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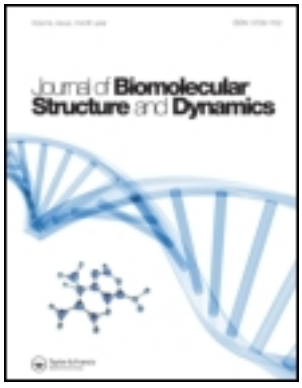

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Appendant structure plays an important role in amyloidogenic cystatin dimerization prior to domain swapping

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It has been hypothesized that prior to protein domain swapping, unfolding occurs in regions important for the stability of the native monomeric structure, which probably increases the possibility of intermolecular interaction. In order to explore the detailed information of the important unfolding regions in cystatin prior to domain swapping, 20 ns molecular dynamic simulations were performed at atomic level with typical amyloidogenic chicken cystatin (cC) mutant I66Q monomer under conditions that enable forming amyloid fibrils in biological experiments. Our results showed that I66Q mutant exhibited relatively large secondary structure changes and obvious expanding tendency of hydrophobic core compared to wild-type cC. More importantly, the appendant structure (AS) showed a large displacement and distortion towards the hydrophobic core in amyloidogenic cystatin. The structural analysis on cystatin monomer suggested that structural changes of the AS might make the hydrophobic core expand more easily. In addition, analysis on docking dimer has shown that the distorted AS was favor to intermolecular interactions between two cystatin monomers. Data from an independent theoretical derived algorithm as well as biological experiments also support this hypothesis.

Keywords: molecular dynamic simulation; domain swapping; appendant structure; amyloidosis; hydrophobic core

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Introduction

More than 20 proteins or their proteolytic products are now known to form amyloid fibrils in human proteins (Revesz et al., 2003). Human cystatin C amyloid angiopathy (HCCAA) is a dominantly inherited disorder characterized by tissue deposition of amyloid fibrils in blood vessels that leads to recurrent hemorrhagic stroke (Sanders et al., 2004). The leucine 68 to glutamine variant of human cystatin C (hCC L68Q) is highly amyloidogenic and can form amyloid deposits spontaneously in the brain arteries of young adults, while wild-type (WT) cystatin forms part of the amyloid deposits in elder patients (Abrahamson, 1996; Olafsson & Grubb, 2000). Besides, it has been reported that both L68Q and WT cystatins could bind to Aβ peptide and affect its amyloid fibril formation in Alzheimer's diseases (Kaeser et al., 2007).

Recent studies have shown that more than ten types of amyloidogenic proteins could form dimer through similar mechanism of 3D domain swapping and then form the amyloid depositions (Jaskolski, 2001). HCC is the first protein whose oligomerization has been proved to be formed via 3D domain swapping (Janowski et al., 2001). Native structure of each hCC molecule consists of a five-stranded anti-parallel β-sheet wrapped around a central helix: (N)-β1-(α)-β2-L1-β3-(AS)-β4-L2-β5-(C), where AS is a broad 'appendant structure' that is unrelated to the compact core of the molecule. Two native disulfide bridges, Cys73-Cys83 and Cys97-Cys117, are located in the C-terminal region of hCC. In morbid state, the members of cystatin dimer exchange β1-(α)-β2 domains with each other, and form a hybrid dimer, which is the basic unit of pathogenic amyloid fibrils. Each subunit of the dimer is composed of a β1-(α)-β2 domain contributed by one molecule and a β3-(AS)-β4-β5 domain contributed by the other protein (Wahlbom et al., 2007). Although an analogous domain swapping

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process, propagating in an open-ended fashion, has been suggested to be the fundamental mechanism of fibrils formation of cystatin amyloid (Janowski et al., 2001), the actual and detailed mechanism of domain swapping remains poorly understood. Recent studies have focused on the unfolding of the monomer before dimer formation. It has been hypothesized that in hCC L68Q, which is a variant in the hydrophobic core, the site mutation causes the destabilization of molecular α/β interface, leading to the structural rearrangements thus resulting in a higher probability of partial unfolding (Calero et al., 2001; Sanders et al., 2004).

Chicken cystatin (cC) has a number of similar structural characteristics to hCC and these two proteins share same canonical cystatin architecture (Calero et al., 2001; Sanders et al., 2004). However, one obvious difference between cC and hCC is that WT cC is thermodynamically stable and its monomers can present in physiological condition, while WT hCC is extremely unstable and easily become dimers which lead to forming amyloid deposits (Bjarnadottir et al., 2001). Thus, cC is more convenient than hCC in the simulated and experimental studies of cystatin dimerization. In our previous experimental study, we have found that the mutant cC I66Q (corresponding to the L68Q variant in HCC) and I108T (also a mutant in the hydrophobic core) showed a series of amyloid properties compared to WT cC (He, Song, Ueyama, Azakami, & Kato, 2005; He et al., 2006; Yu et al., 2010). Structural and dynamic properties of amyloidogenic mutant cC I108T were further investigated with MD simulations at the similar experimental conditions. It was found that the hydrophobic core showed expanding tendency in the unfolding process (Yu et al., 2010). Interestingly, we also found that AS of cC I108T had significant structural distortion with an untwisting inner α-helix compared to WT cC (Yu et al., 2010). Related study on the unfolding of the monomer prior to domain swapping suggested that unfolding occurs in regions that are important for the stability of the native monomeric structure, which probably leads to the subsequent errors in the folding pathway and increases the possibility of intermolecular interactions (Liu & Zhao, 2010). This probably provided a clue to explore the mechanism of cystatin domain swapping occurrence.

In this study, we performed MD simulations of amyloidogenic mutant cC I66Q and WT cC based on our previous researches (He et al., 2006; Yu et al., 2010). We expected to carry out an analysis on the secondary structural fluctuations/changes and further testify the hydrophobic core hypothesis. More importantly, we investigated the possible role of AS that might be one of the crucial domains in the monomeric unfolding process prior to domain swapping. Besides, we tried to explore whether the structural changes of AS could influence the intermolecular interactions between two monomers. In addition, results from MD simulations would be further verified with a theoretical model and biological experiments. These results might help to reveal the possible role of AS in the unfolding process prior to cystatin domain swapping and provide potent clues for exploring the potential critical domains in domain swapping associated with hydrophobic core.

