mesh grid, which had been covered with carbon coated formvar films. After 2 min, excess fluid was drawn out using a filter paper and 1% uranyl acetate was added. After another 2 min, the excess dye was removed. Finally, the grids were viewed with a CEM 902A Zeiss microscope (Oberkochen, Germany).

2.7 Docking

Docking was performed using the graphical interface MGL Tools 1.5.1 (The Molecular Graphics Laboratory, Scripps Research Institute) for Autodock. The executable file vina was used for the actual docking using an exhaustiveness of 20.[36] 2OMP.pdb and 2OMQ.pdb files were used as receptors which correspond to the amyloid forms of LYQLEN and VEALYL segments of insulin, respectively. Visualization of best-obtained docking poses and preparation of the image were carried out using MOE 2010.10 (Chemical Computing Group Inc., Montreal, QC, Canada).

3 Results and discussion

An EDTA containing buffer was used to facilitate amyloid formation. As reported previously, a 24 hours incubation time was sufficient to generate mature insulin fibrils, which were subsequently tested by various techniques (Congo red absorbance, CD, ThT fluorescence, and TEM).[37]

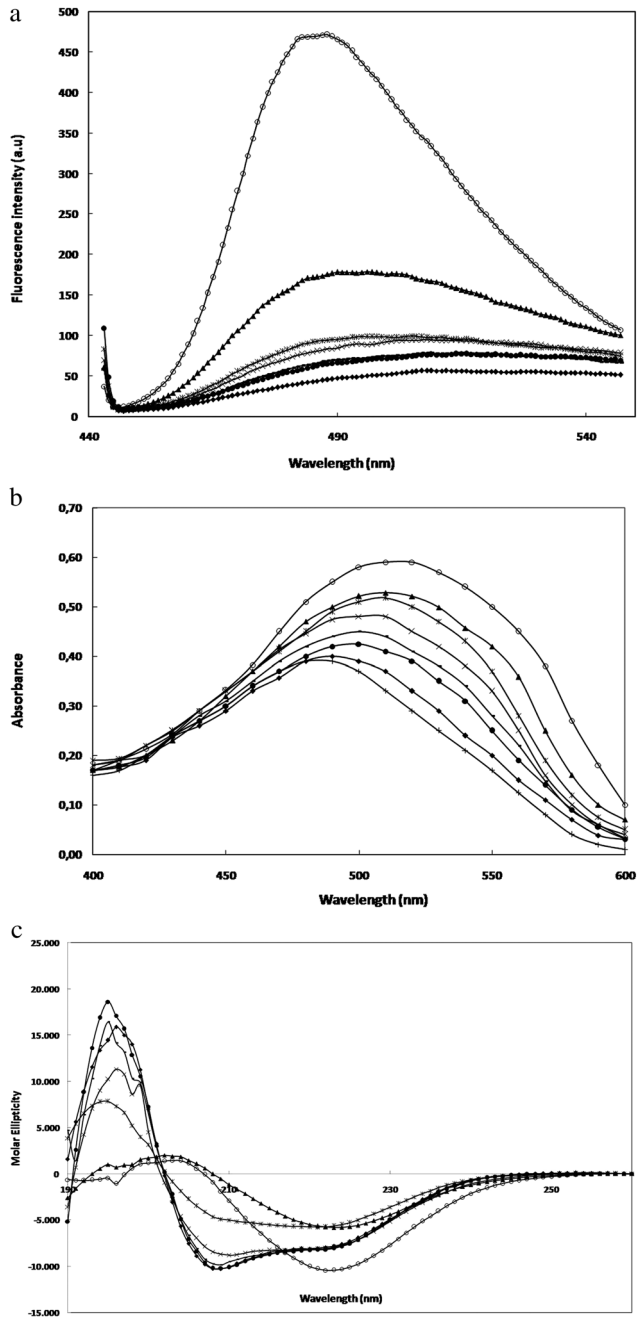


Fig. 1 (a) Fluorescence spectra of the ThT binding assay of insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), and in the presence of curcumin 1 μM (▲), 2 μM (*), 3 μM (×), 4 μM (–), 6 μM (●), 8 μM (◆). (b) Congo red binding assay results for insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), and in the presence of curcumin 1 μM (▲), 2 μM (*), 3 μM (×), 4 μM (–), 6 μM (●), 8 μM (◆). Spectrum of Congo red absorbance (+). (c) Far-UV CD spectra of insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), and in the presence of curcumin 1 μM (▲), 2 μM (*), 3 μM (×), 4 μM (–), 6 μM (●), 8 μM (◆).

