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A quantitative NMR spectroscopic examination of the flexibility of the C-terminal extensions of the molecular chaperones, αA- and αB-crystallin

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ABSTRACT

The principal lens proteins αA- and αB-crystallin are members of the small heat-shock protein (sHsp) family of molecular chaperone proteins. Via their chaperone action, αA- and αB-crystallin play an important role in maintaining lens transparency by preventing crystallin protein aggregation and precipitation. αB-crystallin is found extensively extralenticularly where it is stress inducible and acts as a chaperone to facilitate general protein stabilization. The structure of either αA- or αB-crystallin is not known nor is the mechanism of their chaperone action. Our earlier ^{1}H NMR spectroscopic studies determined that mammalian sHsps have a highly dynamic, polar and unstructured region at their extreme C-terminus (summarized in Carver (1999) Prog. Ret. Eye Res. 18, 431). This C-terminal extension acts as a solubilizing agent for the relatively hydrophobic protein and the complex it makes with its target proteins during chaperone action. In this study, αA- and αB-crystallin were ^{15}N-labelled and their ^{1}H–^{15}N through-bond correlation, heteronuclear single-quantum coherence (HSQC) NMR spectra were assigned via standard methods. ^{1}H–^{15}N spin-lattice (T_1) and spin–spin (T_2) relaxation times were measured for αA- and αB-crystallin in the absence and presence of a bound target protein, reduced α-lactalbumin. ^{1}H–^{15}N Nuclear Overhauser Effect (NOE) values provide an accurate measure, on a residue-by-residue basis, of the backbone flexibility of polypeptides. From measurement of these NOE values, it was determined that the flexibility of the extension in αA- and αB-crystallin increased markedly at the extreme C-terminus. By contrast, upon chaperone interaction of αA-crystallin with reduced α-lactalbumin, flexibility was maintained in the extension but was distributed evenly across all residues in the extension. Two mutants of αB-crystallin in its C-terminal region: (i) I159A and I161A and (ii) K175L, have altered chaperone ability (Treweek et al. (2007) PLoS One 2, e1046). Comparison of ^{1}H–^{15}N NOE values for these mutants with wild type αB-crystallin revealed alteration in flexibility of the extension, particularly at the extremity of K175L αB-crystallin, which may affect chaperone ability.

1. Introduction

One of the major functions of molecular chaperones is to prevent inappropriate protein interactions that would result in protein misfolding and aggregation, particularly under stress conditions, e.g. elevated temperature (Ehrnsperger et al., 1997). In the mammalian lens, α-crystallin, along with the other crystallin proteins, β- and γ-crystallin, serve as structural proteins. α-Crystallin also acts as a molecular chaperone, preventing aggregation of other crystallins and maintaining lens transparency (Horwitz, 1992, 2003). α-Crystallin is composed of two related subunits, αA- and αB-crystallin and is a member of the small heat-shock protein (sHsp) family of molecular chaperones.

Despite α-crystallin being initially regarded as solely a lens protein, αA- and αB-crystallin have since been found in many other

Abbreviations: DTT, dithiothreitol; HSQC, Heteronuclear Single Quantum Coherence; IPTG, isopropyl-1-thio-β-D-galactopyranoside; LB, Luria-Bertani; NMR, Nuclear Magnetic Resonance; NOE, Nuclear Overhauser Effect; NOESY, Nuclear Overhauser Effect Spectroscopy; SDS-PAGE, Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis; T_1, longitudinal (spin-lattice) relaxation time; T_2, transverse (spin–spin) relaxation time; TOCSY, Total Correlation Spectroscopy; WET, Water suppression Enhanced through T_1 effects.

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tissues such as the brain and heart (Bhat and Nagimeni, 1989; Kato et al., 1991a, 1991b; Srinivasan et al., 1992). αB-Crystallin is induced under stress conditions and is involved in many neurodegenerative disorders such as Alzheimer's, Parkinson's and Creutzfeldt-Jakob diseases (Iwaki et al., 1992; Renkawek et al., 1994; Mizutani et al., 1998). The broad extralenticular and cellular distribution of α-crystallin suggests that its chaperone action is important in stabilizing a vast array of proteins.

αA- and αB-Crystallin have a mass of approximately 20 kDa but form very large and heterogeneous assemblies, e.g. the αB-crystallin oligomer has an average mass of around 650 kDa (Haley et al., 1998). Oligomeric α-crystallin is in rapid and dynamic equilibrium with smaller species, (i.e. dimers) (Bova et al., 1999; Sobott et al., 2002) which may be the more chaperone-active species (Carver et al., 2002; Sobott et al., 2002). α-Crystallin interacts with intermediately folded target proteins that are aggregating via a dynamic, nucleation-dependent mechanism (Devlin et al., 2003). The dynamism of both the target protein and its interacting chaperone is proposed to be crucial in facilitating sHsps in their interaction with a wide range of target proteins (van Montfort et al., 2001b; Bova et al., 2002; Carver et al., 2002; Treweek et al., 2003) but has proven to be a barrier to the crystallization of αA- and αB-crystallin (Harding, 1991; Jaenicke, 1994). Progress in this area has been made recently with the crystal structure of the conserved central 'α-crystallin' domain of αB-crystallin and a related sHsp, Hsp20, becoming available (Bagnéris et al., 2009). However, the tertiary and quaternary structure of intact α-crystallin and its mechanism of chaperone action remain elusive.