Materials and methods

Construction of I66Q mutant cC monomer models

The model of the monomeric molecule used in our work was based on the X-ray crystal structure of coordinate monomeric cC (Protein Data Bank [PDB] entry 1CEW), which was obtained from RCSB PDB (Bode et al., 1988). The model of I66Q monomer was constructed using homologous modeling method through the online service of Swiss Model (http://swissmodel.expasy.org/) (Arnold, Bordoli, Kopp, & Schwede, 2006; Guex & Peitsch, 1997).

Molecular dynamics (MD) simulations

All MD simulations were performed using Gromacs 4.0 program (Guex & Peitsch, 1997; Humphrey, Dalke, & Schulten, 1996; Kabsch & Sander, 1983; Massi, Klimov, Thirumalai, & Straub, 2002) at the Supercomputer Center of Liaoning University on dual-core Pentium 2.8G processor of Linux cluster. GROMOS96 43a1 (Bonvin, 2006) force-field parameters were used in all simulations in this study. The integration time steps were 2 fs throughout the whole simulations. A cut-off of 0.9 nm was used for the van der Waals interactions. Long-range electrostatic interactions were calculated with the particle mesh (Ewald method Dobson & Fersht, 1995; Ventura et al., 2004) with a maximum Fast Fourier Transform (FFT) grid spacing of 0.12 nm and cubic interpolation order. Periodic boundary conditions were activated in the simulations. Bonds containing hydrogen atoms were constrained using the SHAKE algorithm with a relative tolerance of 10^{-8}.

Each monomer model was solvated in a water box containing 10,124 SPC water molecules (Ivanova, Sawaya, Gingery, Attinger, & Eisenberg, 2004) and neutralized by adding 14 Cl^- counterions. The minimum distance of a protein atom to the edge of the water box was 1 nm. The solvated and neutralized systems were then energy minimized for 20 ps. Afterwards, the backbone atoms of the structure were fixed, while the side chains and solvent were allowed to move unrestrainedly for further 10 ps, followed by totally unrestrained equilibration for 12 ps, at constant temperature 300 K and 1 atm pressure within a water box using periodic boundary conditions in the NPT ensemble (Isobaric-Isothermal ensemble). After equilibration, the productive run was carried out for 20 ns with the temperature kept constant at 330 K. In all simulations, the temperature was main-

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tained close to the intended values by weak coupling to an external temperature bath with a coupling constant of 0.1 ps. The protein and the rest of the system were coupled separately to the temperature bath. Both of atomic coordinates and atomic velocities were saved every 1 ps. Trajectory coordinates were saved every 0.5 ps for subsequent data analysis.

Automated docking

The online service ZDOCK (http://zdock.bu.edu/) was used to perform docking calculations between two cystatin monomers. ZDOCK searches all possible binding modes in the translational and rotational space between the two monomers, and evaluates each by an energy scoring function. No residue in two proteins was chosen to keep in or out of the binding site. The resulting data were taken from the docking experiments in which the lowest total docking energy (−5.476606e+02) was obtained.

Analysis of the trajectories

The secondary structure of the protein was determined by DSSP program (Kabsch & Sander, 1983). Visual Molecular Dynamics (VMD) and Pymol program (Humphrey et al., 1996) with home-developed scripts were used for system visualizations and other analysis.

Steered molecular dynamics (SMD) simulations

The SMD simulations with constant velocity were performed (Kolodziejczyk et al., 2010). During the SMD simulations, the force was only applied along the pulling direction, which was assigned on disulfide bond for the effective separation of the bond. The trajectories were saved for every 0.5 ps, and steering forces were recorded every 20 fs. Each trajectory along the same pathway was repeated three times.

Theoretical model and algorithm

This algorithm is based on the rules of protein topological information and evolution (Liu & Zhao, 2009, 2010) and comprises three steps: (1) identify remote homologs of the query polypeptide in the GPR; (2) evaluate the opportunity for interchange between the helix-donut and strand-arc zones; and (3) filter of conformational changes allowable under normal conditions (Liu & Zhao, 2010).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting

SDS-PAGE was run according to the method of Laemmli using 15% acrylamide separating gel and 5% stacking gel containing 1% SDS[14]. Electrophoresis was carried out at a constant current of 20 mA for 1.5 h using an electrophoretic Tris-glycine buffer containing 0.1% SDS (He et al., 2006). Western blot analysis was performed using standard methods (He et al., 2006) with Rabbit anti-cC polyclonal antibody.

Results and discussion

Dynamics of I66Q and WT cC secondary structural changes

In our previous experimental study, cystatin fibrils were found favored to be produced in solutions of pH 2, 57 °C (He et al., 2005, 2006). In addition, it has been proved that in MD simulations, high temperature and low pH value can accelerate protein unfolding without changing the pathway of unfolding (Day, Bennion, Ham, & Daggett, 2002; Yu et al., 2010). Therefore, we performed the 20 ns simulations under the extreme conditions with an elevated temperature of 330 K (57 °C) and pH 2 to make the system reach the equilibrated regions at a minimum of simulation time and computational expense. The overall stability of cC monomers was evaluated using the Cα

