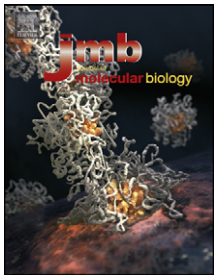

Crowding Modulates the Conformation, Affinity, and Activity of the Components of the Bacterial Disaggregase Machinery

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Abstract

Chaperone-mediated protein aggregate reactivation is a complex reaction that depends on the sequential association of molecular chaperones on their interaction with protein aggregates and on substrate refolding. This process could be modulated by the highly crowded intracellular environment, which is known to affect protein conformational change, enzymatic activity, and protein–protein interactions. Here, we report that molecular crowding shapes the chaperone activity of bacterial disaggregase composed of the DnaK system (DnaK, DnaJ, and GrpE) and the molecular motor ClpB. A combination of biophysical and biochemical methods shows that the excluded volume conditions modify the conformation of DnaK and DnaJ without affecting that of GrpE. These crowding-induced conformational rearrangements activate DnaK, enhance the affinity of DnaK for DnaJ, but not for GrpE, and increase the sensitivity of the chaperone activity to cochaperone concentration, explaining the tight control of their relative intracellular amounts. Furthermore, crowding-mediated disordering of the G/F domain of DnaJ facilitates the reversible formation of intermolecular DnaJ conglomerates. These assemblies could drive the formation of Hsp70 clusters at the aggregate surface with the consequent enhancement of the disaggregation efficiency through their coordinated action via entropic pulling. Finally, crowding helps ClpB to outcompete GrpE for DnaK binding, a key aspect of DnaK/ClpB cooperation given the low affinity of the disaggregase for DnaK. Excluded volume conditions promote the formation of the bichaperone complex that disentangles aggregates, enhancing the efficiency of the disaggregation reaction.

Introduction

Biological macromolecules function within crowded intracellular environments, which are characterized by the presence of high concentrations of proteins and nucleic acids (up to more than 400 g/l) that occupy at least 20–30% of the total volume. The term crowding refers to the exclusion of volume to biomolecules by the presence of other solutes, including macromolecules [1,2]. This environment would increase the thermodynamic activities of macromolecules and strongly reduce their diffusion [3]. Crowding effects using synthetic, uncharged polymers are dominated by steric repulsions that will favor associations and compact conformations [1,4], whereas in the heterogeneous intracellular medium, there are also non-specific chemical interactions that can be associative and repulsive [5,6]. In this crowded intracellular context, newly synthesized polypeptide chains and stressed proteins adopt unstable, non-native conformations prone to aggregation as they attempt to hide their hydrophobic patches from the hydrophilic medium during folding [7,8]. Aggregation is favored in crowded media as protein aggregates exclude less volume than the partially folded monomers that compose them [9]. To avoid this situation, evolution has developed molecular chaperones, which participate in several cellular processes including folding of newly synthesized proteins, remodeling and maintenance of native protein conformations, and reactivation

of protein aggregates [10,11]. The machinery responsible for aggregate reactivation is composed of the corresponding Hsp70 system and a member of the Hsp100 or Hsp110 families. The reactivation process requires formation of transient complexes between a central chaperone, one or several accessory proteins, and protein aggregates.