The effect of curcumin on insulin amyloid formation was investigated by adding various concentrations (1, 2, 3, 4, 6, and 8 μM) of the ligand to the incubation medium. After 24 hours, the resulting structures were characterized. In the presence of curcumin, ThT fluorescence intensities, indicative of amyloid fibrillation,[35] were decreased, and the effect was quite remarkable starting with 3 μM concentration (Fig. 1a). The ligand was also observed to affect the Congo red spectrum, which shows a red shift and increase in absorbance upon binding of the dye to the amyloid structure.[35] As reported in Fig. 1b, a gradual change of the spectral shape and intensity is observed in the presence of increasing amounts of curcumin. It may be noted that the spectrum obtained from the sample containing 8 μM of the ligand almost overlaps with the one corresponding to the dye alone. Based on far-UV CD spectra, the compound seems to be able to preserve the native conformation of insulin (Fig. 1c). While the amyloid structure shows a single deep peak representative of the beta-sheet structure, addition of 1 μM of curcumin decreases the peak depth and with higher concentration of the ligand, the protein spectrum moves closer to the native pattern (typical minima at 208 nm and 222 nm).[37] The anti-amyloidogenic property of curcumin has been reported for several unstructured and structured proteins. In the case of alpha-synuclein, it was found to bind to the monomeric form of the protein and suggested to affect its "reconfiguration rate", *i.e.* to preserve a particular conformation of the peptide, thereby inhibiting the formation of an aggregation-prone intermediate.[23,38] In the case of prion protein[25] and islet amyloid polypeptide,[39] curcumin has been found to interact with intermediate alpha-helical structures. This ligand also prevents the amyloid formation of lysozyme[24] and transthyretin,[40] whose secondary structures are mainly composed of alpha-helices. Since alpha-helices are predominant in native and first intermediate structures of insulin,[33] a potential alpha-helix stabilization effect may be suggested for curcumin. Interestingly, trifluoroethanol (TFE), a well-known alpha-helix stabilizer, has been shown to prevent conversion of insulin to oligomers, but not to amyloid fibrils.[41] TEM images obtained in the presence of 25% TFE showed different fibril morphologies,[41] which seem to contain amorphous structures to some degree, suggesting off pathway species formation. TEM images of insulin incubated under amyloidogenic conditions, in the absence (Fig. 2a) and in the presence (Fig. 2b) of curcumin (4 μM) show a distinct difference in the amount and morphology of formed aggregates. When curcumin was present in the incubation medium, typical fibrils disappeared, and the detected structures resembled amorphous aggregates (Fig. 2b).

Considering the fact that fibril disaggregating propensity has been reported for curcumin (*e.g.* abeta[22] and lysozyme),[24] we assessed the effect of adding this ligand after amyloid fibrillation was completed (24 h), as well as in the earlier stage of the process (4 h). Congo red spectra obtained at these different time points show that the ligand is still capable of causing a decrease in the amount of formed fibrils, even when it is added after the start of amyloid formation (Fig. 3a). Although the red shift

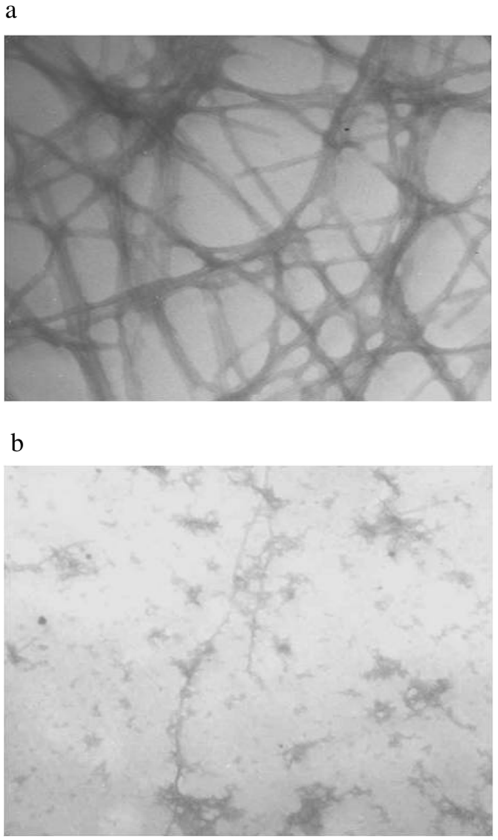


Fig. 2 TEM images obtained from insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (a), and when 4 μM curcumin was added at time point 0 hours (b).

