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Role of domain interactions during the amyloid formation of yeast phosphoglycerate kinase

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ABSTRACT Beta-amyloids are known to be the cause of an increased oxidative stress, which manifests in a higher rate of membrane lipid oxidation in some diseases. There are several proteins that are built up of two structural domains and are deposited full-length in amyloid plaques formed during different diseases. Several publications prove the role of the domain-domain interactions in protein folding, but the effect of the domain interactions on misfolding and amyloid formation has not been tested yet. In this work we show the importance of the inter-domain interactions in amyloid formation. A model protein system based on mutants of the two-domain protein yeast phosphoglycerate kinase was used to study the role of domain interactions in the amyloid formation of multi-domain proteins. After the initiation of the amyloid formation, tryptophan fluorescence spectroscopy was used to detect the structural changes of the two domains from 5 minutes to 4 days. We compared the kinetics of amyloid formation of the individual domains with that of the intact protein. For all mutants, electron micrographs proved the formation of amyloid fibrils after 5 days. We found that the aggregation-coupled conformation changes of the two domains are synchronized in the protein through the domain-domain interactions.
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KEY WORDS

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An important general property of all proteins is that they can fold into their enzymatically active structure. The three-dimensional structural information is coded linearly in the amino acid sequence of the protein. The formation of the native state is guided by non-local interactions between the amino acid side chains. Under stress conditions non-native structures can also form. These misfolded proteins are often prone to aggregate and under certain circumstances instead of being repaired or cleaned up with the help of chaperones and proteases, they can accumulate into protease resistant fibrils or plaques called amyloids. Beta-amyloids were found to be the cause of an increased oxidative stress, which manifests in a higher rate of membrane lipid oxidation in some diseases (Nelson and Alkon 2005). The deposition of amyloid plaques was observed in several diseases (e.g. Creutzfeldt-Jacob disease, Alzheimer disease, Parkinson disease, type II diabetes), and it can lead to tissue degradation and loss of organ function (Serpell et al. 1997). Only a small number of proteins was found to form amyloid plaques *in vivo*. Some of these formed amyloids *in vitro* as well (Dobson 2001). Recently, several non-pathogenic proteins were found to be able to grow amyloids *in vitro* under conditions that strongly destabilize the native structure and favor hydrogen bonding (Fädrich et al. 2001). It has been suggested that the ability to form amyloids under appropriate conditions is a property common to many if not all proteins. Amyloid structures are stabilized by hydrogen bonding between the atoms of the polypeptide backbone as opposed to the interactions between the amino acid side chains stabilizing the native state, thus amyloid formation reflects a polymer nature of the proteins. Some of the proteins observed to be deposited full length *in vivo* in amyloid plaques contain two differently structured and interacting domains. The prion protein, connected to the Creutzfeldt-Jacob disease consists of a structured domain and a second one apparently lacking well-ordered structure. The lysosome deposited in familial amyloidosis and the Ure2 yeast prion protein are both comprised of two interacting structured domains.

Damaschun et al. (2000) have shown that the non-disease-related yeast phosphoglycerate kinase (PGK) is able to form amyloid fibrils *in vitro*. The presence of amyloids was proved by electron microscopy and X-ray scattering a few days after the addition of 190 mM NaCl to the protein denaturated in 10 mM HCl.

Yeast PGK is a 415 residue large monomeric protein. The native state is built up of two domains about equivalent in size, linked by a helical hinge. It has a combined total of 15 α-helices and 15 β-strands. The structure of the domains is similar, consisting of a core of a six-stranded parallel β-sheet that is surrounded by a series of helices, in addition, the C terminal domain has three shorter β-strands. Far UV circular dichroism measurements indicated that this structure is replaced during misfolding and amyloid formation with a fold