In bacteria and yeast, aggregated substrates are remodeled by the Hsp70 system or by the bichaperone Hsp70/Hsp100 complex [10,11]. DnaK is the bacterial Hsp70 protein, which, together with the cochaperone DnaJ and the nucleotide exchange factor (NEF) GrpE, avoids aggregation of partially (un)folded proteins and reactivates aggregates formed by denatured proteins that retain native-like conformations. DnaK is composed of two domains connected by a highly conserved linker that allows their transient interaction: the N-terminal nucleotide binding domain (NBD) that binds and hydrolyzes ATP, and the substrate binding domain formed by a β-sandwich that contains the peptide binding pocket and a helical lid that controls the accessibility of the peptide binding site [12]. DnaK is also an allosteric protein whose functional cycle depends on the nucleotide-controlled switch between at least two conformational states that differ in their affinity for substrates: a compact ATP-state with low affinity, which, after nucleotide hydrolysis, generates an extended ADP state with higher affinity [13,14]. Cycling between both conformations is regulated by DnaJ and the NEF GrpE. First, DnaK interacts with a substrate or with a DnaJ–substrate complex. Then, DnaJ transfers the substrate to DnaK and stimulates its ATPase activity, so that after nucleotide hydrolysis, the ADP state of DnaK forms a stable complex with the bound substrate. Binding of GrpE to this complex accelerates ADP exchange by ATP and induces the opening of the peptide binding site that leads to substrate release, which can fold in solution. When the aggregates are formed by extensively denatured proteins in which the unfolded proteins are enriched in intermolecular β-structure, their reactivation requires the concerted action of the DnaK system and the hexameric disaggregase ClpB (Hsp100). The ability of the DnaK system to work independently or associate with ClpB has been proposed to be controlled by the competition between GrpE and ClpB to bind the NBD of DnaK [15,16]. We have recently characterized the effect of macromolecular crowding on the association properties and activity of ClpB and on its interaction with the DnaK system to form the bichaperone complex [17]. Crowding increases the association constant of the hexamer by 2–3 orders of magnitude, activates the disaggregase, and increases 20–30 times the affinity of DnaK for ClpB. However, the sequential association reactions that take place in the DnaK cycle have been characterized using different methods but always under dilute experimental conditions [18–20].

In this work, we aim to explore how volume exclusion, generated by high concentrations of synthetic crowders such as Ficoll 70 and Dextran 70, regulates the interaction of DnaK with its cochaperones and with ClpB. A specific change in DnaK affinity for these partners could be relevant in directing the chaperone to remodel native and unfolded substrates or to reactivate stable protein aggregates. Our data show that macromolecular crowding modifies the conformation of DnaK and DnaJ, but not that of GrpE, which is reflected in an increased affinity of DnaK only for DnaJ. The G/F domain-dependent and reversible conformational rearrangement of DnaJ under crowding promotes the formation of cochaperone assemblies that might be involved in recruiting DnaK clusters at the aggregate surface and therefore in the regulation of the refolding activity of the DnaK system. Finally, we demonstrate that by enhancing the affinity of DnaK for ClpB, but not for GrpE, crowding facilitates competition of the disaggregase with GrpE for DnaK binding and therefore the formation of the chaperone complex responsible for aggregate reactivation.

Results

Crowding increases the sensitivity of the DnaK system refolding activity to cochaperone concentration

Macromolecular crowding could change the stability of the DnaK–cochaperone complexes and, consequently, alter the refolding activity of the DnaK system. To test this possibility, we followed the effect of two commonly used crowders, Ficoll 70 and Dextran 70, on the chaperone activity of the DnaK system at increasing DnaJ and GrpE concentrations. DnaJ titration was performed in the presence of a fixed concentration of GrpE (0.4 μM), (Fig. 1a and S1). In the absence of crowder, the reactivation rate of luciferase aggregates increased with DnaJ concentration up to 1 μM, as found previously (Fig. 1a) [21]. In contrast, in the presence of 15% crowder, the behavior was biphasic within the cochaperone concentration range employed: at low DnaJ concentrations, that is, at DnaJ:DnaK molar ratios lower than 0.25 for Ficoll 70 and 0.05 for Dextran 70, the initial reactivation rate increased similarly to what was found in the absence of crowder (Fig. 1a), while at higher molar ratios, reactivation was inhibited, more markedly for Dextran 70. As a control of the effect of the increase in sample viscosity, we performed the same experiment at 40% sucrose (wt/vol), which gives bulk viscosity values similar to 15% Ficoll 70. Under these experimental conditions, reactivation of luciferase aggregates was drastically inhibited (only 6% of the