Isomerization as the secret Achilles' heel of long-lived proteins

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Alex J. Guseman and Angela M. Gronenborn[1]

From the Department of Structural Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

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Crystallin proteins, the dominant constituents of the eye lens, are prototypes of long-lived proteins. Such proteins can accumulate harmful modifications over their life span that render them prone to aggregation, which, in the case of lens crystallin, contributes to cataract formation. Lyon *et al.* now explore the structural and functional consequences of amino acid isomerization in α-crystallins using mass spectrometry, molecular dynamics simulations, and other strategies. Their results highlight the potential deleterious effects of these under-detected modifications on protein structural integrity and function.

The average life span of a protein ranges from tens of minutes in simple eukaryotes such as *Saccharomyces cerevisiae* to a few days or longer in mammals (1). In general, cells and organs constantly renew their protein pools to ensure optimal function of the organism. This turnover of molecules involves the removal of damaged/misfolded proteins and their replacement by a fresh supply of newly synthesized proteins (1). However, although mostly true, there are exceptions to this rule: some proteins may exist for decades without renewal—aptly called "long-lived proteins." Naturally, such long-lived proteins are particularly vulnerable to damage over time. Damaging modifications come in various different forms, from bond cleavage to the addition of chemical groups. Although many of these modifications are easily detectable by MS, this is not the case for isomerizations, which result in inversion of amino acid stereocenters or altered polypeptide chain connectivity (2). However, these invisible changes can have drastic effects on protein structure because they may cause conformational changes in the polypeptide backbone by up to 90° (1). New research from Lyon *et al.* (3) now investigates these drastic effects in the case of modifications to eye lens proteins, revealing insights into possible routes of aggregation and encouraging increased attention to these ostensibly minor changes.

Long-lived proteins are often found in biologically inert microenvironments that are isolated from or devoid of the machinery for protein synthesis and degradation. One such environment is the eye lens, home to the long-lived protein family of crystallins (4). Crystallins comprise 90% of the soluble protein in the eye lens and are responsible for ensuring transparency and reducing the refractive index of the lens. Uniquely, these proteins are synthesized *in utero* and must remain functional for decades (4). Of the two crystallin subfamilies (α and β/γ), the α-crystallins function as chaperones to keep members of the β/γ-crystallin subfamily well-behaved. In particular, the α-crystallins are homologs of heat-shock proteins and form higher order oligomeric complexes (Fig. 1*A*) (3, 5, 6) that help rescue damaged β/γ-crystallins (6). However, α-crystallins themselves are susceptible to the same damage as their β/γ cousins (3, 4), rendering their long-term survival in an intact form extremely important.

To investigate how isomerization, potentially accumulating over a lifetime, may affect the structure and function of the two large oligomeric α-crystallin chaperonins, αA- and αB-crystallin, Lyon *et al.* (3) built on previous work in which they identified multiple sites of modifications in α-crystallins purified from a 72-year-old donor lens (7). In the current study, the authors evaluated four modifications in more detail: two involved racemization of serines (Ser-162 and αB Ser-59 in αA-crystallin), and the other two were related to isomerization of aspartates in αB-crystallin (Asp-62 and Asp-109). By employing MS and synthetic peptides, Lyon *et al.* (3) confirmed the presence of these modifications in human lenses and explored the consequences of these changes. They showed that racemization of Ser-162 in the C-terminal tail of αA-crystallin reduces the formation of α-crystallin oligomers, and that this change in multimerization is mediated by a decrease in the affinity of the C-terminal tail for its binding site in the β4/β8 groove on adjacent dimers. A different role is played by the racemization of Ser-59, namely interference with phosphorylation. D-Ser prevents phosphorylation, as shown by enzyme assays with isolated D-Ser- or L-Ser–containing peptides.

In the report by Lyon *et al.* (3), the effect of Asp isomerization was investigated using a combination of native MS and molecular dynamics simulations. Based on the X-ray structure of the αB-crystallin dimer, the authors hypothesized that isomerization of both Asp-62 and Asp-109 would increase the distance between these side chains and their interacting counterparts, resulting in the disruption of salt bridges. Removal of the Asp side chain in a D109A mutant corroborated this notion, because native MS revealed oligomers of higher molecular weight with stoichiometries differing from those found in WT oligomers (Fig. 1*B*).

