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Low crowding agent concentration destabilizes against pressure unfolding

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ABSTRACT

The concentration of macromolecules inside a cell is very high, which can affect the behavior of the enzymes, and consequently influence vital biological processes. This is called macromolecular crowding. Since the most important effect of macromolecular crowding is the excluded volume, we performed pressure experiments, where the volume (as conjugate parameter to the pressure) is the crucial factor. We measured the temperature and pressure stability of bovine serum albumin and lysozyme with various concentrations of crowding agents, dextran, Ficoll™ and lysozyme itself. Our most interesting finding is that low concentration of all the studied crowding agents decreases the pressure stability of the proteins. We explain this by the reduced hydration volume change in the crowded environment. Furthermore, we discuss the volumetric parameters and emphasize the difference between the partial volume of the protein and the volume it influences, and their relation to the excluded volume which is responsible for the macromolecular crowding.

1. Introduction

Although, the interior of a cell is highly crowded due to the presence of large amounts of soluble and insoluble macromolecules including proteins, nucleic acids, ribosomes, and carbohydrates; most of the in vitro protein studies have been carried out in quite dilute solutions (usually less than 1 mg/ml). The question arises: to what extent can these properties be extrapolated to the crowded environment of the cell?

Crowding in the cell means that a significant fraction of the cell's volume is not available to other macromolecules. The concentration of macromolecules in the cytoplasm is in the range of 80–400 mg/ml [1,2]. In physiological fluids all macromolecules collectively occupy 10% to 40% of the total volume [3]. Although the effect of this "macromolecular crowding" is obvious, but often underappreciated. Recently, an increasing amount of evidence suggests that proteins may behave quite differently in this crowded environment. A great impact of this crowding phenomenon was demonstrated on the thermodynamics and kinetics of many biological processes, including protein binding, folding, and aggregation.

Crowding induced stabilization of the native state of the protein was observed in several cases. This was explained by the fact that the native state is more compact. Presence of the crowding agent increased the unfolding temperature of phosphoglycerate kinase [4]. Similarly the heat denaturation temperature of hen egg white lysozyme at pH 2 was increased by 1–3 °C depending on the crowding agent concentration [5]. The most pronounced effect was observed in case of flavodoxin, where the presence of 400 mg/ml crowding agent increased the thermal unfolding temperature by 20 °C [6].

The influence of the crowded environment on the aggregation and fiber forming of proteins depends on several factors. This can explain the variety of the experimental findings. Seelinger et al. studied the fibrillation of the type-2 diabetes mellitus related human islet amyloid polypeptide. Stabilization of the monomeric form and consequently suppression of the fibrillation was found [7]. On the contrary the addition of high concentrations of different polymers dramatically accelerated alpha-synuclein fibrillation in vitro depending on the nature, length and concentration of the polymer [8]. Crowding enhances aggregation propensity of several proteins like apoflavodoxin, creatine kinase [9,10], but almost completely inhibits amyloid formation of lysozyme and stabilizes lysozyme activity [1,11]. The paradoxical results can be understood probably by recalling, that aggregation needs some kind of destabilization of the protein. Native proteins do not aggregate, only intermediate structures do so, which are mostly partially unfolded. Crowding seems to increase the aggregation of these destabilized structures, but their formation from the native state is presumably suppressed by crowding. This can be rationalized by the bigger volume occupied by the destabilized intermediaries.

Enzymatic activity can also be influenced by crowding. Activity of phosphoglycerate kinase was increased in presence of crowding agents. The explanation is that the active conformation is the more compact one, which was favored by the crowded environment [4].

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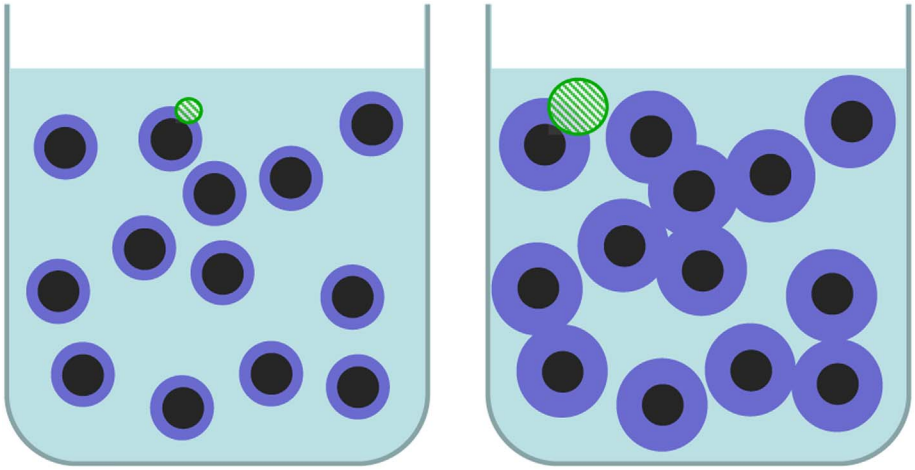

Fig. 1. The excluded volume effect. The crowding agent molecules are indicated by black circles. The black circles and the gray (pink) rings around them together represent the excluded volume. The test protein is shown by a striped circle. As the size of the test protein increases the excluded volume increases also considerably. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Crowding induced stabilization can be explained by the excluded volume effect. The excluded volume is the volume, which is not available for the central point of the test molecule, because other molecules exclude it from this area [12] (Fig. 1).

