Biochimica et Biophysica Acta xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Isomerization of aspartyl residues in crystallins and its influence upon cataract

Noriko Fujii [a,*], Takumi Takata [a], Norihiko Fujii [b], Kenzo Aki [c]

[a] Research Reactor Institute, Kyoto University, Kumatori, Osaka 590-0494, Japan
[b] Radioisotope Research Center, Teikyo University, Kaga Itabashi-ku, Tokyo 173-8605, Japan
[c] Faculty of Pharmaceutical Sciences, Himeji Dokkyo University, Kamiohno, Himeji 670-8524, Japan

ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 25 July 2015
Accepted 3 August 2015
Available online xxxx

Keywords:
D-Amino acid
Aging
Isoaspartate (β-aspartate)
Protein
Cataract
LC–MS/MS

ABSTRACT

Background: Age-related cataracts, which probably form due to insolubilization of lens proteins, can lead to loss of vision. Although the exact reason is unknown, lens protein aggregation may be triggered by increases in PTMs such as D-β-, L-β- and D-α-Asp isomers. These isomers have been observed in aged lens; however, there have been few quantitative and site-specific studies owing to the lack of a quick and precise method for distinguishing between D- and L-Asp in a peptide or protein.
Scope of review: We describe a new method for detecting peptides containing Asp isomers at individual sites in any protein by using an LC–MS/MS system combined with commercial enzymes that specifically react with different isomers. We also summarize current data on the effect of Asp isomerization on lens crystallins.
Major conclusions: The new technique enabled the analysis of isomers of Asp residues in lens proteins precisely and quickly. An extensive proportion of Asp isomerization was observed at all Asp sites of crystallins in the insoluble fraction of aged lens. In addition, D-amino acid substitutions in crystallin-mimic peptides showed altered structural formation and function. These results indicate that isomerization of Asp residues affects the stability, structure and inter-subunit interaction of lens crystallins, which will induce crystallin aggregation and insolubilization, disrupt the associated functions, and ultimately contribute to the onset of senile cataract formation.
General significance: The mechanism underlying the onset of age-related diseases may involve isomerization, whereby D-amino acids are incorporated in the L-amino acid world of life.

1. Background

The eye lens focuses light on the retina. The light is changed to a nerve signal and sent to the brain, resulting in our visual function. To achieve this function, the lens must remain clear throughout the life span of an individual. However, almost all people have a cataract by the age of 80 years. The mechanism of cataract development is not well understood. It is thought that eye lens proteins become abnormally aggregated, initially resulting in clumping that scatters the light and interferes with focusing on the retina, and ultimately becoming a cataract.

Human lens proteins mainly comprise the α-, β-, and γ-crystallin superfamily of proteins [1]. α-Crystallin is a large molecule with a molecular mass of approximately 800 kDa that consists of two kinds of polypeptide: αA and αB. Each αA- or αB-crystallin monomer has a mass of close to 20 kDa; thus, the α-crystallin molecule is a hetero-polymer containing approximately 40-50 subunits. α-Crystallin has chaperone-like activity that inhibits the aggregation of other proteins. β-Crystallin exists as a homo- or hetero-oligomer with a molecular mass of 50–200 kDa, and consists of seven subunits (βA1, βA2, βA3, βA4, βB1, βB2 and βB3), each with a molecular mass of 20–30 kDa. Monomeric γ-crystallin consists of seven subunits (γA, γB, γC, γD, γE, γF and γS), each with a molecular mass of 20 kDa. γ-Crystallin is considered to play an important role in long-term maintenance of the transparency and refractive index of the lens [1].

Because lens proteins are long-lived, various post-translational modifications (PTMs) such as deamidation, isomerization, truncation and cross-linking can take place under physiological conditions and accumulate in the lens during the aging process. Such modifications decrease crystallin solubility, lens transparency, and ultimately lead to the development of a cataract. Although the relationship between PTMs and cataract formation was demonstrated in early studies, it was not known which crystallins — or which amino acids at which sites within these proteins — undergo PTM. To understand the mechanism of PTMs

Abbreviations: WS, water soluble; WI, water insoluble; PTMs, post-translational modifications; RP-HPLC, reverse-phase high-performance liquid chromatography; MS, mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; TOF, time-of-flight; PIMT, L-isoaspartyl methyltransferase; AD, Alzheimer's disease; AMD, age-related macular degeneration.

* Corresponding author at: Research Reactor Institute, Kyoto University, Osaka, Japan.
E-mail address: nfujii@rri.kyoto-u.ac.jp (N. Fujii).

http://dx.doi.org/10.1016/j.bbagen.2015.08.001
0304-4165/

and their effects on lens crystallin, therefore, it is necessary first to identify the specific sites of PTMs in the lens protein.

