Polymer 113 (2017) 39–45

Contents lists available at ScienceDirect

Polymer

journal homepage: www.elsevier.com/locate/polymer

Protein-polyelectrolyte complexes: Molecular dynamics simulations and experimental study

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ARTICLE INFO

Article history:
Received 19 December 2016
Received in revised form
11 February 2017
Accepted 13 February 2017
Available online 15 February 2017

Keywords:
Polyanions
Protein-polyelectrolyte complexes
Molecular dynamics simulation

ABSTRACT

Despite use of polyelectrolytes is considered to be a prospective approach of protein aggregation suppression, owing to formation of soluble protein-polyelectrolyte complexes, the structure of the complexes and mechanism of their formation are not sufficiently understood. The aim of this work was to study the influence of degree of polymerization on the structure and properties of formed complexes. We carried out molecular dynamics simulations of complexes of cationic protein lysozyme with highly charged polyanions – poly(styrene sulfonate) and polyphosphate – of different degree of polymerization. It has been shown that the short charged chains are bound with the protein via a great majority of repeat units, while the long chains have unbound fragments that form charged loops and tails around the protein surface. These loops were earlier suggested to provide stability of the complex. Furthermore, the charge of the complex increased with increasing length of chain. These findings are consistent with the experimentally measured zeta potential. The obtained results help to explain why polyanion protective efficiency against protein aggregation increases with increase of the degree of polymerization.

1. Introductionintroduction

Protein-polyelectrolyte complexes are of great importance in modern science and industry because of their application in bioengineering, pharmacology and biotechnology. Polyelectrolytes can be used for separation or purification of proteins and protein delivery [1–4]. Furthermore, the usage of polyelectrolytes is effective and prospective method for prevention of protein aggregation. It was shown that the addition of a polycation or polyanion in some excess results in formation of water-soluble protein-polyelectrolyte complexes, because electrostatic repulsion between complexes can keep proteins from self-aggregating [5,6]. Moreover, polyelectrolytes are capable of dissolving pre-formed aggregate [7]. Another reason to study the protein-polyelectrolyte interactions is their importance for the living organisms. Thus, many proteins interact with natural polyphosphates [8], including DNA [9,10], and sulfated polymers [11]. Furthermore, introducing of the sulfate group into the protein via posttranslational modification alters interaction of the modified protein with other proteins [12,13].

Proteins and polyelectrolytes interact primarily via electrostatic forces to form complexes, which can have widely varied stoichiometry and architecture. The interaction between protein and polyelectrolytes depends on several factors, such as pH [14], ionic strength [15], protein surface charge distribution [14] and the type of charged group [16], polymerization degree [17], hydrophobicity [17,18], and stiffness [1] of the polyelectrolyte.

One of the main characteristic of polyelectrolytes is the degree of polymerization, which directly influences on efficiency of suppression of the protein aggregation. It has been shown, that the higher degree of polymerization is, the more effective the polyelectrolyte prevents protein aggregation [5,6,16]. Moreover, short chains are not only unable to keep the protein from the aggregation but even destroy protein structure [17].

Since relatively hydrophobic polyelectrolytes can interact with protein due to hydrophobic interactions, hydrophobicity of the polyelectrolyte has significant effect on prevention of protein

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http://dx.doi.org/10.1016/j.polymer.2017.02.047
0032-3861/

aggregation. It has been shown that relatively hydrophobic polyelectrolytes suppress aggregation at lower concentrations than hydrophilic ones [1,6,17,19]. This is likely to be caused by the replacement of adverse intermolecular interaction between hydrophobic patches on the proteins surfaces by hydrophobic interactions between the protein and polyelectrolyte [6]. On the other side, relatively hydrophobic polyelectrolytes, especially short chains, are prone to affect the bound protein structure [16–18].

Molecular modeling of interaction between protein and polyelectrolytes should contribute to understanding of the complex formation mechanisms and the effects of the mentioned above factors such as chain stiffness length, ionic strength, and protein surface charge density. Different methods of the simulations can be useful for protein-polyelectrolyte complexes, including Monte Carlo and coarse-grained Langevin dynamics computer simulations [2,20–23]. These approaches were applied to investigate the driving forces of complex formation [20] and the influence of protein surface charge distribution and environmental conditions [21,22,24] on protein-polyelectrolyte interaction. However, the influence of polyelectrolyte properties on formation of the protein-polyelectrolyte complex has not been investigated by the use of computer modeling. Moreover, investigation of protein structure changes in consequence of interaction with polyelectrolyte requires full-atom simulations that have not been performed yet.

In the present work we aimed to study the influence of polymerization degree of the polyelectrolyte on the structure and properties of the formed complexes by full-atom molecular dynamics simulations. We selected two polyanions differing in the structure and hydrophobicity, and performed simulations of the model cationic protein, lysozyme, with the polyanions with different degree of polymerization. The obtained results were also verified by experimental measurement of the complexes' properties.