NMR spectroscopy has contributed to an understanding of the structure/function aspects of α-crystallin (Carver et al., 1992, 1994; Carver and Lindner, 1998; Carver, 1999; Jehle et al., 2009). Since αA- and αB-crystallin exist as very large, oligomeric complexes, their mass is too large for high-resolution analysis by conventional solution-phase NMR spectroscopy. However, the C-terminal extensions of the α-crystallin subunits that protrude from the domain core of the molecule are polar, unstructured, highly flexible and solvent accessible and, as a result, give rise to well-resolved NMR spectra (Carver et al., 1992, 1994, 1995a; Carver and Lindner, 1998; Carver, 1999). In fact, these regions have flexibility comparable to that of a peptide of the same length of around 10 to 12 amino acids (Esposito et al., 1998). The primary role of the C-terminal extension is proposed to be to solubilize the oligomeric chaperone, which has a relatively high degree of exposed hydrophobicity, and also the chaperone-target protein complex (Carver and Lindner, 1998; Lindner et al., 1998, 2000). Such polar, short, unstructured C-terminal extensions are a common feature of other mammalian sHsps (Carver et al., 1995b; van de Klundert et al., 1998; Smulders et al., 2002; Ghahghaei et al., 2009) and other molecular chaperones also have regions of flexibility which impart malleability and facilitate interaction with a wide range of proteins (Tompa and Csermely, 2004). In sHsps, the C-terminal extensions have been the focus of various functional studies which show that they are critical to chaperone activity (Kelley et al., 1993; Takemoto et al., 1993; Andley et al., 1996; Smulders et al., 1996; Lindner et al., 1998, 2000; Fernando and Heikkila, 2000; Kamei et al., 2000; Treweek et al., 2007; Morris et al., 2008) and also to oligomeric assembly (Kim et al., 1998; van Montfort et al., 2001a; White et al., 2006; Treweek et al., 2007).

Recently, NMR structural characterization of the domain core of αB-crystallin has been achieved via a combined solid and solution-state NMR study of αB-crystallin and its 'α-crystallin' domain (Jehle et al., 2009). It was concluded that this region adopts a β-sheet structure in both species that was comparable to the arrangements derived from crystal structures of well-ordered and oligomeric, non-mammalian sHsps (Kim et al., 1998; van Montfort et al., 2001a). Fig. 1 shows the putative structure of the αB-crystallin monomer as modelled on the crystal structures of two non-mammalian sHsps (Ghosh et al., 2005). The β-sheet containing 'α-crystallin' domain is flanked by the N-terminal region and the C-terminal region which includes the C-terminal extension at its extremity.

In this study, uniform ^{15}N-labelling of wild type and mutant α-crystallins allowed us to quantify the flexibility of the C-terminal regions of αA- and αB-crystallin under non-chaperone and chaperone-interacting conditions via ^{1}H–^{15}N relaxation time and Nuclear Overhauser Effect (NOE) measurements. Such NMR studies are a powerful tool for examining the backbone dynamics of proteins in solution (Kay et al., 1989; Clore et al., 1990; Redfield et al., 1992). Upon uniform ^{15}N-labelling of proteins, data can be obtained on the motion of the backbone amides (Clore et al., 1990). The set of three experiments described in this study (^{1}H–^{15}N T_1 and T_2 relaxation time and steady-state NOE measurements) are routinely used to investigate the amplitude and range of local molecular motions of backbone amides (Barbato et al., 1992; Cho et al., 1996). T_1 relaxation times, during which energy of a spin is dissipated within its surrounding lattice, provide information on motions occurring with a frequency of 10^8–10^{12} s^{-1} whereas T_2 (spin–spin) relaxation times are also sensitive to motions occurring over micro- or milliseconds, and therefore they reflect molecular motion over a large timescale (Kay et al., 1989). ^{1}H–^{15}N NOE measurements provide information on the motion of individual N–H bond vectors with their sign and magnitude being indicative of their mobility relative to the overall molecular tumbling rate of the protein and also to internal dynamics. Thus, positive NOE values occur for N–H bond vectors that are tumbling with a correlation time comparable to the overall protein, i.e. they are reflective of folded or structured regions within the protein. Large negative NOE values are indicative of large amplitude motions for N–H bond vectors that occur on a faster timescale and arise from unstructured regions of the protein i.e., regions with correlation times that are much shorter than those from structured regions of proteins.

Our previous study (Treweek et al., 2007) investigated the structural and functional effects of mutations of selected residues in the C-terminal extension of αB-crystallin. As a result, we identified that K175L and the double residue mutant, I159A/I161A αB-crystallin, had altered chaperone activity toward target proteins undergoing amorphous and fibrillar aggregation (Treweek et al., 2007). Using NMR spectroscopy, we investigated herein whether this flexibility was affected (compared to the wild type protein) in these two C-terminal mutants in order to provide a possible

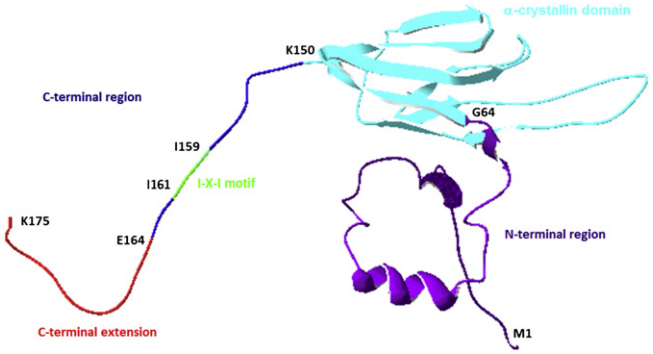

Fig. 1. Homology model of the αB-crystallin monomer (Ghosh et al., 2005). The three distinct structural regions of the protein are indicated, i.e. the N-terminal region, α-crystallin domain and C-terminal region. At the latter's extremity is the flexible C-terminal extension which is the focus of this study. The conserved I-X-I motif in sHsps is indicated.