indicative of the presence of amyloid occurs when curcumin is added at the end of the process, the maximum absorbance is lower. ThT spectra also show a markedly lower intensity upon curcumin addition at all time points (Fig. 3b). More subtle differences could be detected in the CD spectra: addition of curcumin at 4 h results in a spectrum pattern which is closer to the native structure of insulin, but curcumin added at 24 h seems to have only lessened the amount of beta-sheet containing structures (Fig. 3c). TEM images also indicate fewer mature fibrils and a population of intermediate structures (possibly oligomers) upon late addition of curcumin (Fig. 3d and e).

Maximum absorbance of ThT was also checked at different time points in the presence and in the absence of 4 μM curcumin (Fig. 4). The results indicate effectiveness of curcumin in suppressing amyloid formation at all stages, including when pre-formed aggregates were added to the incubation medium.

Overall, these results, combined with previous reports, suggest an interesting potential of curcumin as an anti-amyloid agent. However, it should be noted that from a therapeutic point of view, possible toxicity of amyloid-related structures is of importance. Oligomeric species,[42] and even segments derived from disaggregated fibrils[43] may show higher toxicity than fibrils; small molecules that lengthen the lag phase of amyloid formation could also cause higher toxicity.[44] As such, further cell-based studies are needed to assess reduction of toxicity by curcumin. Indeed, the beneficial effects of curcumin in enhancing cell viability upon exposure to pathogenic amyloid species that originated from other proteins have been demonstrated (*e.g.* ref. 45).

Curcumin has been reported to be unstable at neutral pH, a condition which leads to proton removal from its phenol groups, while it has a relatively higher stability under acidic conditions (as used in the present study), due to its conjugated diene structure.[46,47] Although degradation cannot be ruled out, specially over long incubation periods and at high temperatures, we assume that the curcumin structure in its bound form under our experimental conditions is preserved, as observed in the case of curcumin binding to albumin at alkaline pH.[48] However, considering the importance of the physiological pH, and with regard to previous experiments that have been performed with curcumin at neutral pH,[23,38] a complementary experiment was done at pH 7.5. When curcumin was present in the medium with concentrations of 1, 2, 4, and 8 μM, the amount of beta-sheets that were formed after 24 hours under amyloidogenic conditions decreased (CD spectra in Fig. S1†). TEM images also show a decrease in fibrillation in the presence of 4 and 8 μM curcumin (Fig. S2a and b† respectively) in comparison with the sample devoid of curcumin (Fig. S2c†). However, when comparing these results with what was obtained under acidic pH, the effect of curcumin appears less pronounced. CD spectra show that the native structure of insulin has not been preserved, and TEM images indicate retained capacity for fibril formation. Altogether, these results indicate the general potential of curcumin to prevent amyloid formation under a variety of conditions. In order to enhance curcumin stability under neutral pH, formulations have been recently proposed,[49,50] which could be used to further assess anti-amyloid capacity of the compound in future studies.

The ability of curcumin to bind to amyloid fibrils has been directly demonstrated by its co-crystallization with an amyloid segment of the tau protein.[51] The ligand was found to be positioned parallel to the fiber axis, and this seemed to have affected the arrangement of steric zippers.[51] In a different approach for understanding the mechanism of action of curcumin related to the newly formed beta structures, a theoretical docking experiment was performed on amyloid insulin segments VEALYL and LYQLEN. These are two amyloidogenic segments of insulin, whose crystallized fibrils have been characterized.[52] In order to check the potential beta-sheet breaking property of curcumin, the two beta strands of each segment, originally interacting by backbone hydrogen bonds, were moved apart to a distance of about 7 Å. This repositioning was done in order to accommodate enough space for a putative ligand. The result of docking the compound in these structures is shown in Fig. 5a and b. In both segments, curcumin is able to position itself between the strands, and make several hydrogen bonds with the peptide backbones. In the VEALYL segment, putative hydrogen bonds are observed with backbones of Y of one strand and (possibly) L4 of the facing strand, as well as the side chain of E, while a hydrophobic contact may occur between the L4 side chain and one aromatic ring of curcumin (Fig. 5a). In the case of the