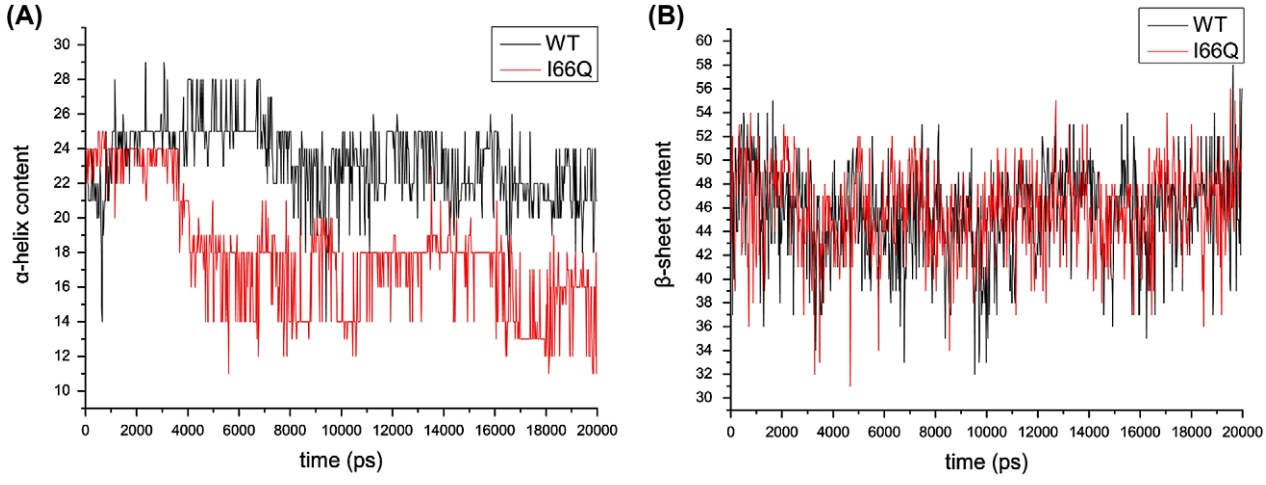

Figure 1. Contents of α-helix (A) and β-sheet (B) in I66Q mutant (red) and WT (black) cC as a function of simulation time.

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root-mean-square deviation (RMSD) relative to the corresponding initial structure. The RMSD value reached a relative equilibrium region at 3 ns in both I66Q and WT cC (data not shown). After 3 ns, the overall RMSD value of I66Q mutant was 0.32 nm, higher than that of WT cC (0.25 nm). The increased structural change during the MD calculations of cC I66Q suggested a secondary structure change, which was probably related to amyloid fibril formation.

Generally, a typical characteristic of amyloid protein is the β-sheet content increasing while α-helix content decreasing in the secondary structure (Janowski et al., 2001). In order to explore detailed information on the secondary structure changes of cC I66Q during the whole simulations, we defined the content of either α-helix or β-sheet as a function of simulation time by DSSP algorithm (Figure 1). The α-helix content in cC I66Q dramatically decreased to 14% (15 residues), while it was slightly altered in WT cC (20%, 22 residues). This simulation result was well corresponding to the X-ray crystallography and CD spectroscopy data in previous experimental studies (Table 1). On the other hand, the β-sheet content was not altered obviously both in I66Q (43%) and WT cC (44%), although the β-sheet content showed dramatic fluctuations between 31 and 58 residues during the whole 20 ns simulations. Interestingly, the experimental data showed that the β-sheet contents of I66Q and WT cC were 42 and 45%, respectively (Table 1). One possible explanation to the difference between the simulation and experimental result is that the β-sheet structure might be affected by certain changing structural factors which keep the dynamic equilibrium of the β-sheet forming and unfolding process in our single monomer MD simulation system. It seems that the dynamic equilibrium of I66Q cC may tend to β-sheet forming through intermolecular interaction in multi-molecule system, while the dynamic equilibrium of WT cC may tend to β-sheet unfolding. In our parallel experiment, another amyloidogenic mutant cC I108T also showed similar characteristics (Yu et al., 2010). Thus, the decreasing of α-helix and the dramatic fluctuations of β-sheet might be the basis of intermolecular domain swapping of cystatins.

The hydrophobic core had an apparent expanding tendency in I66Q cC

The interior hydrophobic core of cystatin provides the structural stability favoring the native state during the amyloid protein unfolding. To analyze the behavior of the hydrophobic core in amyloidogenic mutant cC I66Q, the structure drift of the hydrophobic core throughout whole simulations was determined. It was found that although both hydrophobic cores of I66Q and WT cCs were in a relatively stable state, the hydrophobic core of I66Q was more flexible and freer (Figure 2). The accessible surface areas of the hydrophobic core of I66Q mutant was 37.49 nm^2, larger than that of WT cC (30.59 nm^2). As expected, these properties of cCs was coincided with the previous studies of their human homologs (Liu et al., 2007). In addition, this expanding tendency of I66Q hydrophobic core was more notable than that of another amyloidogenic mutant cC I108T shown in our previous study (Yu et al., 2010).

The structure of AS showed obvious deviation and distortion in I66Q cC

The mutation in the hydrophobic core induced the increasing instability of the amyloidogenic mutant I66Q and caused more apparent amyloidogenic characteristics on secondary structure compared to WT cC. In order to find the crucial domain which induces the instability of I66Q cC, we analyzed the residue averages of the root mean square fluctuations (RMSF) during the MD simulations. The RMSF values were basically similar in most residues of both I66Q mutant and WT cC, except for the residues in the AS region (Figure 3(A)). Quantitative analysis showed that α2-helix which involved in AS was nearly destroyed in I66Q (decreased from 6 residues to 0, calculated by DSSP algorithm), while it remained unchanged in WT cC (six residues at the final state). Therein α2-helix unfolding into coil was responsible for the holistic α-helix content decreasing in I66Q cC. The similar activity of AS was also observed from another amyloidogenic mutant I108T (Yu et al., 2010).

To obtain more information about the behavior of AS in I66Q, we applied a visual analysis by superimposing alignment with VMD program between I66Q and

Table 1. Content of α-helix and β-sheet in MD simulations compared with the experimental data.

	MDs[a]		Experimental[b]	
	α-helix content (%)	β-sheet content (%)	α-helix content (%)	β-sheet content (%)
WT	20	44	21[c]	42[c]
I66Q	14	43	14[d]	45[d]
I108T	16	43	17[d]	48[d]

[a]330 K, pH = 2.
[b]298 K, pH = 7.
[c]The data determined by X-ray (Bode et al., 1988).
[d]The data determined by CD spectroscopy (He et al., 2005, 2006).