According to the excluded volume effect theory, any reaction that amplifies the available volume will be stimulated by macromolecular crowding [13]. It was suggested that crowding may have a stabilizing effect on the folded protein indirectly, because the native state is more compact. Therefore, the general belief was that crowding enhances protein stabilization against denaturation by heat, cold or chemical denaturants. However, as it was pointed out recently, macromolecular crowding has both positive and negative effects on protein folding, structure and stability. The negative effect is represented by promotion of aggregation and amyloid formation [2]. These negative effects of the macromolecular crowding can be understood by considering that a fibrous state may also be more compact than the native one, which leads to favoring aggregation over correct folding [14].

Experimentally crowding is studied in vitro mostly by adding high concentration of crowding agents to the dilute solution of the target protein. These crowding agents can be hard particles like dextran or relatively open structures like Ficoll [13]. Another possibility is to use proteins (other than the test protein) [7]. The aim is to select a crowding agent, which does not have any specific interaction with the test protein, and allows measuring purely the excluded volume effect.

One of the most widely used crowding agents is Ficoll™, which is a copolymer of sucrose and epichlorohydrin. It is uncharged, highly soluble in water and commonly used to adjust density and viscosity of solutions [15]. Dextran is a polymer of D-glucopyranose. It is quite flexible and linear with less than 5% branching.

Despite of the large number of chemical, biochemical, and biophysical investigations performed as a function of temperature, the pressure is still a less known and rarely used thermodynamic parameter. Pressure as a thermodynamic parameter is conjugated to volume. This means, that pressure experiments provide more insightful information compared to temperature or chemically-induced conformational changes, since pressure induced effects can be related to volume changes [16–20].

Pressure favors the conformation with smaller volume [17,21]. Therefore, it is important to understand how the volume of a protein is composed. In case of proteins in a solution, the partial volume of the protein is defined as the volume increase of the system due to insertion of a small amount of a protein over the number of moles of the added protein [16]:

$$v_{protein} = \left(\frac{\partial V}{\partial n}\right)\Big|_{n_j,p,T} \quad (1)$$

here V is the volume of the solution and n is the number of moles of the solute added [16].

This implies that the volume of the protein molecule cannot be obtained simply as the interior of a certain compartment, but it is composed of three factors:

$$V_{\text{protein}} = V_{\text{atom}} + V_{\text{void}} + \Delta V_{\text{hydration}}, \quad (2)$$

where the two first terms are volumes of the atoms and of the voids in the interior of the folded protein [16,22]. The latter is due to incomplete packing of the side chains. The third term is associated with the interaction of the protein with the solvent. It is known that the solvent has altered density in the surrounding of the protein. This tightly packed layer is only a few angstroms in size [23]. The volume of water in this shell is smaller than the volume of the same amount of water molecules in the bulk phase. This leads to the remarkable feature of $\Delta V_{\text{hydration}}$ namely that it gives a negative contribution.

Considering the pressure effect on proteins, three major effects can be observed depending on the magnitude of the pressure. Elastic effects already appear at the smallest pressure values. These are reversible distortions of the primary and secondary bonds. The compression of the primary chemical bonds is very small; their contribution to the volume change of the system is negligible. Compression of the hydrogen bonds can lead to the distortion of the molecular conformation, which can reduce the size of the internal cavities in the protein.

Typically if the pressure reaches 200 MPa the intermolecular interactions and the tertiary structure are destabilized [24–26].

Higher pressure can unfold the proteins. The typical pressure required for unfolding is around 500 MPa but this varies from protein to protein, in the range of 100 MPa to 1 GPa or even higher pressures in special cases [27]. The voids disappear if the protein unfolds. Since the $\Delta V_{\text{hydration}}$ is proportional to the surface of the protein, the absolute value of this term increases too, which means this term becomes more negative. Both the void volume and the change in the hydration volume contribute to the volume decrease at the pressure unfolding. Which one of these factors is more important, was the subject of a long debate [28,29]. From our perspective both of them might have their distinct role, and their relative contribution can vary according to the actual situation. Although pressure is well-known to unfold proteins, to our knowledge there are only a few studies reported on the effect of crowding on pressure-induced unfolding [30,31]. Wang et al. found that in crowded solutions high-pressure unfolding of staphylococcal nuclease was impeded [30]. According to the results of Zai et al.