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Colonic amyloidosis, computational analysis of the major amyloidogenic species, Serum Amyloid A

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ABSTRACT

Amyloidosis is characterized by misfolding of proteins. The clinical gastrointestinal manifestations of amyloidosis may mimic other disease, such as inflammatory bowel disease or colonic cancer. As these patients have a high risk for bleeding and poor wound healing following surgery it is important to diagnose them correctly and do a careful preoperative assessment. The most common form of colonic amyloidosis is caused by Serum Amyloid A (SAA), an acute phase protein of unknown function. It is expressed in response to inflammation and the increased levels may lead to amyloidosis. The main treatment is to suppress the acute phase response and thereby reduce production of SAA.

As no structure for SAA is available we aim to perform an in silico assessment of its structural and fibrillation properties. In the paper we propose an ab initio model of the structure of SAA, which consists of a five membered helical bundle with a fold related to the tetratricopeptide repeat domain. As there are uncertainties relating to the packing of the helices, each helical region is subjected to triplicate molecular dynamics simulations to assess the integrity of the structural region. The first helix, stretching from residues 1 to 13, is the least stable according to the simulations; almost all of the helical conformation is lost during the 10 ns simulations, whereas the other helices maintain portions that remain in an helical conformation in at least 80% of the simulations. All helices are also subjected to a single 100 ns simulation to investigate how the secondary structure develops over time. In them helix 1 adopts a β-hairpin structure similar to other fibril forming proteins. The β-hairpin can in turn multimerise and form a mature fibril structure. The mechanism behind the conformational transition appears to be driven by interactions of side chains of charged residues, particularly Arginine 1. It exchanges interaction partners in the simulation and stabilizes intermediate conformations on the folding pathway to the final β-hairpin.

1. Introduction

Amyloidosis is characterized by misfolding of proteins. More than 20 proteins can form fibrils in vivo, where the fibrils can disrupt tissue structure and impair function of organs. The common feature of the proteins associated with amyloidosis is that they can assume a non-native beta sheet structure that builds up the fibril, regardless of their native structure (Nelson and Eisenberg, 2006). One of the most well studied fibril forming proteins is the Amyloid β-peptide which makes up the core of the neuronal plaques in Alzheimer's disease. It is a cleavage product of the amyloid precursor protein. In the native protein it is part of a transmembrane helix, but upon cleavage it is released from the cell (O'Brien and Wong, 2011). As the helix normally is partly buried in a lipid environment it is destabilized in an aqueous solution and can adopt an extended conformation which in turn can self-associate into oligomers which can form mature fibril structures (Fandrich et al., 2011). The fibrils can be cleared by macrophages, but in the disease state the build-up of fibrils is faster than the clearance. There are dual mechanisms behind the toxicity by fibrillar proteins, the oligomers formed on the pathway to fibrils have been shown to be cytotoxic and the mature fibrils will eventually lead to mechanical damage to the surrounding cells, causing cell-death (Gotz et al., 2011).

Serum Amyloid A (SAA) is an acute phase protein, expressed in response to inflammation, such as inflammatory bowel disease (IBD) or Crohn's disease (Uhlar and Whitehead, 1999), where the increased levels may lead to fibril formation (De Beer et al., 1982). The disease is referred to as Amyloid A amyloidosis. The function of SAA in not known but it is associated with high density lipoparticles (HDL), where it is believed to be involved in regulation of cholesterol levels (Kisilevsky and Manley, 2012; Uhlar and Whitehead, 1999). Amyloid A amyloidosis is the most common form of colonic amyloidosis (Uhlar and Whitehead, 1999). The tertiary structure of the protein is as elusive as its function, yet circular dichroism

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Table 1
Properties of the peptides subjected to molecular dynamics simulations.

	Residues	Charge	Ions added
Helix 1	1–13	−1	1 Na^+
Helix 2	14–31	0	–
Helix 3	32–48	+2	2 Cl^-
Helix 4	50–70	−1	1 Na^+
Helix 5	73–89	−3	3 Na^+

measurements indicate that it has a mostly alpha helical structure with no traces of β-sheet content (Egashira et al., 2011). The major portion of the SAA fibrils is made up of the first 66 or 76 N-terminal residues of mature SAA (Husebekk et al., 1985).

The clinical gastrointestinal (GI) manifestations of amyloidosis may mimic other diseases, such as IBD or colonic cancer (Chen et al., 2002; De Casa and Bocian, 1965; Ebert and Nagar, 2008). Deposits are most commonly located in the descending and rectosigmoid colon (Seliger et al., 1971). The most common findings radiologically are luminal narrowing, loss of haustrations and thickening of mucosal wall (Ebert and Nagar, 2008). Motility disorders can be present in the large intestine, initially they appear as constipation which progresses to diarrhea.

A careful preoperative assessment should be performed in patients with GI amyloidosis. These patients have a high risk for bleeding and poor wound healing following surgery (Mardinger et al., 1999). In bowel surgery anastomic dehiscence is a risk, it may be related to the presence of amyloid deposits in the resection margins (Johnson et al., 1982).