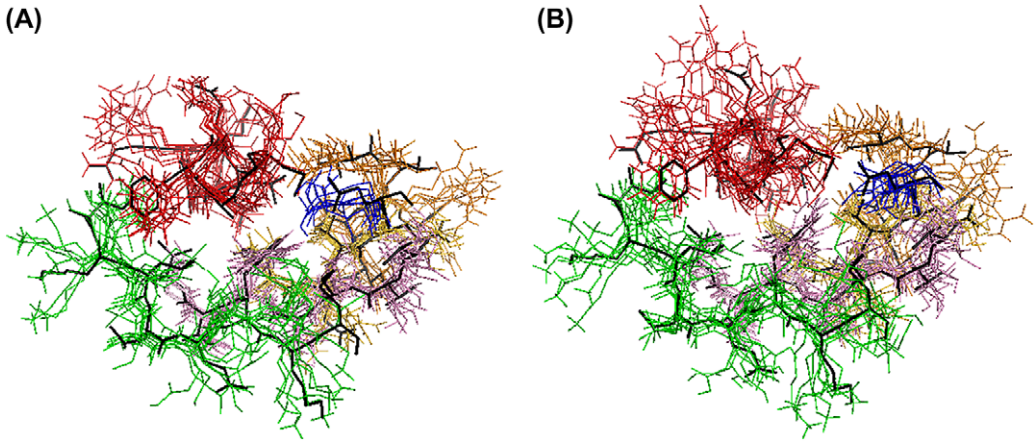

Figure 2. Superimpositions of the hydrophobic core snapshots of WT cC (A) and I66Q cC (B), respectively (black for the reference structure, red for α1, orange for β2, gold for β3, pink for β4, green for β5, and blue for the 66 residue).

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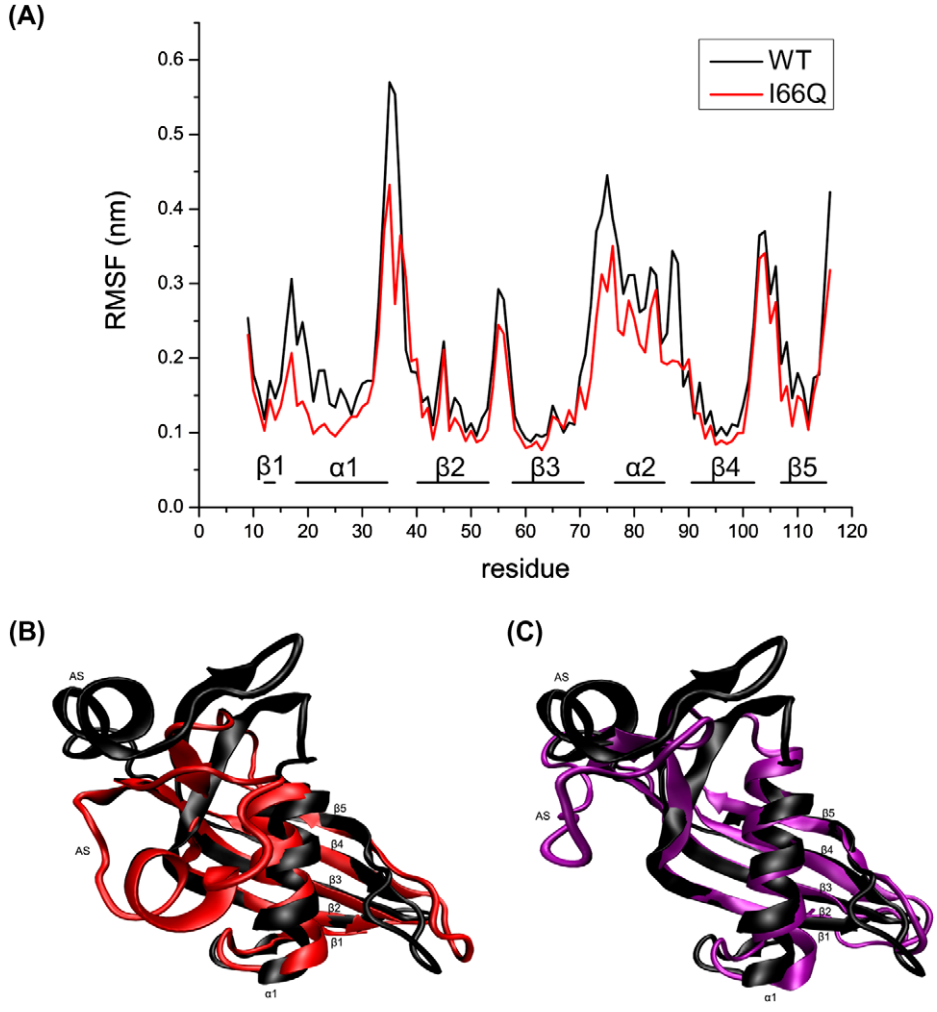

Figure 3. (A) The residue averages of the RMSF for the Cα atoms of I66Q (red) and WT (black) cC during the 20 ns MD simulations; (B) superposition of the Cα atoms of WT (black) and I66Q (red) cC structures at the end of the simulation; and (C) superposition of the Cα atoms of WT cC (black) and WT hCC (purple) structures.

WT cCs. It was shown that AS of cC I66Q had significant structural distortion to the center of globular protein with an inner α-helix untwisting compared to the WT cC (Figure 3(B)). The residue shifts of AS were further calculated and found that AS in I66Q had a large displacement with 0.26 nm referred to WT cC. The large displacement of AS was also observed in another amyloidogenic mutant I108T (0.27 nm) (Yu et al., 2010), though they were not completely the same. Interestingly, the result of structural alignment has shown that AS in WT hCC monomer (constructed using homologous modeling method based on 3GAX.pdb (Kolodziejczyk et al., 2010) and performed with 10 ns MD simulation to reach the equilibration in our study) mainly adopted a random coil structure and distorted towards its hydrophobic core compared to its chicken homologs (Figure 3(C)). Together, these observations give a hint to explore the possible role of the AS in cystatin dimerization.

