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Lens β-crystallins: The role of deamidation and related modifications in aging and cataract

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ABSTRACT

Crystallins are the major proteins in the lens of the eye and function to maintain transparency of the lens. Of the human crystallins, α, β, and γ, the β-crystallins remain the most elusive in their structural significance due to their greater number of subunits and possible oligomer formations. The β-crystallins are also heavily modified during aging. This review focuses on the functional significance of deamidation and the related modifications of racemization and isomerization, the major modifications in β-crystallins of the aged human lens. Elucidating the role of these modifications in cataract formation has been slow, because they are analytically among the most difficult post-translational modifications to study. Recent results suggest that many amides deamidate to similar extent in normal aged and cataractous lenses, while others may undergo greater deamidation in cataract. Mimicking deamidation at critical structural regions induces structural changes that disrupt the stability of the β-crystallins and lead to their aggregation *in vitro*. Deamidations at the surface disrupt interactions with other crystallins. Additionally, the α-crystallin chaperone is unable to completely prevent deamidated β-crystallins from insolubilization. Therefore, deamidation of β-crystallins may enhance their precipitation and light scattering *in vivo* contributing to cataract formation.

Future experiments are needed to quantify differences in deamidation rates at all Asn and Gln residues within crystallins from aged and cataractous lenses, as well as racemization and isomerization which potentially perturb protein structure greater than deamidation alone. Quantitative data is greatly needed to investigate the importance of these major age-related modifications in cataract formation.

1. Introduction

Several recent reviews have focused on the numerous modifications of crystallins associated with aging and cataracts (Moreau and King, 2012; Sharma and Santhoshkumar, 2009; Wilmarth et al., 2010). The aim of this review is to focus on the mechanism and functional significance of deamidation, the major modification in crystallins of the aged human lens. We will review several studies examining the functional significance of deamidation, with special emphasis on how deamidation alters β-crystallin structure. We will also discuss the related modifications of racemization and isomerization, as well as non-enzymatic cleavages that can occur during such processes. These modifications have been described in lens for nearly 40 years (Van Kleef et al., 1975). However, elucidating their role in cataract formation has been slow, because they are analytically among the most difficult post-translational modifications to study. We will review recent progress in detecting and quantifying these modifications.

2. Mechanism of deamidation and associated modifications in lens

Deamidation, which converts an amide to an acid, is a ubiquitous protein modification. Not surprisingly, it is the major modification to long-lived β-crystallins in aged and cataractous lenses. Deamidation at L-Asn residues in proteins occurs primarily by formation of a L-succinimide ring intermediate that forms via attack of the backbone amide on the side chain carbonyl to displace ammonia (Fig. 1A, step A). Following its formation, the L-succinimide ring is hydrolyzed, leaving a carboxylate group that introduces a negative charge in the protein in a newly formed L-Asp residue (Fig. 1A, step B). This hydrolysis can also result in the

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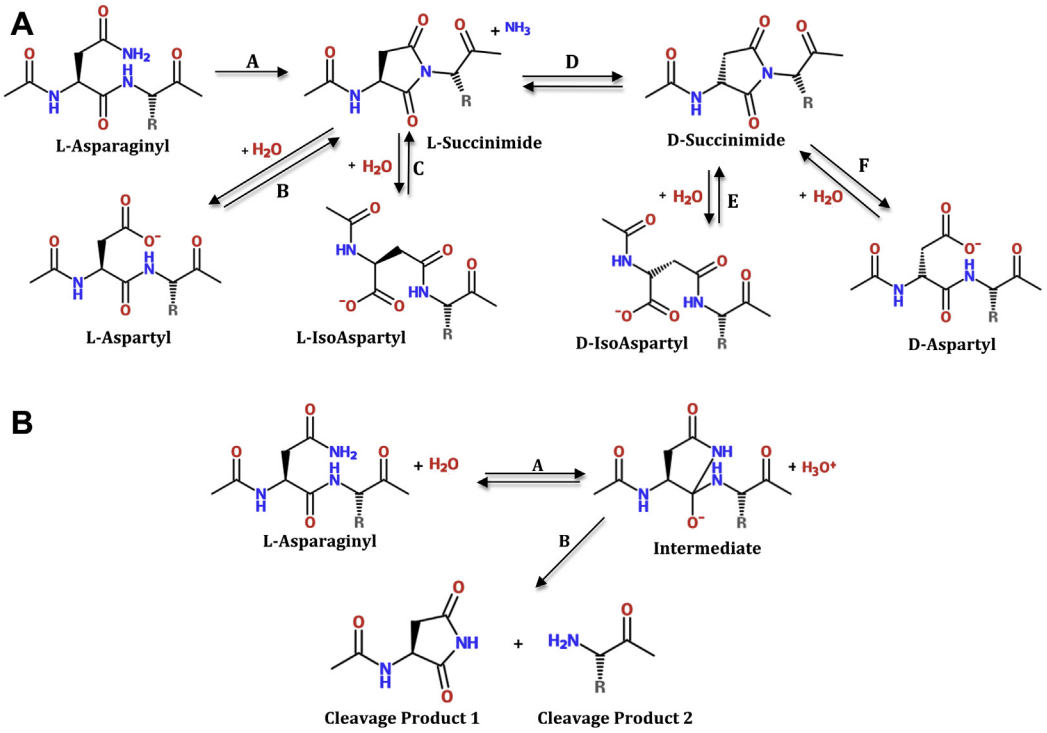