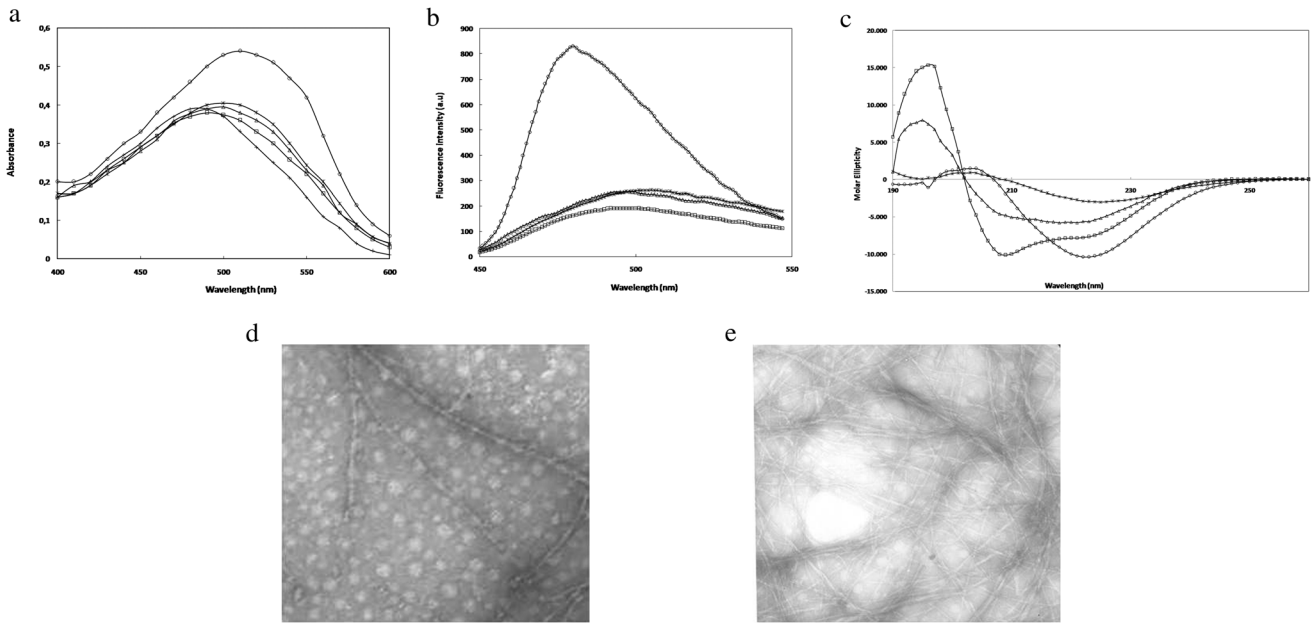


Fig. 3 (a) Congo red binding assay for insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), when 4 μM curcumin was added at time point 0 hours (□), 4 hours (Δ), and 24 hours (*). The spectrum of Congo red absorbance (+). (b) Fluorescence spectra of the ThT binding assay for insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), when 4 μM curcumin was added at time point 0 hours (□), 4 hours (Δ), and 24 hours (*). (c) Far-UV CD spectra of insulin after 24 hours incubation under amyloidogenic conditions in the absence of curcumin (○), when 4 μM curcumin was added at time point 0 hours (□), 4 hours (Δ), and 24 hours (*). (d) TEM image obtained from insulin after 24 hours incubation under amyloidogenic conditions when 4 μM curcumin was added at time point 4 hours. (e) TEM image obtained from insulin after 24 hours incubation under amyloidogenic conditions, when 4 μM curcumin was added at time point 24 hours.