The distorted conformation of AS in amyloidogenic cC could increase the contact between AS and the hydrophobic core

Previous study has shown that the AS is a slightly hydrophobic structure (Yu et al., 2010). This suggests that if the distorted AS is close enough to the hydrophobic core of the protein, it will be very likely to induce the instability of the hydrophobic core in amyloidogenic mutant I66Q. As shown in Figure 4(A), the residue distance between AS

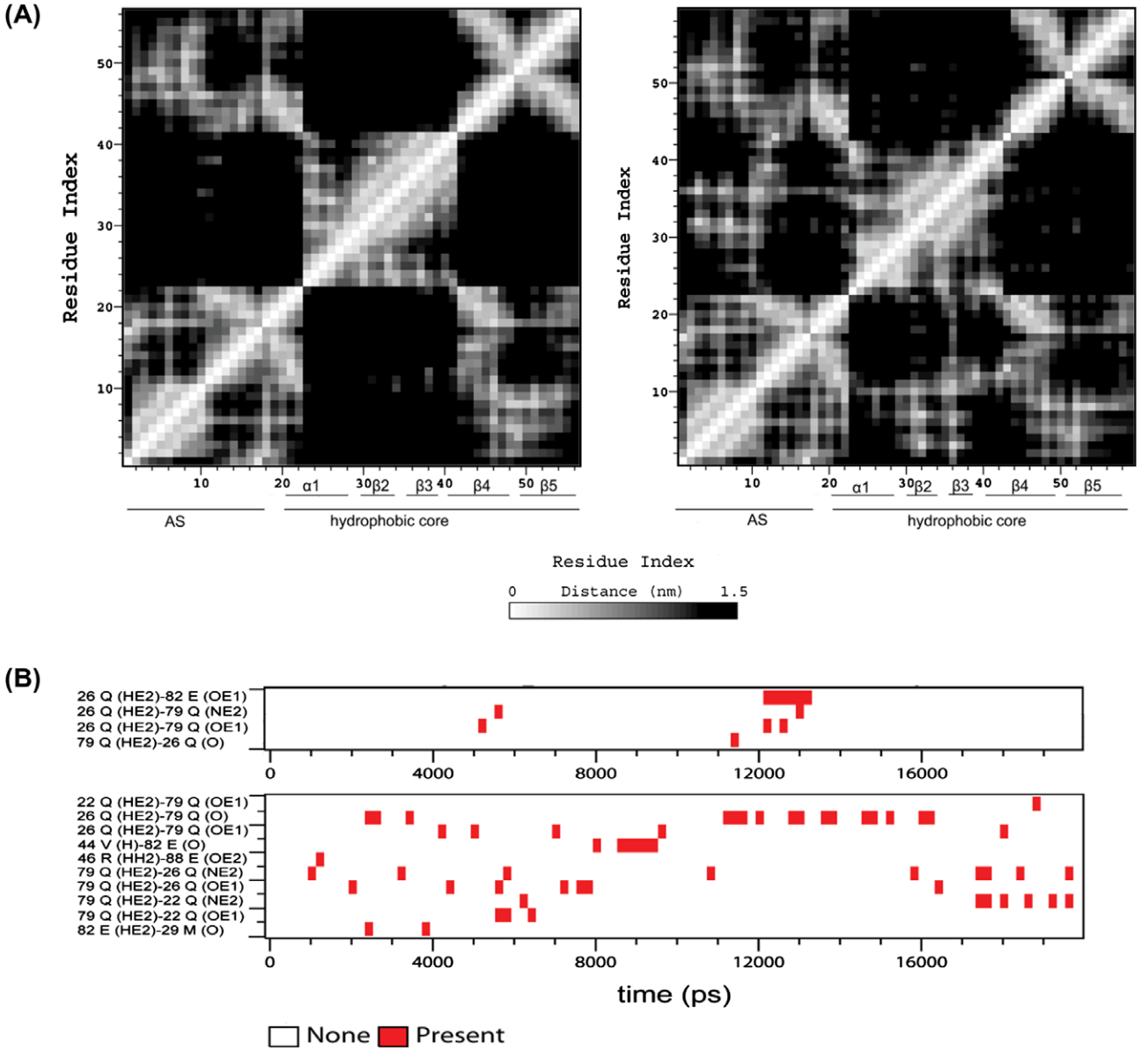

Figure 4. (A) Distance matrix intramolecule in WT cC (left) and I66Q cC (right) and (B) existence of possible hydrogen bonds between AS and hydrophobic core residues during MD simulations.

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and the hydrophobic core was obviously shorter in I66Q than that in WT cC. Quantitatively, the number of contacted residues within a cut-off distance of 0.6 nm (defined according to the radial distribution function(Massi et al., 2002) in I66Q was 6 pairs, whereas no such contacted residues has been found in WT cC. Furthermore, we analyzed the hydrogen bonds between AS and hydrophobic core residues during MD simulations. The number of hydrogen bonds was 10 in I66Q, but only 4 in WT cC. Also, most of the hydrogen bond occupancy in I66Q was longer than that in WT cC during the MD simulations (Figure 4(B)). These results indicated an increased contact between AS and residues in β1-α1-β2-β3 of hydrophobic core in mutant cC I66Q. Since mutation in site 66 has already induced instability of hydrophobic core, the increased contact between AS and the hydrophobic core would aggravate such instability, which might influence the subsequent domain swapping occurrence.

