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Chaperone-like activity of synthetic polyanions can be higher than the activity of natural chaperones at elevated temperature

Pavel I. Semenyuk [a, *], Lidia P. Kurochkina [a, b], Nikolai B. Gusev [c], Vladimir A. Izumrudov [d, e], Vladimir I. Muronetz [a, f]

[a] Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russia
[b] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia
[c] School of Biology, Lomonosov Moscow State University, Moscow, Russia
[d] Faculty of Chemistry, Lomonosov Moscow State University, Moscow, Russia
[e] Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences, Moscow, Russia
[f] Faculty of Bioengineering and Bioinformatics, Lomonosov Moscow State University, Moscow, Russia

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ABSTRACT

Polyelectrolytes are a prospective tool for protection of proteins against aggregation. We compared synthetic polyanion, poly(styrene sulfonate), and natural chaperones of different types, namely, GroEL-like chaperonin from *Pseudomonas aeruginosa* phage EL and human small heat shock protein HspB5 (αB-crystallin), in their ability to prevent aggregation of client proteins. At 45 °C, all three agents efficiently suppressed thermal aggregation of phage endolysin. At higher temperatures, HspB5 and poly(styrene sulfonate) also inhibited endolysin aggregation, though polyanion became less efficient than HspB5 at 55 °C and 60 °C. However, the polyanion completely protected another protein, glyceraldehyde-3-phosphate dehydrogenase, even at 60 °C, in contrast to both natural chaperones whose effect disappeared at 50–55 °C. These results provide a platform for the development of artificial chaperones based on synthetic polyelectrolytes.

1. Introduction

The search of an effective method of protein stabilization and prevention of protein aggregation is an important problem in modern biochemistry, bioengineering, and biotechnology. At present, proteins are increasingly used as drugs [1] and as biosensors [2,3]. In such systems, enzymes have to be stabilized against various stresses. Amyloid protein aggregation is a key event in the development of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases and prion infections [4–7]. Maybe the most important problem for current bioengineering and molecular biology is that of inclusion bodies formation. Insoluble inclusion bodies are accumulated under overproduction of recombinant proteins in bacterial cells due to the lack of appropriate chaperones [8].

Chaperones play a crucial role in all the above-mentioned processes. Thus, amyloid aggregation can be inhibited or prevented by chaperones [9–11]. A range of chaperones is used for folding or refolding of recombinant proteins [8,12]. However, the natural chaperone systems are extremely complex and difficult to analyze; in addition, different chaperones can induce completely different effects [11,13]. Furthermore, a complicated chaperone system can be affected by many stresses, such as heat or chemical shocks and many other factors.

Recently, we succeeded in preventing protein aggregation by the use of polyelectrolytes [14–17]. The most efficient of these, namely sulfated and sulfonated polyanions, were able to disrupt pre-formed protein aggregates with partial recovery of the enzyme activity [18]. According to the proposed model, polyelectrolyte chains form a charged coat around the protein and thus stabilize it against aggregation. It is noteworthy that the most efficient stabilization required tight binding restricted by a number of oppositely charged groups on the protein surface [19]. In this case, unbound

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PSS, poly(styrene sulfonate).

* Corresponding author. Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, 119234 Moscow, Leninskye gory 1, bld. 40, Russia.
E-mail address: psemenyuk@belozersky.msu.ru (P.I. Semenyuk).

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fragments of polyelectrolyte chains formed a charged loops and tails stabilizing complex in solution. Using molecular dynamics simulations [20] and experimental data [19,21], we and other groups [22,23] investigated a mechanism of protein-polyelectrolyte interaction and revealed the main factors including ion pair formation, release of counterions [24] and hydrophobic interactions [25]. Thus, an ability of the polyelectrolyte to participate in hydrophobic interactions with the protein enhances its anti-aggregation efficiency [17,18,26].

In the present study, we compared linear synthetic polyanion, poly(styrene sulfonate), and two natural chaperones of different types regarding their ability to prevent aggregation of client proteins. GroEL-like chaperonin from phage EL [27,28] was selected as a model chaperonin which are probably the most complex machines for protein folding and refolding within the ATP hydrolysis cycle and are also able to protect unfolded protein against aggregation [29,30]. HspB5, also known as αB-crystallin, was selected as a member of the small heat shock proteins family, which plays an important role in the protection of cellular proteins from aggregation under heat shock, but are not able themselves to fold client proteins in an ATP-dependent manner [31,32]. The antiaggregation efficiency of the above-mentioned chaperones and polyanion at elevated temperatures was compared. Two different proteins, namely, small monomeric protein phage endolysin (34 kDa) known as a specific substrate for phage chaperonin [27], and oligomeric glyceraldehyde-3-phosphate dehydrogenase (144 kDa), were used as model protein-substrates.