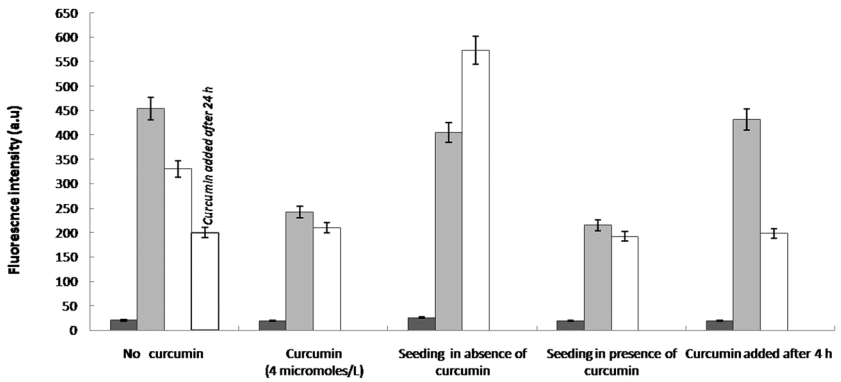


Fig. 4 Maximum ThT emission measured for insulin incubated under amyloidogenic conditions at different time points: 0 hours (■), 4 hours (■), and 24 hours (□), in the presence and in the absence of curcumin, as indicated in the figure. In the first set of experiments, ThT maximal emission was monitored in the absence of curcumin at the three time points, then 4 μM of the compound was added to the incubation medium and ThT maximal emission was measured (■). Seeding was done with the addition of pre-formed aggregates (obtained after 2 hours) at time point zero.

LYQLEN segment, hydrogen bonds are formed between curcumin and backbones of L1 and Q from one strand, and L4 from the facing strand (Fig. 5b). Overall, this result is suggestive of the potential role of curcumin in preventing formation of beta-sheets by steric hindrance and providing alternative hydrogen bonds. With regard to the importance of steric zippers in fibril formation,[51,52] another docking was performed on the complete VEALYL structure. In a similar manner to the previous experiment, the side chains were moved apart to a distance of about 7 Å from each other. As shown in Fig. 5c, a putative hydrogen bond could be formed with the Y residue, while most other (potential) interactions would be of hydrophobic nature. Considering the fact that curcumin may be able to interact with the native structure of insulin, a final approach consisted of using this structure. Both the R-state and the T-state of insulin were used to this end (Fig. 5d and e respectively). The main interaction position was found to involve the B chain in both structures. In the R-state, curcumin has an extended conformation, which enables it to span over a large part of the B chain. This positioning occurs in a location similar to the one found previously (by docking) for potential anti-amyloidogenic benzofuranone ligands[37] and includes V12 of the B chain, which is