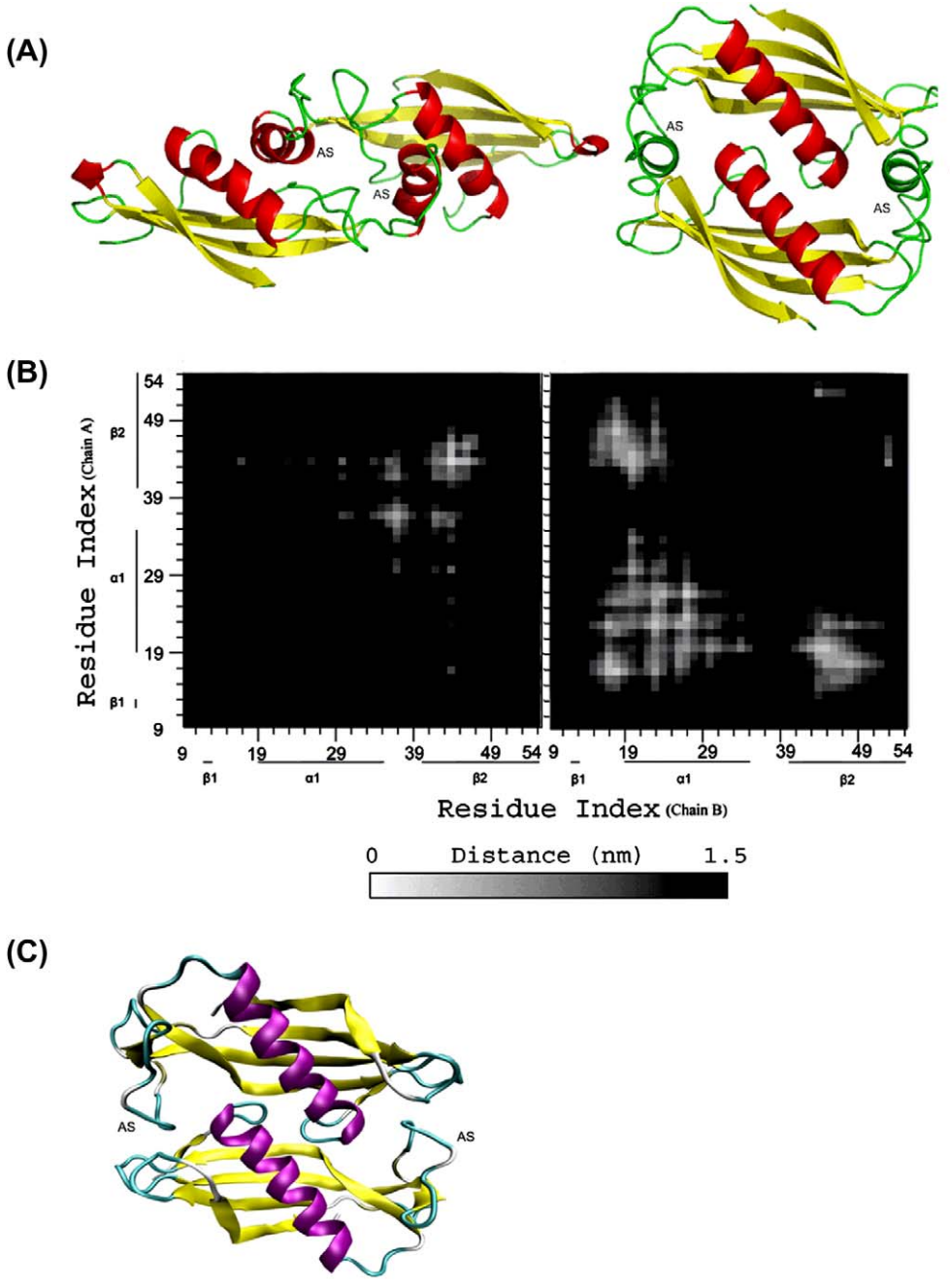

Figure 5. (A) Docking structure of WT (left) and I66Q (right) cCs; (B) distance matrix of β1-α1-β2 group between chain A and chain B in docking dimer of WT (left) and I66Q (right) cCs; and (C) docking structure of WT hCCs.

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The distorted conformation of AS in amyloidogenic cC had a tendency to increase the intermolecular contact between two monomers

Docking has been widely used for predicting the 3D structure of a complex based on its known constituents. To obtain more information about AS in the process of dimerization, we performed the docking calculations between two cC monomers (named as chains A and B in the docking dimer structure) after simulations. This would give us some information about whether the distorted conformation of AS in amyloidogenic cC had a tendency to increase the intermolecular contact between two monomers.

Docking structure with the highest score (lowest total docking energy) was selected. The AS, located in the center of the docking structure of WT cC, would hinder the two monomers approaching and cause a steric effect that influenced the interactions between the two monomers (Figure 5(A), left). However, in the docking structure of I66Q, the large displacement and distortion of AS could give much spatial room to allow the two β1-α1-β2 groups get close to each other (Figure 5(A), right). The distance between two β1-α1-β2 groups was further calculated for each residue in the docking structure. The residue distance in I66Q mutant was obviously much shorter than that in WT cC, indicating that intermolecular contacts between residues in I66Q were much more than those in WT cC (Figure 5(B)). Quantitatively, the number of contacted residues within a cut-off distance of 0.6 nm in I66Q was 18 pairs, which is six times higher than that in WT cC (three pairs). It was noteworthy that in the docking dimer of I66Q, the hydrophobic residues of AS could form a new small hydrophobic core with β2 domain of the other monomer, which might have a competitive effect with the old expanded hydrophobic cores. This effect would be helpful for the monomer unfolding and dimer formation in multi-molecule system if this docking dimer had existed in reality.

Interestingly, the similar observation as cC I66Q was also obtained from WT hCC docking dimer which had the highest score (Figure 5(C)). It is notable that the X-ray crystal structure of hCC dimer (1G96) has shown that AS in one molecule distorted to the hydrophobic core and the α2-helix in AS is also absent (Janowski et al., 2001), which is similar to those in amyloidogenic cC found in our study. This suggested that the role of distorted AS structure in amyloidogenic cystatin could be conserved from chick cystatin to its human homolog. Also, this might give a hint to explain why WT hCC has easily become a domain-swapped dimer, which leads to the formation of amyloid deposits, but WT cC is thermodynamically stable.

