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Similarly charged polyelectrolyte can be the most efficient suppressor of the protein aggregation

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ABSTRACT

Recently we succeeded in preventing aggregation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) using polyelectrolytes. In the present work, the range of the model proteins has been extended at the expense of lysozyme and alpha-lactalbumin that are noticeably differed by isoelectric points. Experiments were performed both at higher and lower pH values than pI. In all cases, highly charged polycation or polyanion were capable of aggregation suppression. Furthermore, GAPDH was protected efficiently by both polyanion and polycation in all selected conditions. Noteworthy, in many cases protein protection was achieved by similarly charged polyelectrolyte, and the higher level of protection by polycation was achieved at lower pH despite the weakening of the Coulomb attraction. According to molecular dynamics simulations, polycation bound only negatively charged sites and formed loops and tails around protein surface. The unbound fragments seem to determine the high solubility of the complex and hence, are favorable the protein aggregation protection. The reported results could be important as a platform for the development of artificial chaperones.

1.Introduction

Since proteins are widely used in modern biochemistry, bioengineering, and pharmacy, stabilization of protein against aggregation is an important problem. Thus, overproduction of recombinant proteins in bacterial cells often leads to aggregation and formation of inclusion bodies [1]. The unwanted protein aggregation is frequently inherent in the production of protein-based drugs, the number of which seems to increase in future [2]. Protein aggregation is also involved in the development of neurodegenerative diseases such as Alzheimer's, Parkinson's and Huntington's diseases and prion infections [3–5].

Many approaches were described for protein aggregation suppression, including as point mutation [6] and the use of chaperones [7–9]. However, these methods are complex and protein-specific [10,11]. Thus, cellular chaperon system consists in many different proteins [12] whose effect on aggregation can be different and even opposite [13,14], that hinders the regulation.

The use of synthetic highly charged polyelectrolytes was recently suggested as the efficient tools to prevent the heat-induced aggregation [15–17]. It was shown that the addition of a polycation or polyanion, depending on the charge of protein under current conditions, results in the formation of water-soluble polyelectrolyte/protein complexes. The binding and efficiency of aggregation suppression depend on many factors such as protein isoelectric point and pH value [18,19], ionic strength of the buffer [20], a type of charged groups of polyelectrolyte [17], polyelectrolyte hydrophobicity [21,22] and degree of polymerization [22]. It was also shown that long hydrophilic charged chains do not induce inactivation of the bound protein in contrast to short and relatively hydrophobic (i.e. able to hydrophobic interactions) polyelectrolytes [17,23,24]. However, amphiphilic polyelectrolytes are able to stabilize membrane proteins in aqueous solution [25] and can be used to prevent aggregation during protein renaturation [26]. Finally, polyelectrolytes were found to be able to dissolute the protein aggregates, specifically inclusion bodies [27,28].

Despite numerous publications on protein-polyelectrolyte

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interactions (see for example the review Ref. [29]), the specificity of the polyelectrolyte's action is still understood incompletely. In the present work, we aimed to verify the statement that the use of polyelectrolytes is a universal approach to prevent protein aggregation and hence polyelectrolytes could be an artificial substitute for chaperones with a wide range of substrates. Since these interactions depend on charges of the reagents, we selected proteins with different isoelectric point and tested their behavior at different pH in the presence of polycation or polyanion.

2. Materials and methods

2.1. Proteins and polymers

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was isolated from the rabbit muscle and purified by Scopes method [30]. The purified protein was characterized by the specific activity of 90 μmol NADH/min per mg protein and the A_{280}/A_{260} ratio of 1.1. The purity of the isolated protein was tested using SDS-PAGE. Immediately prior to the experiments, GAPDH sulfate ammonium suspension was dialyzed against 1000-fold volume of 10 mM potassium phosphate buffer with the desired pH. Concentration of the enzyme was measured spectrophotometrically using $A_{280}^{0.1\%}$ value of 1.0.

Chicken egg lysozyme was purchased from Sigma-Aldrich. Alpha-lactalbumin from cow milk was kindly provided by Prof. Thomas Haertle from INRA, Nantes.

Samples of sodium dextran sulfate with a molecular weight of 5 kDa containing approximately 30 sulfate groups per molecule (DS) and poly(*N*-ethyl-4-vinylpyridinium) bromide (PEVP) with a degree of polymerization of 1600 were purchased from Sigma-Aldrich. Polyanion concentrations were expressed in terms of molar concentration of charged groups.