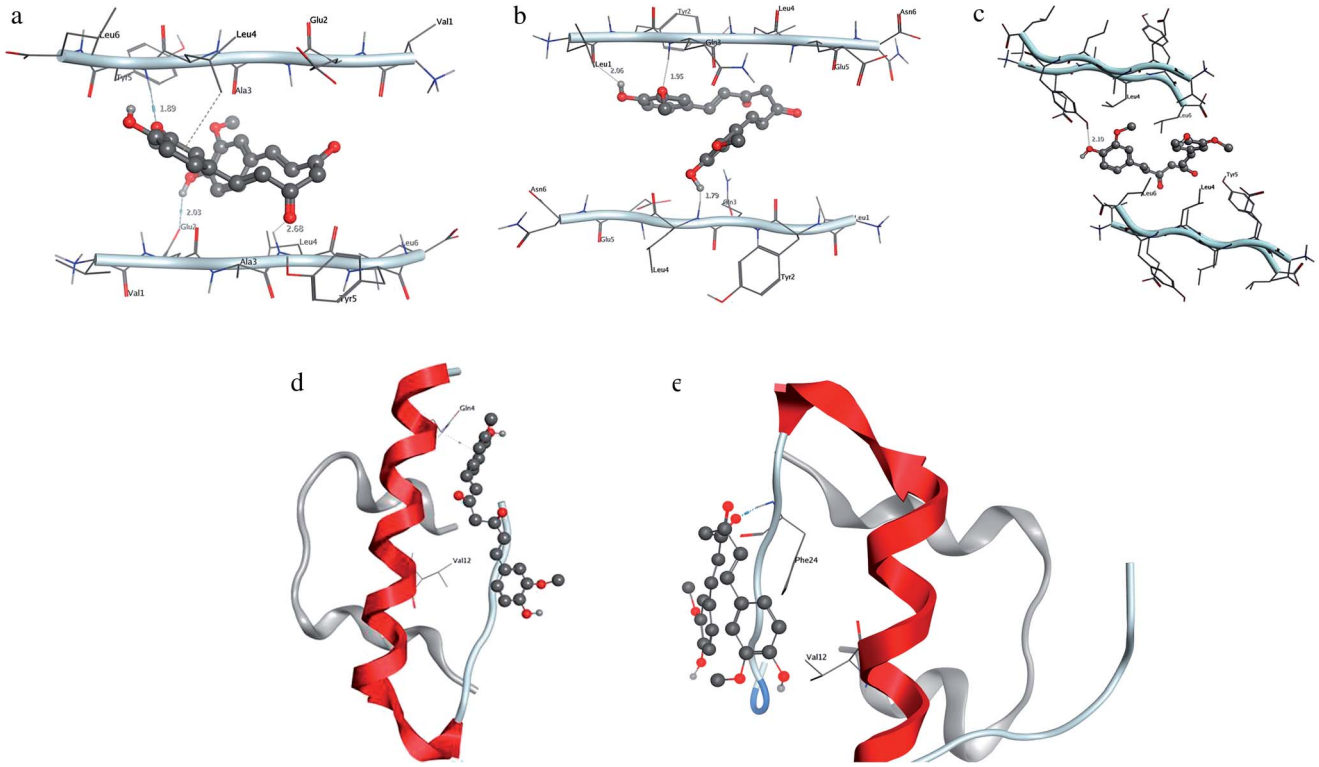


Fig. 5 Results of curcumin docking into insulin. (a) The VEALYL segment, (b) the LYQLEN segment, (c) the complete VEALYL dimeric structure as reported in the 2OMQ.pdb structure, (d) the native insulin structure in the R-state, and (e) the native insulin structure in the T-state.

the starting residue of the VEALYL segment. In the T-state, the curcumin main docking pose is bent, but again, V12 of the B-chain is close to the ligand. However, the other end of the ligand may interact with the C-terminal region of the B-chain.

So far, a number of compounds have been investigated with regard to their anti-amyloidogenic potential toward insulin. The small molecules in this category include carbohydrate-based structures such as trehalose,[53] the so-called stress molecules (ectoine, betaine and citrulline),[53] as well as aromatic compounds such as benzofuranones,[37] and the flavonoid quercetin.[54] Small stress molecules were effective in inhibiting amyloid fibril formation while preserving the native structure of insulin to some extent. However, when seeding was carried out, only ectoine suppressed fibril formation. These compounds are suggested to act as general stabilizers of the protein structure, acting by interaction with hydrophilic parts of their targets, thereby preventing formation of an amyloid prone structure.[53] A more recent study has indicated that trehalose may cause insulin fibrils to remain in a state of higher toxicity by prolonging the lag phase of the fibrillation process.[44] Benzofuranone derivatives were not found to be effective in preserving the native conformation of insulin and were suggested to act as potential beta-sheet breakers.[37] Interestingly, quercetin, which is a polyphenol compound, was shown to decrease fibril formation of insulin, act on pre-formed fibrils and change the morphology of aggregates to amorphous species,[54] which is, to some extent, similar to our observations related to curcumin.

In conclusion, this study has shown the anti-amyloidogenic potential of curcumin toward insulin, which is exerted by inhibition of fibril formation, as well as disruption of pre-formed fibrils. The extensive reported observations on control of amyloid fibrillation of a number of proteins by curcumin under both *in vitro* and *in vivo* conditions, suggest a strong potential therapeutic role of this natural product in control and prevention of amyloid disorders.

Acknowledgements

We would like to thank the members of the Biomaterial Laboratory of IBB, University of Tehran, for their nice collaboration in this project, as well as Dr Marjan Sabbaghian for her practical advice and recommendations related to this study. This work was supported by a grant from the Iranian Academy of Medical Science.

References

1 G. G. Glenner, E. D. Eanes, H. A. Bladen, R. P. Linke and J. D. Termine, *J. Histochem. Cytochem.*, 1974, **22**, 1141–1158.
2 R. Cabriolu, D. Kashchiev and S. Auer, *Biophys. J.*, 2011, **101**, 2232–2241.
3 M. Calamai, F. Chiti and C. M. Dobson, *Biophys. J.*, 2005, **89**, 4201–4210.
4 E. I. Agorogiannis, G. I. Agorogiannis, A. Papadimitriou and G. M. Hadjigeorgiou, *Neuropathol. Appl. Neurobiol.*, 2004, **30**, 215–224.