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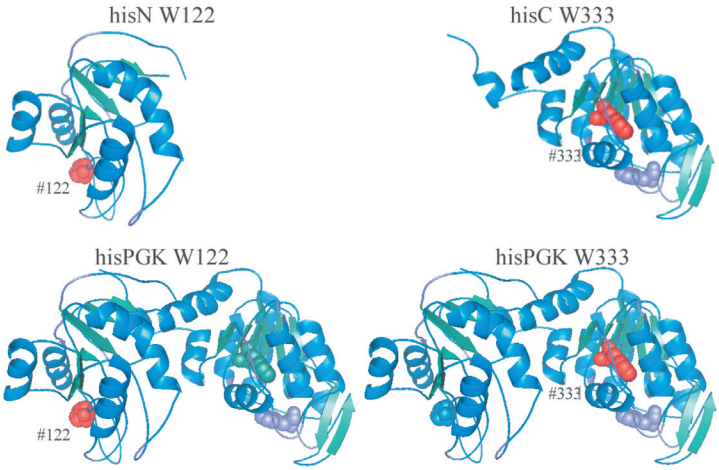

PGK WT	hisN W122	hisPGK W122	hisC W333	hisPGK W333
full sequence	*residues 1-185*	full sequence	*residues 186-415*	full sequence
No his-tag	*his-tagged*	*his-tagged*	*his-tagged*	*his-tagged*
Y122	*W122*	*W122*		Y122
W308		*F308*	*F308*	*F308*
W333		*F333*	*F333*	W333

Figure 1. 3D representation of the PGK variants used in our model protein system (Watson et al. 1982). The location where the single tryptophan reporters were introduced is indicated in red. Constructing the model system involved the mutation of the residues Trp 308, Trp 333, and Tyr 122, these are shown as "spacefill". Alpha helices are colored blue, and beta sheets green. Sequence differences between the wild type yeast PGK and the mutants are listed in italics in the table.

that has significantly higher β-sheet content. Enrichment of β-sheet structure is a general feature of amyloid growth.

Several studies address the role of domain stability and inter-domain contacts in the folding reaction. Yeast PGK has been used successfully as model for the investigation of domain interactions (Beechem et al. 1995). Despite of its possible importance, the effect of the domain interactions on misfolding and amyloid formation has not been tested yet. Here we show that contacts between the two domains of PGK have a strong effect on the misfolding pathway during amyloid formation. These also influence the details of the final fibrillar structure formed.

Materials and Methods

Mutants of the histidine tagged variant of yeast PGK (hisPGK), the histidine tagged variant of the N terminal domain (1-186) of yeast PGK (hisN) and the histidine tagged variant of the C terminal domain (187-412) of yeast PGK (hisC) were constructed, expressed, purified and stored as described earlier (Osváth et al. 2003).

The mutants were acid unfolded by dialysis against 10 mM HCl prior to the experiment. Misfolding was initiated by the addition of 200 mM NaCl to the acid unfolded (10 mM HCl) protein. Kinetic changes were recorded using tryptophan fluorescence from 5 minutes to 4 days. Fluorescence spectra and manual mixing experiments were measured on an Edinburgh Analytical Instruments C-900 luminometer (Edinburgh, Scotland, UK).

For every point of the kinetics, the fluorescence emission spectrum was recorded from 300 nm to 400 nm with 0.5 nm steps. Fluorescence was excited at 295 nm with 5 nm spectral width while detection bandwidth was 1 nm.

Results

A model system (Fig. 1) comprised of two mutant pairs of single tryptophan mutants of yeast PGK was used to investigate the importance of the domain interactions in amyloid formation. The model system comprises single tryptophan mutants of the isolated N and C domains and the corresponding mutants of the intact protein. Fluorescence measurements allowed the comparison of the structural changes in the vicinity of the tryptophan residues of the isolated domains and that of the whole protein during the amyloid formation.

Misfolding was initiated by adding 200 mM NaCl to the protein unfolded in 10 mM HCl. Following this, amyloids grew from all variants of the yeast PGK studied in this work. The presence of amyloid fibrils was checked after 5 days using electron microscopy. The amyloids visualized this way were of roughly identical size for all mutants. Figure 2 shows the spectral shift accompanying the misfolding, aggregation and fibril formation process 17 minutes, 3 hours and 4 days after initiating amyloid formation.

In order to describe quantitatively the time dependence of the shift of the spectrum during amyloid formation, the ratio of the fluorescence intensity integrated between 310-320 nm and 360-370 nm was calculated from the individual spectra.

