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Review

Macromolecular crowding: Macromolecules friend or foe

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ABSTRACT

Background: Cellular interior is known to be densely crowded due to the presence of soluble and insoluble macromolecules, which altogether occupy ~40% of the total cellular volume. This results in altered biological properties of macromolecules.
Scope of Review: Macromolecular crowding is observed to have both positive and negative effects on protein folding, structure, stability and function. Significant data has been accumulated so far on both the aspects. However, most of the review articles so far have focused on the positive aspect of macromolecular crowding and not much attention has been paid on the deleterious aspect of crowding on macromolecules. In order to have a complete knowledge of the effect of macromolecular crowding on proteins and enzymes, it is important to look into both the aspects of crowding to determine its precise role under physiological conditions. To fill the gap in the understanding of the effect of macromolecular crowding on proteins and enzymes, this review article focuses on the deleterious influence of crowding on macromolecules.
Major Conclusions: Macromolecular crowding is not always good but also has several deleterious effects on various macromolecular properties. Taken together, the properties of biological macromolecules in vivo appears to be finely regulated by the nature and level of the intracellular crowdedness in order to perform their biological functions appropriately.
General Significance: The information provided here gives an understanding of the role played by the nature and level of cellular crowdedness in intensifying and/or alleviating the burden of various proteopathies.

1. Introduction

The present understanding of various biological processes has been acquired through investigations largely made under dilute experimental conditions where the total macromolecular concentration never exceeds 10 g/l. However, biological macromolecules are known to evolve and function under crowded intracellular environments consisting of a plethora of both soluble and insoluble macromolecules like proteins, nucleic acids, ribosomes and carbohydrates with their sum concentration reaching around several hundred g/l. For example, the total concentration of protein and RNA inside the bacterium, *Escherichia coli* is in the range of 300–400 g/l [1]. Altogether, these macromolecules occupy a significant fraction (~40%) of the total cellular volume [2], making it virtually unavailable to the other macromolecules present. Such media are termed 'crowded' or 'volume-occupied' rather than 'concentrated', because no single species of macromolecule is necessarily present at a high concentration. In fact, the level of crowdedness varies among different cell types and cellular compartments. Human lens contains approximately 340 g/l protein [3]; the red blood cells contain about 350 g/l hemoglobin [4]; while the total protein content in the mitochondrial matrix may reach up to 500 g/l [5]. Macromolecular crowding is observed not only in the cellular interior but also in the extracellular matrix of tissues. For example, blood plasma contains ~80 g/l protein, a concentration high enough to cause significant crowding effects [2]. The degree of volume occupancy by these macromolecules is expected to have major thermodynamic and kinetic consequences on the properties of macromolecules present in the cell [6–9]. The term 'macromolecular crowding' connotes the non-specific influence of steric repulsions on specific reactions, and processes that occur in highly volume-occupied media [10]. It was Minton and Wilf [11] who brought the influence of crowding on macromolecules to the forefront in terms of theory and experiment and coined the term "macromolecular crowding" in 1981.

The influence of macromolecular crowding on various properties of macromolecules has been examined in depth by adding high concentrations of inert synthetic or natural macromolecules, termed crowding agents or crowders, to the system in vitro to create an in vivo like scenario [2]. It is generally believed that macromolecular crowding (i) enhances protein stabilization against denaturation by heat, cold or denaturant. It has been argued that macromolecular crowding stabilizes globular proteins due to excluded volume effect because the native state occupies less space than the denatured state [12–15]; (ii) alters the reaction rates depending on the nature of reactions (diffusion-limited or transition-state-limited). Since, macromolecular crowding decreases

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the diffusion of macromolecules, the rate of diffusion-limited reactions is decreased with the increasing crowder concentration [16,17]. However, the rate of transition-state-limited reactions is increased as crowding is expected to enhance the relative abundance of the transition state complex [18]; (iii) increases the catalytic activity of enzymes, either due to alteration in the conformation of the enzyme to a higher activity state [19–22] or due to an increase in the effective concentration of the enzyme resulting from decrease in the amount of the available free water [20]; (iv) increases protein–protein association leading to oligomer formation depending on the conformation of monomer [18,23–26]; and (v) inhibits aggregation of β-rich proteins [27,28]. All these effects of crowding on macromolecules have been described as "positive effect" in this article.