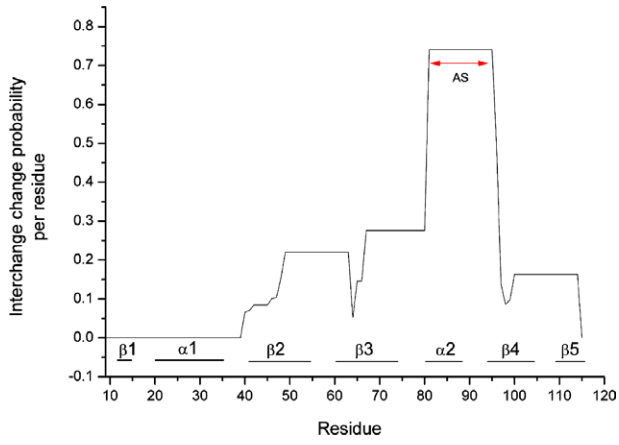

Figure 6. The per residue capability of cC native structure in switching on pathogenic protein misfolding predicted by independent algorithm. Coordinates cC monomer structure (PDB code: 1A67_A) was used as input file for prediction. The predication was insensitive to slight homologous difference. Region with the highest interchange change probability was predicted to be sites where pathogenic conformational changes were triggered.

An independent investigation predicted that switch region of cC was involved in AS

It is interesting to find that AS might be one of the key domains which affect the dimerization of cC from the study of MD simulations. Combined with other previous studies, it was suggested that both AS and the hydrophobic core might be important for cC's amyloid properties. In our recent research, it was found that amyloid protein misfolding was triggered at certain region which can be predicted with an algorithm based on the basic rule of protein topological information and evolution (Liu & Zhao, 2009, 2010). It is noteworthy that this switch region prediction method is suitable for most proteins in body fluid and with a high accuracy of 94% (Liu & Zhao, 2010). Thus, in order to verify the finding obtained above, we further investigate the switch region of cC with this independent theoretical deriving approach. Miraculously, it was shown that both AS and the hydrophobic core of cC were essential and especially residues 81–95 involved in AS were predicted to be the

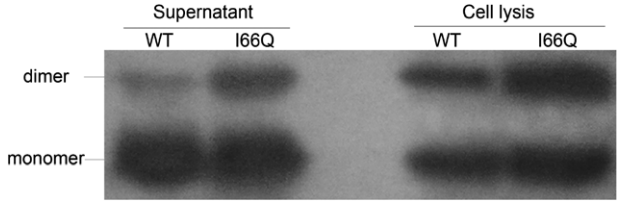

Figure 7. Western blot analysis on supernatant and cell lysis with anti-cystatin polyclonal antibodies. WT, yeast expressed wild recombinant cC; I66Q, yeast expressed wild recombinant cC mutant I66Q. The ratio of dimer to monomer was indicated.

switch region of cC in triggering pathogenic protein misfolding (Figure 6). The results of our MD simulation and the prediction were matched with each other, and strongly suggested the important role of AS in amyloidogenic cC. As the disease is due to the aggregation of morbid cC dimer, we believe that AS plays a vital pathogenic role through influencing the binding mode of protein–protein interaction.

Experimental investigation indicated that dimers can be formed more easily between unfolded cC

As mentioned above, an unintelligible phenomenon between cC and hCC is that WT cC is thermodynamically stable and its monomers can present in physiological condition but hCC cannot (Bjarnadottir et al., 2001; Sanders et al., 2004). Even though Szymańska et al. (2009) had speculated that the turn-formation propensity of WT hCC might contribute to their ability to form domain-swapped dimers, why cC is not easy to dimerize is still unknown. Based on our assumption about the role of AS in dimerazation, it seemed that the unfolded or misfolded WT cC would more easily form dimer than its mature form does. To verify this deduction, we performed molecular biological experiment using yeast modeling system expressed recombinant cC. The transformed yeasts carrying WT and mutant I66Q cDNAs were cultivated at 30 °C for 3 days, and then the supernatant of the cultivation medium and cell lyses were directly subjected to SDS-PAGE respectively. Western blotting results showed that in contrast to amyloidogenic cC I66Q, less dimers were detected for WT cC protein samples as expected (Figure 7). Interestingly, our data also showed that dimers from cell lysis