The finding by Lyon *et al.* (3) that Ser and Asp isomerization in α-crystallins interferes with the formation of native oligo-

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[1] To whom correspondence should be addressed. E-mail: amg100@pitt.edu.

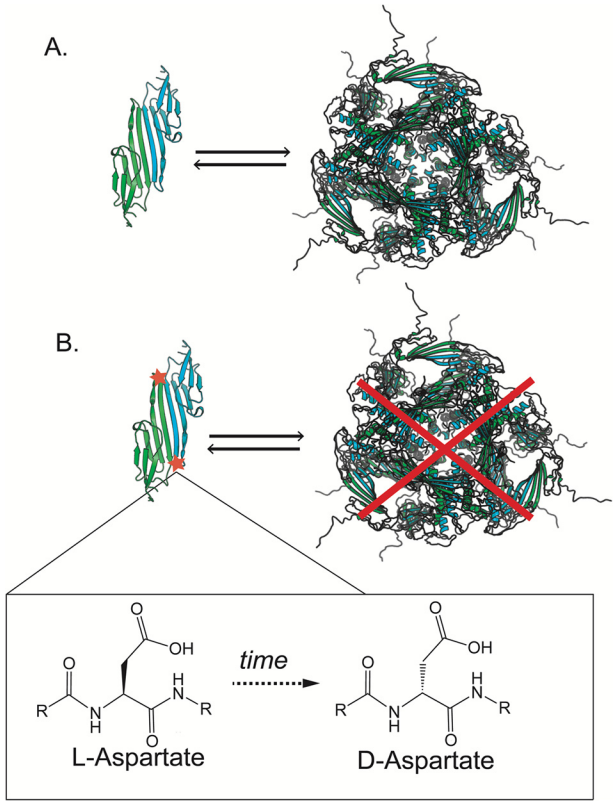

Figure 1. *A,* structure of the αB-crystallin dimer (PDB code 2KLR) and a model of the assembled 24-mer (PDB code 3J07). *B,* isomerization of Asp-109, one of the modifications explored by Lyon *et al.* (3), at the oligomer interface inhibits assembly into the functional quaternary structure.

mers has important implications for eye lens physiology. As chaperones, if α-crystallins are impaired in forming their native functional quaternary structures, they lose their ability to interact with and sequester damaged β/γ-crystallins, enhancing the probability for damaged β/γ crystallins to aggregate and contribute to cataract formation.

Given that isomerization of amino acids in α-crystallins, as shown by Lyon *et al.* (3), has biological consequences in the eye lens, similar "aging" of proteins may also play a role in other age-related diseases. Even though the susceptibility of an average protein to individual amino acid isomerization may be small, given the slow rate of isomerization (8), long-lived proteins, by their nature, are not "average" in terms of susceptibility. Collagen and elastin may be examples of these kinds of proteins. For long-lived proteins in nondividing cells, such as neurons, racemization may have devastating effects because neurons cannot easily be replaced, like a cataractous eye lens. Furthermore, discovering such damage by commonly used approaches is not straightforward, and these changes in crystallin and other long-lived proteins may elude detection by generic proteomics. Thus, the importance of the findings by Lyon *et al.* (3) stems from their identification of amino acid racemization in α-crystallins and the potential functional impact of this modification for other long-lived proteins. It therefore may be prudent to specifically search and test whether isomerizations are present in more proteins as they age and/or whether these chemical changes play a role in other protein deposition diseases associated with aging.

The ever-increasing capabilities of present-day methodologies, such as mass spectrometry and NMR, with the latter being the only tool available that can directly detect and identify amino acid isomerization, will play important roles in this quest, although NMR's sensitivity is still wanting in a native setting. Devising advanced analytical tools, combined with targeted chemical approaches for tailored detection of these hidden modifications, will aid toward charting strategies to address challenges linked to age-associated protein damage and the invariable decline in organismal function over time.

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