doi:10.1016/j.jmb.2011.07.016 J. Mol. Biol. (2011) **413**, 297–309

Contents lists available at www.sciencedirect.com

Journal of Molecular Biology

journal homepage: http://ees.elsevier.com.jmb

αB-Crystallin Polydispersity Is a Consequence of Unbiased Quaternary Dynamics

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Received 25 April 2011;
received in revised form
8 July 2011;
accepted 11 July 2011
Available online
3 August 2011

Edited by A. G. Palmer III

Keywords:
Polydispersity;
mass spectrometry;
small heat shock proteins (sHSPs);
molecular chaperones;
thermodynamics and kinetics

The inherent heterogeneity of many protein assemblies complicates characterization of their structure and dynamics, as most biophysical techniques require homogeneous preparations of isolated components. For this reason, quantitative studies of the molecular chaperone αB-crystallin, which populates a range of interconverting oligomeric states, have been difficult, and the physicochemical basis for its polydispersity has remained unknown. Here, we perform mass spectrometry experiments to study αB-crystallin and extract detailed information as to its oligomeric distribution and exchange of subunits under a range of conditions. This allows a determination of the thermodynamic and kinetic parameters that govern the polydisperse ensemble and enables the construction of a simple energy profile for oligomerization. We find that the quaternary structure and dynamics of the protein can be explained using a simple model with just two oligomer-independent interactions (i.e., interactions that are energetically identical in all oligomers from 10mers to 40mers) between constituent monomers. As such, the distribution of oligomers is governed purely by the dynamics of individual monomers. This provides a new means for understanding the polydispersity of αB-crystallin and a framework for interrogating other heterogeneous protein assemblies.

Introduction

The vast majority of proteins perform their cellular functions as multimeric assemblies,[1] with interactions ranging from the robust to the fleetingly transient.[2] The preponderance of these protein assemblies is currently thought to exist in one principal oligomeric state, reflective of a defined structure having evolved to perform a particular cellular function.[3] Seemingly contrary to this paradigm, however, some proteins instead populate an ensemble of stoichiometries at equilibrium.

Perhaps the most famous example of such polydispersity is the vertebrate small heat shock protein (sHSP) α-crystallin, which exists in two isoforms (αA and αB) of high sequence similarly.[4] The α-crystallins populate all possible oligomers from between 10 and 40 subunits,[5] a remarkable heterogeneity that has impeded high-resolution structural analysis.[6] Nevertheless, structures of α-crystallin dimers, generated by removal of approximately

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Abbreviations used: sHSP, small heat shock protein; MS, mass spectrometry; CID, collision-induced dissociation.

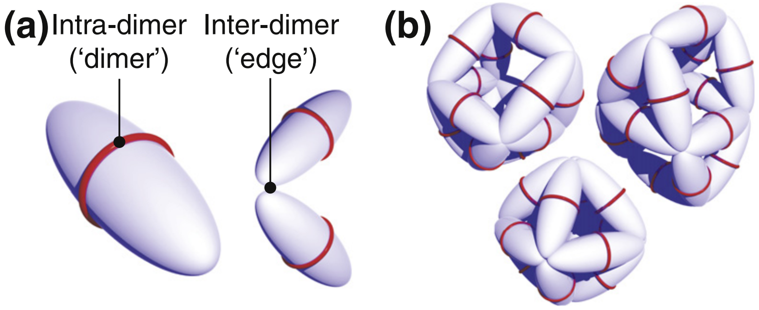

Fig. 1. The oligomeric organization of the sHSPs. (a) The protomeric dimers of αB-crystallin can be represented as ellipsoids, are stabilized by an intra-dimer interface, and assemble into oligomers via inter-dimer interactions. In the model described in this work, these interfaces are termed dimer and edge interactions, respectively, with their strengths described by the corresponding free energies ΔG_d and ΔG_e. (b) These dimers assemble into an ensemble of globular oligomers spanning at least 30 subunits at equilibrium and of unknown structure.

70 N- and 15 C-terminal residues, have been reported.[7–10] These dimers therefore represent the building block of the oligomers[11] and assemble into an ensemble of globular particles.[6,12–14] α-Crystallin structures are therefore stabilized by both intra- and inter-dimer interactions (Fig. 1a).

Despite these structural insights, the origins of the variable stoichiometry of the α-crystallins, vital to the role of this protein in the eye lens,[15,16] are not understood. In addition, the oligomers are known to be very dynamic, with oligomers readily exchanging subunits[17,18] in a process associated with their protective function.[19,20] αB-crystallin is found in many tissues,[15] where, along with other sHSPs, it is central to the maintenance of protein homeostasis.[21] Its molecular chaperone function is to bind and maintain the solubility of unfolded proteins,[22,23] thereby preventing their accumulation into potentially pathogenic aggregates.[24] αB-crystallin malfunction is consequently associated with a range of protein deposition diseases, from cataract formation to Alzheimer's disease.[25] For an appreciation of the mechanism by which αB-crystallin functions as a chaperone, a quantitative understanding of the origins of both the quaternary dynamics and heterogeneity is required.

Nanoelectrospray mass spectrometry (MS) is an emerging structural biology approach, reliably determining the stoichiometries of protein assemblies with unrivalled accuracy.[26–28] Furthermore, through benefiting from very high separation efficiency, it is particularly valuable in the study of polydisperse proteins, allowing the assessment of the oligomers they populate.[29] Though performed in the gas phase, such quantification directly reflects the distribution observed in solution, but with dramatically improved mass resolution.[30–32] This approach has resulted in the quantification of the relative populations of the different oligomers comprising the polydisperse ensemble of the α-crystallins.[5,30,31] Furthermore, the large amount of data obtainable by means of MS in real time makes it well suited to investigating the quaternary dynamics of oligomeric proteins,[33] such as the subunit exchange of the α-crystallins.[31]

Here, we use MS to obtain oligomeric distributions and subunit exchange kinetics of αB-crystallin over a wide range of pH values and temperatures to describe fully the thermodynamic and kinetic basis for its polydispersity. We show that, remarkably, this property can be understood in terms of just two monomer-level interactions, intra- and inter-dimer, which are independent of oligomer size. Furthermore, we demonstrate within the context of this model that formation of α-crystallin oligomer distributions can be explained by the quaternary dynamics associated with single monomers "hopping" between oligomers. As such, the seemingly complex organization of this protein can be rationalized in terms of very simple physical principles, shedding light on how other polydisperse proteins might assemble.

Results

Determining oligomeric distributions and dynamics by means of MS

MS approaches have proven very useful for interrogating the polydisperse αB-crystallin ensemble.[5,30,31,34] A nanoelectrospray mass spectrum of αB-crystallin at pH 7, under conditions optimized for the ionization and transmission of intact protein assemblies, reveals a broad region of signal between 8,000 and 14,000 m/z (Fig. 2a), as is typical for this protein.[5,30,31,34] The extensive overlap of charge states, stemming from the wide range of copopulated stoichiometries, renders the spectrum essentially uninterpretable. To improve the identification of the individual species that comprise the oligomeric ensemble, we employed collision-induced dissociation (CID).[35,36] In this experiment, all ions are accelerated into an argon-filled cell to effect their activation. A spectrum obtained at a high acceleration voltage shows a compact series of peaks at low m/z and a multitude of peaks at 16,000 m/z and above (Fig. 2b). These correspond to monomers and complementary "stripped" oligomers, that is,