2.2. Protein aggregation assay

Aggregation of glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) was induced by heating at 60 °C. The extent of the aggregation in the absence and in the presence of the polyanions was estimated by measuring the turbidity. The protein was injected into a heated polyanion solution to the final GAPDH concentration of 0.1 mg/ml, and the increase in the optical density at 320 nm was measured during 5 min. The values determined for each mixture of GAPDH with polyanion were normalized to GAPDH solution of the same concentration in the same conditions. In other words, the ratio 0 and 1 correspond to complete suppression of thermal aggregation and the absence of any effect, respectively. 10 mM potassium phosphate buffer, pH 6.5, 7.5, or 9.0 was used.

To estimate aggregation level of **lysozyme**, we adopted dynamic light scattering (DLS). ZetaSizer Nano ZS instrument (Malvern Instruments Ltd.) with the scattering angle of 173° was used. The samples were incubated for 10 min at 75 °C, pH 7.5 or pH 9.0, and at 80 °C, pH 11. In all cases, protein concentration was 1 mg/ml.

Alpha-lactalbumin aggregation was also estimated using DLS. The protein solution, as well as mixtures of protein with polyelectrolytes, were incubated at 85 °C for 15 min. Protein concentration was 1 mg/ml. Potassium phosphate buffer, pH 6.5 was used.

2.3. Molecular modeling

The structure of lysozyme used as a model protein was retrieved from PDB database, PDB ID 4nhi. The PDB2PQR server [31] was used for protein protonation at different pH. APBS tools [32] was used for visualization of the electrostatic potential.

The topology for PEVP molecule was obtained with the use of RED III tools [33]. Geometry optimization of the monomer was performed in Firefly QC package [34], which is partially based on the GAMESS (US) [35] source code.

Molecular dynamics simulations were performed with the use of GROMACS 5.0 software [36]. The GROMOS 54a7 force field was used. We performed a set of simulations of the system containing one protein and 5 polycation ($PEVP_{18}$) molecules in random orientations. Polycation charges were compensated with Cl^- ions. Energy minimization for the protein structure alone was performed before main dynamics. PEVP molecule alone also was optimized by 1-ns simulation in water, and the final structure was used for the box construction. Then water molecules were added and equilibrated for a 2 ps. The length of main simulations was 50 ns? The choice of the simulations duration was based on time when a number of protein-dendrimer salt bridges reached a plateau. The temperature of the box was set to 300 K using v-rescale thermostat. Pressure coupling was performed with Berendsen algorithm.

The analysis of the obtained trajectories was performed in two steps. First of all, we visually looked for polycation loops and tails around protein surface. We defined loops and tails as relatively long (more than five monomers) parts of polycation chains bound to the protein that are not bound to the protein. Then we calculated time dependencies of a number of salt bridges between protein and PEVP with a threshold of 0.35 nm. All monomers of polycation chains bound to protein were divided into two groups – bound to protein and non-bound. The percentage of bound monomers was calculated and averaged for the last 5 ns?

3. Results

In order to verify whether suppression of protein aggregation by polyelectrolytes is a versatile approach, three model proteins with different isoelectric point (pI) were used: glyceraldehyde-3-phosphate dehydrogenase (GAPDH, pI 8.5), lysozyme (pI 9.3), and alpha-lactalbumin (pI 4.9). Experiments were performed at different pH, both higher and lower than the isoelectric point of the proteins. Thus the surface charge of proteins was changed, and consequently, protein interaction with polyelectrolytes also changed. In all cases, we investigated the influence of polyanion dextran sulfate (DS) as well as polycation poly(*N*-ethyl-4-vinylpyridinium) bromide (PEVP). Both DS and PEVP chains bear the charge in practically all repeat units in the whole operative pH region.

The treatment of lysozyme with polycation PEVP allowed us to suppress thermal aggregation of the protein at all selected pH, namely 7.5, 9.0, and 11.0. The hydrodynamic diameter of native lysozyme particles was 4–7 nm depending on pH value and increased up to 500–1000 nm after incubation at 75–80 °C for of 10 min (Fig. 1, black curves). Small portions of added PEVP did not affect the size of the particles, while an increase of PEVP concentration caused a decrease of the diameter of complexes, indicating suppression of aggregation by polycation. Thus, in the presence of only 12.5 μM of PEVP at pH 7.5 the system contains no large particles. In other words, the aggregation was prevented completely in these conditions (Fig. 1A).

PEVP concentration required for complete suppression of aggregation depended on pH value. Thus, at pH 7.5 addition of 8 μM of PEVP was enough for complete preventing aggregation (Fig. 1A), whilst 1 mM of PEVP was required at pH 9.0 (Fig. 1B). At pH 11.0, the complete aggregation suppression was not achieved even by addition of 8 mM of PEVP when relatively large complexes with the size of 250 nm were formed. Nevertheless, they were smaller than 1000-nm aggregates of native protein under the same conditions reflecting a noticeable suppression (Fig. 1C). It is noteworthy that contrary to the expectations, suppression of aggregation by