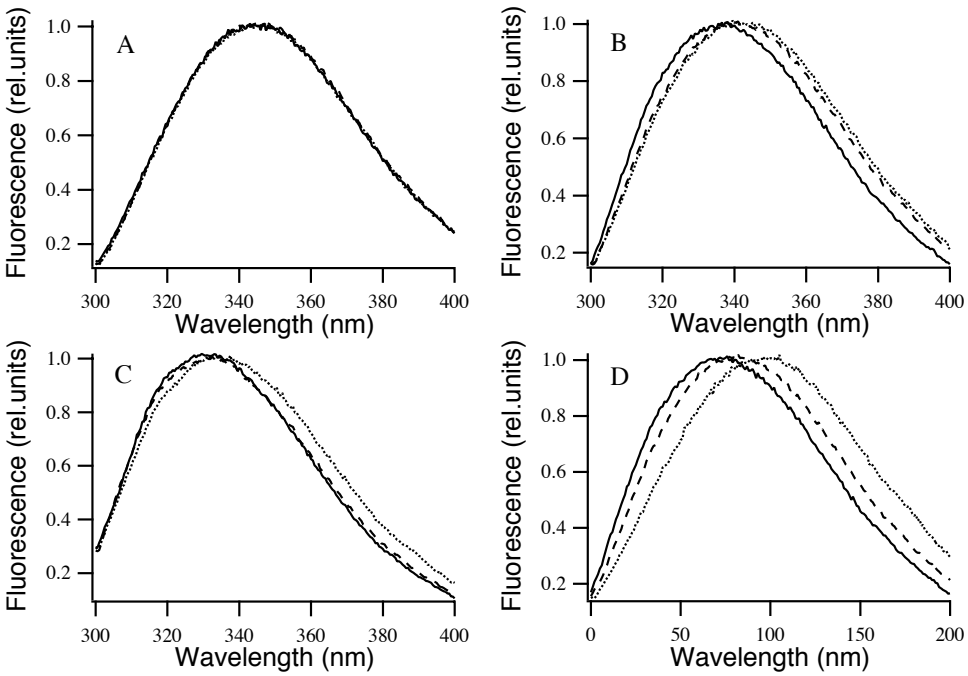

Figure 2. Changes of tryptophan fluorescence emission spectra during the misfolding and amyloid formation of hisN W122 (A), hisC W333 (B), hisPGK W122 (C) and hisPGK W333 (D). Spectra were recorded 17 minutes (dotted line), 3 hours (dashed line) and 4 days (continuous line) after initiating misfolding by the addition of 200 mM NaCl to the samples containing 80 ± 4 μM acid unfolded protein. Tryptophan fluorescence was excited at 295 nm with a spectral width of 5 nm and detected with 1 nm bandwidth.