2. Materials and methods

2.1. Proteins

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was isolated from rabbit skeletal muscle and purified by Scopes method [33]. The purified protein was characterized by specific activity of 80 μmol NADH/min per mg of the protein. Before experiments, suspension of GAPDH in ammonium sulfate was dialyzed against the 1000-fold volume of 10 mM potassium phosphate buffer, pH 7.5.

Phage EL **endolysin** (gene product 188 of phage EL genomic DNA) and phage **EL chaperonin** (gene product 146) were expressed in *E. coli* BL21(DE3) cells and purified as described earlier [27].

Small heat shock protein **HspB5** was expressed and purified as described earlier [34].

Proteins concentration was determined spectrophotometrically using $A_{280}^{0.1\%}$ values of 1.0, 1.7, 0.58, and 0.7 for GAPDH, endolysin, EL chaperonin, and HspB5, respectively. The concentration of the chaperonin was expressed in terms of molar concentration of tetradecamer. The concentration of HspB5 was expressed in terms of molar concentration of monomer since its oligomeric behavior is complex and dependent on protein concentration.

2.2. Polyanions

Sodium poly(styrene sulfonate) was purchased from Sigma-Aldrich. Two samples with a polymerization degree of 155 and 1700 were used. The concentration of the polyanions was expressed usually in terms of molar concentration of charged chains. Mainly we also specified a concentration in terms of mg/ml and sometimes in terms of molar concentrations of charged groups.

2.3. Protein aggregation assay

Kinetics of the protein aggregation at different temperatures was studied using dynamic light scattering on ZetaSizer NanoZS instrument (Malvern Instruments) with a laser wavelength of 633 nm and scattering angle of 173°. Concentration of endolysin and GAPDH were 0.1 mg/ml, that is 3 μM for endolysin and 2.8 μM for CAPDH monomers (0.7 μM for tetramer).

2.4. Thermal stability determination

Thermal stability of EL chaperonin was analyzed using differential scanning calorimetry on DASM-4 microcalorimeter with 0.47-ml capillary platinum cells. Protein concentration was equal to 0.5 mg/ml, scanning rate was 2 °C/min. The second heating curve was used as an instrumental baseline due to irreversible denaturation of the EL chaperonin. The chemical baseline was calculated and subtracted with the use of Arina 2 software (Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Russia).

Thermal stability of HspB5 was determined using dynamic light scattering by heating up to 75 °C with average heating rate of 0.5 °C/min. Protein concentration was 0.1 mg/ml.

3. Results

We studied the influence of two different natural chaperones and poly(styrene sulfonate) anion (PSS-1700) on the thermal aggregation of phage endolysin at various temperatures using dynamic light scattering (Fig. 1). Both natural chaperones, namely, the

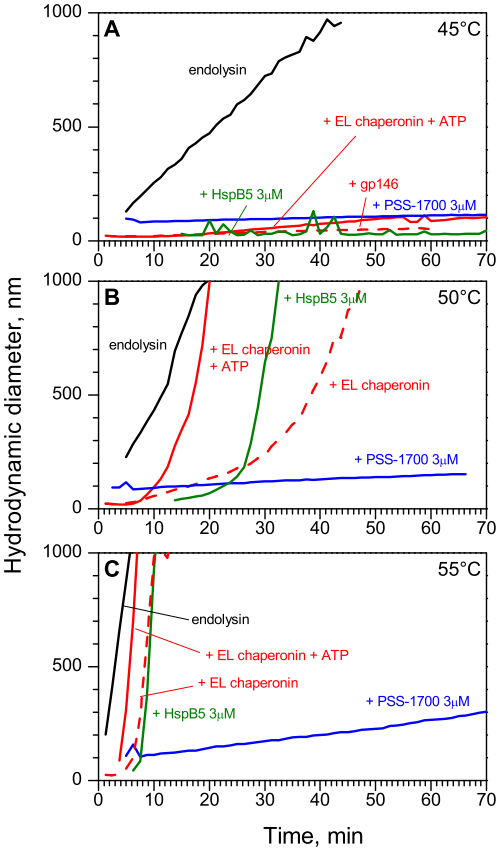

Fig. 1. Thermal aggregation of endolysin measured at 45 °C (A), 50 °C (B), and 55 °C (C) alone (black line) and in the presence of EL chaperonin with ATP (solid red line), EL chaperonin without ATP (dashed red line), HspB5 (green line), and PSS-1700 (blue line). Concentration of all chaperones was 3 μM that corresponded to 2.5 mg/ml for EL chaperonin, 0.06 mg/ml for HspB5, and 1 mg/ml for PSS-1700. Concentration of endolysin was 3 μM (0.1 mg/ml). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)