5 A. J. Thompson and C. J. Barrow, *Curr. Med. Chem.*, 2002, **9**, 1751–1762.
6 N. Gregersen, L. Bolund and P. Bross, *Mol. Biotechnol.*, 2005, **31**, 141–150.
7 D. J. Selkoe, *Nature*, 2003, **426**, 900–904.
8 B. Bulic, M. Pickhardt, B. Schmidt, E. M. Mandelkow, H. Waldmann and E. Mandelkow, *Angew. Chem., Int. Ed.*, 2009, **48**, 1740–1752.
9 A. R. A. Ladiwala, J. S. Dordick and P. M. Tessier, *J. Biol. Chem.*, 2011, **286**, 3209–3218.
10 F. Chiti and C. M. Dobson, *Annu. Rev. Biochem.*, 2006, **75**, 333–366.
11 J. Madine, E. Jack, P. G. Stockley, S. E. Radford, L. C. Serpell and D. A. Middleton, *J. Am. Chem. Soc.*, 2008, **130**, 14990–15001.
12 J. L. Jimenez, E. J. Nettleton, M. Bouchard, C. V. Robinson, C. M. Dobson and H. R. Saibil, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 9196–9201.
13 H. A. Lashuel, D. Hartley, B. M. Petre, T. Walz and P. T. Lansbury, *Nature*, 2002, **418**, 291.
14 M. Bucciantini, E. Giannoni, F. Chiti, F. Baroni, L. Formigli, J. s. Zurdo, N. Taddei, G. Ramponi, C. M. Dobson and M. Stefani, *Nature*, 2002, **416**, 507–511.
15 S. Vilasi, R. Sarcina, R. Maritato, A. De Simone, G. Irace and I. Sirangelo, *PLoS One*, 2011, **6**, e22076.
16 I. Sirangelo, C. Iannuzzi, S. Vilasi, G. Irace, G. Giuberti, G. Misso, A. D'Alessandro, A. Abbruzzese and M. Caraglia, *J. Cell. Physiol.*, 2009, **221**, 412–423.
17 S. A. Hudson, H. Ecroyd, F. C. Dehle, I. F. Musgrave and J. A. Carver, *J. Mol. Biol.*, 2009, **392**, 689–700.
18 I. S. Moreira, P. A. Fernandes and M. J. Ramos, *Proteins*, 2007, **68**, 803–812.
19 O. S. Makin, E. Atkins, P. Sikorski, J. Johansson and L. C. Serpell, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 315.
20 L. Vitagliano, F. Stanzione, A. De Simone and L. Esposito, *Biopolymers*, 2009, **91**, 1161–1171.
21 B. Permanne, C. Adessi, S. Fraga, M. J. Frossard, G. P. Saborio and C. Soto, *J. Neural Transm., Suppl.*, 2002, 293–301.
22 F. Yang, G. P. Lim, A. N. Begum, O. J. Ubeda, M. R. Simmons, S. S. Ambegaokar, P. P. Chen, R. Kayed, C. G. Glabe, S. A. Frautschy and G. M. Cole, *J. Biol. Chem.*, 2005, **280**, 5892–5901.
23 N. Pandey, J. Strider, W. C. Nolan, S. X. Yan and J. E. Galvin, *Acta Neuropathol.*, 2008, **115**, 479–489.
24 S. S. Wang, K. N. Liu and W. H. Lee, *Biophys. Chem.*, 2009, **144**, 78–87.
25 I. Hafner-Bratkovic, J. Gaspersic, L. M. Smid, M. Bresjanac and R. Jerala, *J. Neurochem.*, 2008, **104**, 1553–1564.
26 B. B. Aggarwal, C. Sundaram, N. Malani and H. Ichikawa, *The molecular targets and therapeutic uses of curcumin in health and disease*, 2007, pp. 1–75.
27 P. Basnet and N. Skalko-Basnet, *Molecules*, 2011, **16**, 4567–4598.
28 J. Brange, L. Andersen, E. D. Laursen, G. Meyn and E. Rasmussen, *J. Pharm. Sci.*, 1997, **86**, 517–525.
29 R. Woods, J. Alarcón, E. McVey and R. Pettis, *J. Diabetes Sci. Technol.*, 2012, **6**, 265.