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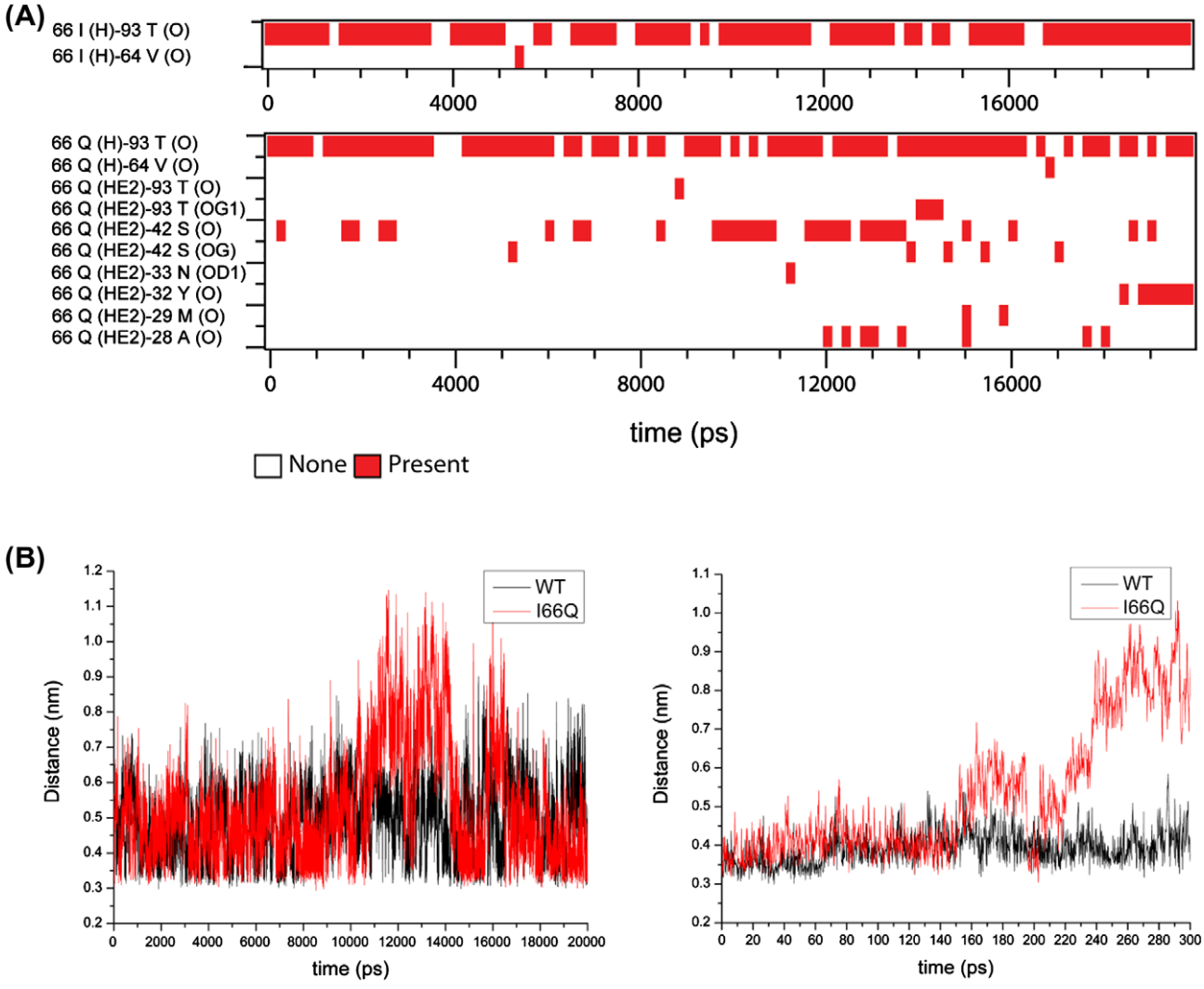

Figure 8. (A) Existence of possible hydrogen bonds between residue 66 and adjacent residues during MD simulations; (B) the disulfide bond distance between Cys71 and Cys81 as a function of MD simulation time; (C) the disulfide bond distance between Cys71 and Cys81 as a function of cv-SMD simulation time.

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were much more than those from supernatant both in WT cC and I66Q mutant. Since only matured protein could be secreted into cultivation medium and unfolded/misfolded protein should be remained in the cell, this result indicated that unfolded structure of cC was beneficial for its dimerization.

Possible reason which led to the distortion of AS domain in I66Q cC

Site 66 is far distant from the AS both in sequence and in structure, which indicates that I66Q mutation is unlikely to cause the significant changes of AS directly. Hence it becomes crucial to explore how Gln 66 interacts with adjacent residues and how these residues contribute to AS distortion, or if the amino acid substitution disturbs the hydrogen bond network of cC. As shown in Figure 4 (B), both the number and existence time of hydrogen bonds between AS and hydrophobic core residues in I66Q mutant were obviously more than those in WT cC. Also, the hydrogen bonds around Gln66 in I66Q mutant has been increased approximately six times compared to original WT cC (Figure 8(A)). These results might give some hints to explore the detailed mechanism. Moreover, it is noteworthy that the disulfide bond within the AS (Cys71-Cys81) in cC I66Q was dramatically fluctuated from 10 ns, while that in WT cC was relatively stable (Figure 8(B)). In order to further analyze the strength and stability of this disulfide, a constant velocity SMD (cv-SMD) approach was introduced in this study. It was found that the stability of the disulfide bond Cys71-Cys81 in I66Q was apparently lower than that in WT cC (Figure 8(C)). We assumed this might be one important reason which led to the distortion of AS domain.

Conclusion

Protein aggregation is one of the typical features of many endogenous conformation diseases. The aggregation-prone regions, which usually tend to be blocked in the native state (Dobson, 1999), determine the tendency of proteins to aggregate and form amyloid fibrils (Ivanova et al., 2004; Ventura et al., 2004). In the first step of such pathogenic structural changes, misfolding occurs in domains important for the stability of the native structure. This process destabilizes the normal protein conformation in monomer, while exposing the previously hidden aggregation-prone regions, leading to the subsequent errors in the folding pathway which would increase the intermolecular aggregation of the protein (Liu & Zhao, 2010).

In this study, we performed the 20 ns MD simulations on cC to investigate the unfolding process prior to domain swapping. A major finding was that the distorted AS domain shifted close to the expanded hydrophobic core in amyloidogenic cC I66Q monomer compared to WT cC, which is probably caused by the disulfide bond strength changes. Our data suggested that the distortion of AS with large hydrophobic surface to the hydrophobic core is likely to enhance the hydrophobic interactions between them and make the hydrophobic core expand more easily. The displacement and distortion of AS seem to give much more room to the two intermolecular β1-α1-β2 groups and increased the contacts between two monomers in the docking dimer. In addition, the MD studies of hCC and another amyloidogenic cC I108T monomer have shown the similar phenomenon. Moreover, the prediction of theoretical deriving and the result of 'wet' experiment were in accordance with our assumption obtained from MD simulations. Totally, these results suggested that the AS could be an important switch region in dimerization of cC besides the hydrophobic core.

Nevertheless, our study is still limited to cC monomers prior to domain swapping. To confirm our hypothesis and address more detailed mechanism about the role of the AS domain in cC dimerization, it is necessary to screen a series of related mutants to find the key factors using more quantitative MD simulation analysis approaches (e.g. free energy, potential energy, and water molecular interaction) and biological experiments.

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