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E.V. SIDORIN, V.A. KHOMENKO, T.F. SOLOV'EVA

The effect of pH on the chaperone activity of Skp from *Yersinia pseudotuberculosis*

The aim of this study was to evaluate the effect of pH on the behavior Skp of Yersinia pseudotuberculosis in solution and its manifestation of chaperone activity. Commercial samples of Fc- and Fab-fragments of human IgG were used as protein substrates for binding to Skp chaperone. The aggregation kinetics of rSkp, Fc- and Fab-fragments and protein substrates in the presence of a chaperone in acidic, neutral, and basic solutions was studied using the dynamic light scattering method. The obtained results demonstrate the pH-dependent character of the chaperone activities of rSkp. The most stable low-molecular complexes (RH up to 10 nm) between protein substrates and chaperone are formed atacidic pH values of the medium. In the case of alkalinization of the reaction medium, the chaperone activity of rSkp decreases, chaperone forms weakly stable complexes with Fc- and Fab-fragments of human IgG,which do not exclude further self-association and aggregation of protein substrates

Key words: Chaperone Skp; Yersinia pseudotuberculosis; Fc- and Fab-fragments of human IgG; aggregation of proteins; protein–protein interactions; dynamic light scattering

Chaperones perform different functions: they can help fold and inhibit aggregation of the unfolded protein; accompany the newly synthesized protein to the site of its localization in the cell, supporting in the unfolded, active for translocation state; prevent lethal nonspecific association of proteins under stress conditions for the cells. Incorrect protein folding in the cell and/or aggregation thereof is the cause of destructive human diseases, such as prion infections, Alzheimer's disease, and type 2 diabetes. In this regard, the study of the properties and mechanisms of chaperone functioning represents not only fundamental, but also of practical interest, associated with the search for approaches and the creation of drugs for the prevention and treatment of neurodegenerative diseases and to enhance the body's resistance to stress. One of the most important periplasmic chaperones of gram-negative bacteria is Skp protein. Along with the ability to interact with the outer membrane proteins as a chaperone, Skp proteins have other properties that can be biologically and physiologically significant. They exhibit lipopolysaccharide- and DNA-binding activity [1-4] and are chemoattractants for monocytes and polymorphonuclear leukocytes [5].Previously, we showed that Skp *Yersinia pseudotuberculosis* bound human and rabbit IgG by a non-immune manner (bypassing the antigen-binding sites of IgG (antibodies)) both in the form monomer (Skp) [6] and in the form homotrimer (Skp_3) [7].

The aim of this study was to evaluate the effect of pH on the behavior of Skp *Y. pseudotuberculosis* in solution, to determine the qualitative and quantitative characteristics of its chaperone- and immunoglobulin-binding activities. Protein Skp was expressed in *E. coli*, and was isolated from the cells and purified as described previously [7]. Commercial samples of Fc- and Fab-fragments of human IgG were used as protein substrates for binding to Skp chaperone.

* SIDORIN Evgeniy Viktorovich – PhD, Researcher, KHOMENKO Valentina Alexandrovna – PhD, Senior Researcher, Solov'eva Tamara Fedorovna – DSc, Principal Researcher (G.B. Elyakov Pacific Institute of Bioorganic Chemistry, FEB RAS, Vladivostok, Russia). *E-mail: sev1972@mail.ru

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According to dynamic light scattering (DLS), recombinant Skp (rSkp) in sodium acetate buffer, pH 5.0 had has exhibits a monomodal particle size distribution with a hydrodynamic radius (R_H) of 3.6 ± 0.3 nm. When the pH of the medium changes from acidic to alkaline, rSkp aggregation is observed, and its rate increases as pH approaches the isoelectric point of the protein (*pI* = 9.33). Within 6 days after isolation, rSkp in the buffer, pH 5.0 had a Z-average hydrodynamic radius (Z-average) of only 6.4 nm.Already in the first 30 minutes after the transfer of the chaperone to solutions with neutral and alkaline pH values of 6.7 and 7.9, its Z-average sharply increased to 61 and 155 nm, respectively, and by 24 hours was 341 and 383 nm, respectively.

Fc- and Fab-fragments of human IgG (Mr = 52 kDa) in PBS showed a monomodal particle size distribution with R_H = 3.3 ± 0.3 nm, the Z-average values of the samples were 14 and 11 nm, respectively. In buffer solutions with a lower ionic strength and pH values of 5.1, 6.7 and 7.9, these proteins showed an increase in Z-average, distribution width (multimodal distribution), and relative content (%) of particles with R_H greater than 10 nm. Such behavior indicates self-association and subsequent aggregation of Fc- and Fab-fragments of human IgG under the test conditions. In the presence of rSkp, the rate of these processes decreased significantly: an increase in the relative content (%) of particles with R_H greater than 10 nm and the Z-average of the samples was slowed down. The experiments carried out by us showed a significant influence of the pH of the medium on the rSkp chaperone activity. At acidic pH of the solution (under the conditions of which rSkp is most stable) the chaperone formed complexes with Fc and Fab that retained the stability for up to 20 hours and had R_H to 10 nm. In solutions with neutral and alkaline pH values (6.7 and 7.9), the rSkp chaperone activity decreased markedly. The low molecular weight Skp-Fc, -Fab complexes (R_H up to 10 nm), which dominated in the solution for the first 30 minutes (98.2-99.8%), were replaced in the course of time by coarse particles with R_H from 10 to above 200 nm: at pH 6.7 to 24 hours in the solution the particles larger than 200 nm predominated (63.3 and 100% by volume for Skp-Fc and Skp -Fab, respectively), and at pH 7.9 only particles larger than 200 nm were present.

Chaperone activity of Skp in respect of Fc- and Fab fragments was different. At pH 7.9, rSkp bound Fc to form small size complexes (R_H to 10 nm) that were stable for 4 hours, whereas the interaction of the chaperone with Fab under these conditions resulted in the formation of particles with R_H in the range of 10-200 nm.This may be the result of the differences between Fc and Fab in their binding strength to rSkp.Using biosensor analysis based on the plasmon resonance method, we have shown that K_A rSkp with the Fc fragment is greater than with the Fab fragment in 1.4 and 2.2 times at pH 7.0 (9.7×10^3 M^{-1} and 6.9×10^3 M^{-1}) and at pH 8.0 (2.0×10^6 M^{-1} and 9.1×10^5 M^{-1}), respectively.

In recent years, more information has appeared in the literature that the structural transformations of proteins in cells caused by changes in the pH of the medium increase or decrease the activity of chaperone proteins [8-10]. The results obtained in this work also demonstrate that the chaperone activity of Skp *Y. pseudotuberculosis* is pH dependent. Recombinant Skp stably binds Fc- and Fab-fragments of human IgG at low pH, thereby preventing their irreversible aggregation, but when neutralizing the pH of the medium, chaperone forms weakly stable complexes with them, which do not exclude further self-association and aggregation of protein substrates.

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