In contrast to the facts described above, there exists large volume of data that oppose the findings (Fig. 1). All these opposing effects are described as "hostile or negative effect" in the entire manuscript. For instance, macromolecular crowding has been demonstrated to have a destabilizing influence on the stability of Myoglobin [29]. Recent investigations made by Fan et al. suggested a decrease in the activity of recombinant human brain-type creatine kinase under crowded conditions [30]. Furthermore, in a systematic study by Dobson and coworkers, crowding was found to disrupt the refolding of reduced lysozyme and caused aggregation [31,32]. All these evidences suggest that the stabilizing or positive effect of crowding on macromolecules is not universally true. Therefore, in addition to the understanding of the positive effects of crowding on macromolecular properties, it is important to have a knowledge of the hostile effects of macromolecular crowding as well so as to have a complete picture of the effect macromolecular crowding has on macromolecular properties. This review article is therefore, designed to give a collective knowledge on almost all the

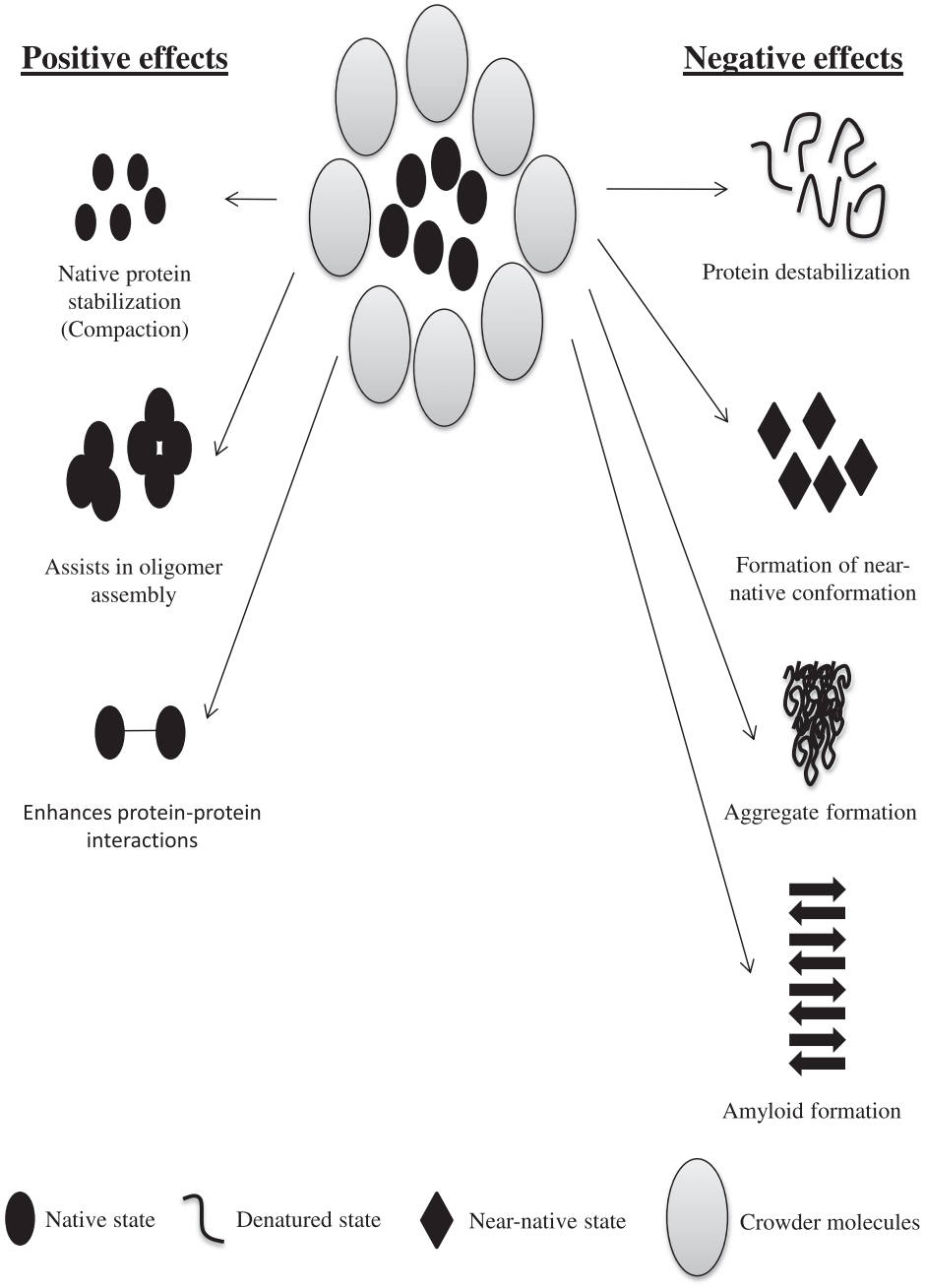

Fig. 1. Consequences of macromolecular crowding on proteins. Illustration of both the positive and negative effects of macromolecular crowding on proteins.