2. Materials and methods

2.1. Structures for molecular dynamics simulations

Lysozyme (PDB ID 4nhi) was selected as a model protein for molecular dynamics simulations. APBS tools [25] were used to obtain electrostatic potential for visualization of the protein surface.

Polyphosphate and poly(styrene sulfonate) with degree of polymerization of 5, 18, and 45 were used. Polyanion molecules were parameterized by using the RED III tools [26]. Geometry optimization of the monomer was performed in the Firefly QC package [27], which is partially based on the GAMESS (US) [28] source code.

2.2. Molecular dynamics simulations and analysis

Molecular dynamics simulations of the interaction between lysozyme and polyelectrolytes were carried out using the GROMACS 5.0 molecular dynamics software package [29]. The GROMOS 54a7 force field was used [30].

Each simulation systems consisted of one molecule of lysozyme and several molecules of polyphosphate or poly(styrene sulfonate). Depending on the length of polyelectrolyte we added different number of chains in such way that the total number of monomers in the box was equal to 90. Therefore we added 2, 5 or 18 chains, having length of 45, 18 and 5 respectively. The system for each length of polyelectrolytes was simulated three times with different random start positions of the chains in the box. Polyelectrolytes were initially equilibrated in solvent for 1 ns. Optimization and relaxation of the protein structure also were performed by using molecular dynamics simulations for 1 ns. These optimized structures of protein and polyanions were used in main simulations. Polyelectrolytes were placed in random orientation in the box with protein, where the minimal distance to the edge of the box was 2.5, 2 or 1.5 nm depending on the length of the added polyelectrolytes (the degree of polymerization of 45, 18, and 5 respectively). The length of added polyelectrolytes did not exceed the corresponding box dimensions. All systems were solvated in TIP3P water molecules [31] in a rectangular box with a minimal distance between the solute and the box walls of 5 Å. All systems were neutralized by adding the necessary amount of Na^+ ions.

The systems were simulated at constant temperature of 300 K using Velocity rescale method and at a constant pressure of 1 bar using Berendsen's method. Before simulations, energy minimization was performed using steepest descent algorithm. Then equilibration of the solvent was conducted for 0.2 ps All simulations were carried out for 50 ns with a 2 fs time step. The choice of the simulations duration was based on time when a number of protein-polyelectrolyte bonds reached a plateau.

The analysis of the obtained trajectories was performed by using the GROMACS 5.0 molecular dynamics software package. We calculated time dependences of the number of hydrogen bonds and ion pairs between protein and polyelectrolytes with a threshold of 0.35 nm. We analyzed the change of hydrophilic and hydrophobic solvent accessible surface area of protein-polyelectrolyte complex. All monomers of polyelectrolyte chains bound to protein were divided in two groups – bound to protein and non-bound. The number of bound and non-bound monomers was calculated and averaged for the last 5 ns. Non-bound chains were excluded from the analyses. Charge of protein-polyelectrolyte complex was computed as the averaged total protein and bound chains charge for the last 5 ns. To probe the contribution of counterions release, we calculated a number of Na^+ ions within 0.5 nm of polyanion chains averaged for the last 5 ns, and then averaged the values for polyanion chains bound to the protein.

2.3. Determination of the complexes' size and zeta potential

Lysozyme was purchased from Sigma-Aldrich. The protein was dissolved in 10 mM potassium phosphate buffer, pH 7.5.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was isolated from the rabbit muscle and purified by Scopes method [32]. The purified protein was characterized by the specific activity of 80 μmol NADH/min per mg of protein. Before the experiments, GAPDH sulfate ammonium suspension was dialyzed against 1000-fold volume of 10 mM potassium phosphate buffer, pH 6.5.

Concentration of the proteins was measured spectrophotometrically using $A_{280}^{0.1\%}$ value of 2.3 for lysozyme and $A_{280}^{0.1\%}$ value of 1.0 for GAPDH.

Samples of sodium poly(styrene sulfonate) with polymerization degree of 20, 77, 155 and 1700 (PSS_{20}, PSS_{77}, PSS_{155}, PSS_{1700}, respectively) were purchased from Sigma–Aldrich (USA).

The size and zeta potential of the complexes was determined by dynamic light scattering using a ZetaSizer Nano ZS instrument (Malvern Instruments Ltd., UK) with the scattering angle of 173°. All measurements were performed at 25 °C. Lysozyme concentration was 1 mg/ml, and concentrations of polyanions were 2 mM. In the experiments with GAPDH the protein concentration was 0.5 mg/ml, concentrations of polyanions were 0.35 mM. Before the analysis, the proteins were incubated with polyanions for 5 min.

3. Results

Investigation of interaction of polyanions of different polymerization degrees and protein was carried out by using the method of