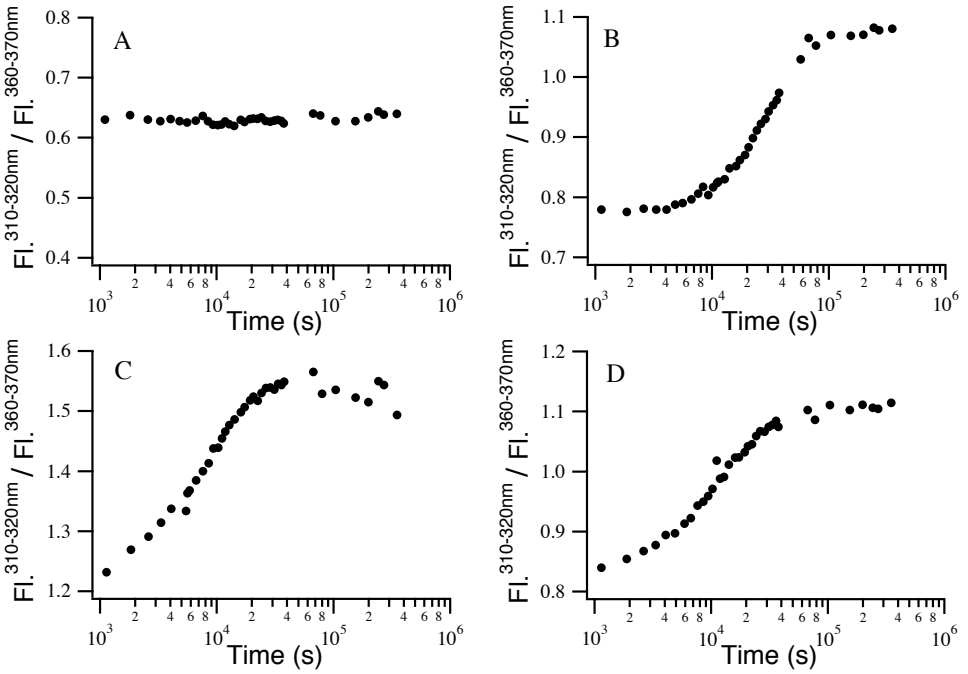

Figure 3. Conformation changes in the four mutants of the model system during amyloid formation. Structural changes were monitored by the blue shift of the fluorescence emission of the single tryptophan residues quantified as the ratio of the intensities emitted in the 310-320 nm and 360-370 nm regions. Fluorescence was excited at 295 nm with 5 nm bandwidth and detected with 1 nm spectral width.

Figure 3 shows the time dependence of the calculated ratio from 5 minutes to 4 days. hisPGK W122, hisC W333 and hisPGK W333 all show a blue shift in the 10^3 to $5{\cdot}10^4$ s region, but hisPGK W122 shows no spectral changes. After $5{\cdot}10^4$ s no further changes were observed in either of the mutants. The kinetics of the spectral change is very different for both domains from the corresponding intact protein, but the tryptophan residues placed at positions 122 and 333 report a similar kinetic course for the misfolding of the complete enzyme.

Discussion

Although there are several diseases in which two-domain proteins are deposited full length in amyloid plaques, the role of domain-domain interactions during misfolding and amyloid formation of proteins has been overlooked. A possible reason for this could be that the domain structure of the native state is destabilized and probably eventually abolished during misfolding and amyloid formation, thus one could expect, that domain interactions bear no importance in amyloid formation. Contrary to this expectation, here we show that contacts between the two unfolded domains of yeast PGK have a strong effect on the amyloid fibril formation. The presence of the complete polypeptide domain also influences the architecture of the final fibrillar structure. This can be of great importance for the *in vivo* scavenging mechanisms, since the resistance to proteases may depend on the molecular details of the formed structure. Our findings can be rationalized if we take into account that the folding pathways of proteins depend strongly on the stability of the folding domains relative to the domain interactions. Since misfolding often occurs from a destabilized native state of the protein or a collapsed partly folded intermediate, the misfolding pathways of the destabilized domains can be easily determined by the domain interactions. This is in perfect agreement with the findings that one structural domain of the human lysozyme deposited in fatal familial insomnia is destabilized in all its amyloidogenic mutants.

Misfolding and amyloid formation of yeast PGK was studied by manual mixing measurements using two single tryptophan mutant pairs designed to compare the misfolding properties of the individual domains and of the domains within the complete protein. Fluorescence emission spectra revealed that the tryptophan labels are more solvent exposed in the intermediate formed 5 minutes after the initiation of the amyloid formation than in the native state, but less exposed than in the acid unfolded state. Our results are in accord with the findings of Modler et al. (2003) who have proved the presence of a partially refolded intermediate at this stage of the yeast PGK misfolding. This partially folded intermediate is the starting point for protein aggregation and amyloid formation. The tryptophan emission spectra measured 4 days after initializing misfolding were found to be different for the individual domains and the corresponding complete proteins, thus the final structure adopted within the amyloid depends sensitively on the presence of the other domain.

We found that the two individual domains show different misfolding kinetics, but in the corresponding mutants of the complete protein misfolding proceeds differently from the individual domains, and similarly to each other. This shows that long-range interactions play a decisive effect on the misfolding pathway. All amyloids have a characteristic β-sheet structure regardless of the sequence of the protein. Our results however show that the sequence specific contacts such as the long-range interactions between different parts of the polypeptide chain are of great importance in all steps of the misfolding and amyloid growth. Domain interactions also determine the conformation of the polypeptide chain adopted in the amyloid fibrils by influencing the early misfolding steps.

In conclusion we can say that: Amyloid-like fibrils grew from mutants of the individual domains and the complete protein under the same conditions.

Interactions between the N and C terminal polypeptide regions influenced the amyloid formation and influenced the final tertiary structure formed as well.

Conformational changes were different for the individual domains, but in the complete protein these changes were synchronized by the interactions between the C and N terminal domains of the protein.

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