The main treatment of GI amyloidosis is to suppress the acute phase response and thereby reduce the production of SAA. Up to date there is no specific treatment for GI complications of amyloidosis available (Sattianayagam et al., 2009). Morbidities include gastrointestinal bleeding in up to 22% of this patient group (Kumar et al., 2001).

In this study we aim to investigate if the existing information about the structure can be extended by computational techniques, and if possible search for the core amyloidogenic sequence of SAA. A previous study proposed a model for SAA based on homology modeling based on a template with very low sequence identity to SAA, 18% to the first 66 residues and even less for the rest of the protein, which introduces a fair amount of uncertainty into the results (Stevens, 2004). The emergence of ab initio protein folding algorithms allows stepping outside the boundary of homologous proteins with experimentally determined structures in the prediction of protein structure (Zhang, 2008).

2. Methods

PSIPRED was used to predict the secondary structure of the sequence (Jones, 1999).

I-Tasser web server (Zhang, 2008) was used to build an ab initio model of the mature chain of SAA (Uniprot ID: P02735), ranging from amino acid 19 to 122, corresponding to 104 residues. The numbering scheme used in this paper will refer to the first residue of the mature chain as number 1.

Each helical region from the ab initio model, using the coordinates assigned to each residue from the model building process (see Table 1 for details) were subjected to molecular dynamics (MD) simulations in the GROMACS software version 4.5.5 (Van Der Spoel et al., 2005).

The OPLS-AA/L all-atom force field was used (Kaminski et al., 2001). Each system was neutralized by adding sodium or chloride ions until the systems net charge is zero, see Table 1 for charges and the number of added ions. A cubic box was used and the edges

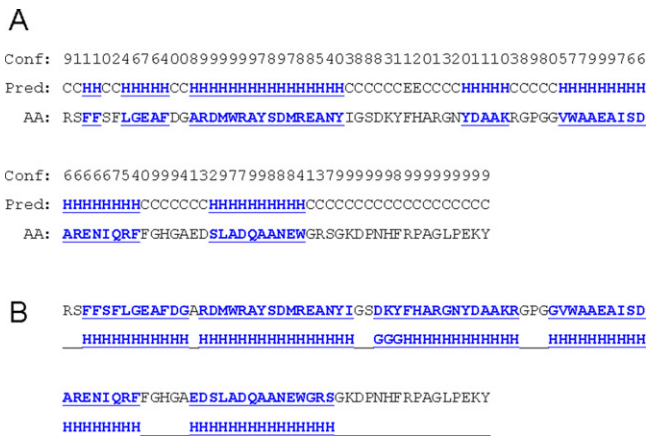

Fig. 1. (A) PSIPRED prediction of secondary structure for SAA. Conf: confidence of prediction, Pred: predicted secondary structure, C: coil, H: helix, E: strand. (B) DSSP calculated secondary structure of I-Tasser model; H: helix; G: 3/10 helix; _, coil.

distanced 1 nm from the protein and filled with SPC/E modeled water molecules (Kusalik and Svishchev, 1994).

The systems were energy minimized using position restraints and all were terminated due to fulfillment of the energy criteria, that the maximum force should be less than 1000 kJ/mol/nm, prior to that the maximum number of iterations were reached. The equilibration of the systems is divided in two phases, first by keeping the number of particles, volume and temperature constant for 100 ps. In the second part the simulation is continued for another 100 ps but this time the pressure is kept constant instead of volume. The equilibration also employs position restraints.

The MD analysis is run in triplicates for 10 ns for each system at 300 K, starting velocities are taken from the equilibration simulation and no position restraints are used. Each simulation was allowed to evolve individually by using different random numbers to initiate the calculations. The time step are 2 fs and all run associated data are stored each 2 ps. The simulation uses Particle-Mesh Ewald electrostatics (Darden et al., 1999), velocity-rescaling temperature coupling (Bussi et al., 2007) and Parrinello–Rahman pressure coupling (Parrinello and Rahman, 1981).

To further explore the conformational properties of each region, a longer MD simulation of 100 ns was performed, the only differing run parameter, except the longer timescale is that run associated data are saved each 10 ps.

The results are analyzed by GROMACS internal analytical tools and the DSSP algorithm for secondary structure assessment of the trajectories (Kabsch and Sander, 1983).

3. Results

The secondary structure predictions indicate an almost all helical protein with 5 helical regions, assuming at least 4 residues are required to form a helix. Helix 1 is predicted with low probability and could be extended toward the N-terminus where two residues with predicted coil conformation separate a two residue segment of helical residues. See Fig. 1A for the full prediction.

The highest scoring I-Tasser ab initio prediction with a C-score of −2.69, is shown in Fig. 2. A C-score of that magnitude indicates uncertainty of the correct topology of the model. The structure contains five helical regions that pack against each other to form a helical bundle. The structure is available as supplemental material to this article in pdb-format. In Fig. 1B the secondary structure content of the model determined by the DSSP algorithm is presented to allow for a direct comparison with the PSIPRED prediction.

In Fig. 3A is the average root mean square distance during the triplicate 10 ns MD simulations for each helical segment to its