30 F. Dische, C. Wernstedt, G. Westermark, P. Westermark, M. Pepys, J. Rennie, S. Gilbey and P. Watkins, *Diabetologia*, 1988, **31**, 158–161.
31 Y. Shikama, J. Kitazawa, N. Yagihashi, O. Uehara, Y. Murata, N. Yajima, R. Wada and S. Yagihashi, *Intern. Med.*, 2010, **49**, 397–401.
32 A. Ahmad, I. S. Millett, S. Doniach, V. N. Uversky and A. L. Fink, *Biochemistry*, 2003, **42**, 11404–11416.
33 S. Yamamoto and H. Watarai, *Chirality*, 2012, **24**, 97–103.
34 L. Nielsen, S. Frokjaer, J. Brange, V. N. Uversky and A. L. Fink, *Biochemistry*, 2001, **40**, 8397–8409.
35 M. R. Nilsson, *Methods*, 2004, **34**, 151–160.
36 O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455–461.
37 A. Rabiee, A. Ebrahim-Habibi, L. Navidpour, D. Morshedi, A. Ghasemi, M. Sabbaghian, M. Nemati-Lay and M. Nemat-Gorgani, *Chem. Biol. Drug Des.*, 2011, **78**, 659–666.
38 B. Ahmad and L. J. Lapidus, *J. Biol. Chem.*, 2012, **287**, 9193–9199.
39 M. Daval, S. Bedrood, T. Gurlo, C. J. Huang, S. Costes, P. C. Butler and R. Langen, *Amyloid*, 2010, **17**, 118–128.
40 R. Pullakhandam, P. Srinivas, M. K. Nair and G. B. Reddy, *Arch. Biochem. Biophys.*, 2009, **485**, 115–119.
41 V. Banerjee and K. Das, *Colloids Surf., B*, 2012, **92**, 142–150.
42 E. Kachooei, A. A. Moosavi-Movahedi, F. Khodagholi, H. Ramshini, F. Shaerzadeh and N. Sheibani, *PLoS One*, 2012, **7**, e41344.
43 C. L. Heldt, D. Kurouski, M. Sorci, E. Grafeld, I. K. Lednev and G. Belfort, *Biophys. J.*, 2011, **100**, 2792–2800.
44 S. Vilasi, C. Iannuzzi, M. Portaccio, G. Irace and I. Sirangelo, *Biochemistry*, 2008, **47**, 1789–1796.
45 K.-N. Liu, C.-M. Lai, Y.-T. Lee, S.-N. Wang, R. P. Chen, J.-S. Jan, H.-S. Liu and S. S.-S. Wang, *Biochim. Biophys. Acta, Gen. Subj.*, 2012, **1820**, 1774–1786.
46 Y. J. Wang, M. H. Pan, A. L. Cheng, L. I. Lin, Y. S. Ho, C. Y. Hsieh and J. K. Lin, *J. Pharm. Biomed. Anal.*, 1997, **15**, 1867–1876.
47 B. Zebib, Z. Mouloungui and V. Noirot, *Bioinorg. Chem. Appl.*, 2010, 292760.
48 F. Zsila, Z. Bikàdi and M. Simonyi, *Biochem. Biophys. Res. Commun.*, 2003, **301**, 776–782.
49 A. Kaniyoor and S. Ramaprabhu, *J. Nanosci. Nanotechnol.*, 2012, **12**, 8323–8329.
50 M. G. O'Toole, R. M. Henderson, P. A. Soucy, B. H. Fasciotto, P. J. Hoblitzell, R. S. Keynton, W. D. Ehringer and A. S. Gobin, *Biomacromolecules*, 2012, **13**, 2309–2314.
51 M. Landau, M. R. Sawaya, K. F. Faull, A. Laganowsky, L. Jiang, S. A. Sievers, J. Liu, J. R. Barrio and D. Eisenberg, *PLos Biol.*, 2011, **9**, e1001080.
52 M. R. Sawaya, S. Sambashivan, R. Nelson, M. I. Ivanova, S. A. Sievers, M. I. Apostol, M. J. Thompson, M. Balbirnie, J. J. W. Wiltzius and H. T. McFarlane, *Nature*, 2007, **447**, 453–457.
53 A. Arora, C. Ha and C. B. Park, *FEBS Lett.*, 2004, **564**, 121–125.
54 J. B. Wang, Y. M. Wang and C. M. Zeng, *Biochem. Biophys. Res. Commun.*, 2011, **415**, 675–679.