Fig. 1. A. Reactions of the labile Asn/Asp residues in proteins from aged and cataractous lenses. Step A, formation of a succinimide ring structure and loss of ammonia. Step B, hydrolysis of succinimide ring and formation of L-Asp. Note: L-asp residues can also undergo the reverse reaction to form the succinimide ring. Step C, alternate hydrolysis of succinimide ring to form L-isoAsp. Step D, racemization of L-succinimide ring to the D-succinimide ring form. Steps E and F, hydrolysis of D- succinimde ring to either D-isoAsp or D-Asp, respectively. B. Backbone cleavage at Asn/Asp (shown for Asn). The cleavage reaction is in competition with formation of the succinimide ring shown in Fig. 1A.

formation of an L-isoAsp (Fig. 1A, step C), where the protein backbone is elongated by insertion of an extra methylene group in the peptide backbone. The process also induces racemization, since the L-succinimide intermediate can undergo conversion to D-succinimide (Fig. 1A, step D), followed by hydrolysis to either D-Asp (Fig. 1A, step E) or D-isoAsp (Fig. 1A, step F) (Fujii et al., 2012; Geiger and Clarke, 1987). The new Asp and isoAsp moieties introduce destabilizing negative charges, and production of racemized D-isoAsp and D-Asp would be expected to further perturb protein structure. Asp residues can also undergo similar succinimide ring formation and conversion to D-Asp, L-isoAsp, and D-isoAsp. A competing reaction with deamidation is backbone cleavage at Asn (Fig. 1B) further discussed below.

The mechanism of Gln deamidation in lens is less well-understood, but may occur via a similar mechanism involving a slower-forming glutarimide intermediate (Robinson and Robinson, 2004b), or through simple hydrolysis of Gln amides during the long lifespan of lens proteins. Gln residues can also undergo enzymatic deamidation due to transglutaminase, activity, which is capable of causing deamidation of Gln residues in βB2- and βB3-crystallins (Boros et al., 2008). Regardless of the mechanism, deamidation at Gln occurs less frequently than at Asn in lens crystallins (Hains and Truscott, 2010; Wilmarth et al., 2006).

Another feature of deamidation is that it occurs more readily at certain Asn and Gln residues than others (Hooi et al., 2012b; Lapko et al., 2002; Robinson and Robinson, 2004a; Wright, 1991; Xie et al., 2000). This may be due to both adjacent amino acids residues (Robinson and Robinson, 2004b) and the location of amides in unstructured regions where the succinimide or glutarimide ring structures can form more readily, or on the surface of crystallins where hydrolysis could occur more ready.

3. Deamidation in aged human lens

Crystallins are extensively modified during normal aging and cataracts (Hains and Truscott, 2007, 2010; Harrington et al., 2004; Hooi et al., 2012a; Lampi et al., 1998; Lund et al., 1996; Ma et al., 1998; Miesbauer et al., 1994; Srivastava and Srivastava, 2003; Takemoto, 1998b; Wilmarth et al., 2006; Zhang et al., 2003). The use of mass spectrometry has unambiguously identified deamidation as the cause for the increase in acidic crystallins in early reports from the human lens (de Jong et al., 1988; Groenen et al., 1990; Van Kleef et al., 1975; Voorter et al., 1988, 1987). Many of these studies targeted the analysis of deamidation in individual purified crystallins or globally at individual amides within crystallins (Hanson et al., 2000; Lampi et al., 1998; Lapko et al., 2002; Lund et al., 1996; Miesbauer et al., 1994; Srivastava and Srivastava, 2003; Takemoto and Boyle, 2000). When lenses are homogenized, increasing proportions of the proteins isolated from the inner-most "nuclear" region become insoluble in water with increasing age (Liu and Liang, 2007). The long-standing hypothesis is that the accumulation of post-translational modification to crystallins is the cause of this insolubilization. This does not, however, exclude the possibility that the modifications occurred after the aggregation event.

More recently, global approaches have been used to study lens deamidation. Wilmarth et al. (2006) reported that deamidation of Asn and Gln were the major modifications identified in several human cataractous and aged lenses, totaling 66% of the total modifications tabulated when water-soluble and water-insoluble fractions were analyzed by 2D LC/MS and the relative extent of modifications estimated by spectral counting. This suggested that deamidation is one of the major, if not the major modification in