2. Determination of the specific sites of PTMs in the aged lens

The specific sites of deamidation of asparagine (Asn) or glutamine (Gln) residues [2–7], oxidation of methionine (Met), tryptophan (Trp) or cysteine [2–4,8,9], disulfide bonding of cysteine [3], phosphorylation of serine (Ser) or threonine (Thr) [6,8,10] and advanced glycation end products [11] have been identified in all crystallins alongside recent advances in analytical methods, as summarized in detail by Sharma et al. in an excellent review [12]. Spontaneous N- and C-terminal truncations of crystallins [13–16], peptide bond cleavage at Ser residues [17,18], and cross-linking have been detected in aging crystallins. In addition to these modifications, cross-linking through dehydroalanine and dehydrobutyrine has been reported by Wang et al. [19]. Identification of the specific sites of these modifications has been mostly achieved by simple mass analysis. For example, the formation of Asp or Glu from Asn or Gln, respectively, by a deamidation induces a +1 mass shift, and the oxidation of Met or Trp generates, respectively, a +16 or +32 mass shift. Therefore, many oxidation and deamidation sites in crystallins have been reported, and further in vitro research has suggested the biological effect of these modifications on lens crystallins [7,12,20]. Recent progress in proteomics analysis has shown that oxidation is not associated with aging but related to cataract formation [9,20]. As well as the above modifications, the isomerization and inversion of amino acids are important PTMs because they are directly associated with changes in protein structure. Nevertheless, they have not attracted much attention among the PTMs of proteins, probably because the detection of D-isomers is not possible by mass analysis as the mass of D- and L-isomer-containing peptides are exactly the same. Thus, the detection and quantitation of D-isomers in protein is more difficult and more complicated than other PTMs. This is the principal reason why there have been few studies of D-isomers in protein. However, some specific D-Asp sites have been identified in αA- [21], αB- [22] and β-crystallins [23], while one specific D-Ser site has been determined in αA-crystallin [24].

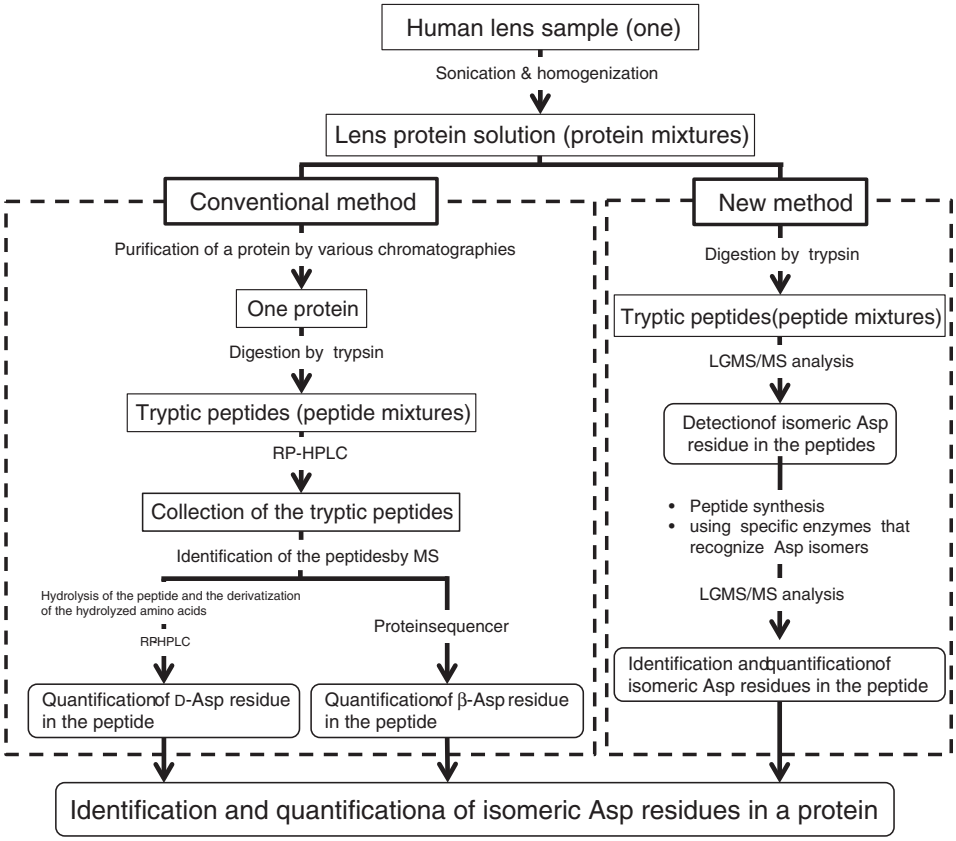
3. Determination of the specific sites of D-Asp in aged lens

3.1. Conventional methods

Until relatively recently, the determination of specific D-Asp sites in lens proteins had been achieved by the following complicated steps, as shown on the left side of Scheme 1:

(i) Crystallin is purified from lenses by multiple steps of column chromatography.
(ii) Each protein is digested with a protease such as trypsin.
(iii) The resulting peptides are separated by reverse-phase high-performance liquid chromatography (RP-HPLC).
(iv) The peptides are identified by mass analysis and/or protein sequencing.
(v) The α or β isomer of the identified peptides is determined by Edman degradation reaction.
(vi) The D/L ratios of the Asp residues are independently determined after the hydrolysis of peptides with 6 N HCl and derivatization to the diastereoisomers.
(vii) The diastereoisomers are analyzed by RP-HPLC, and the D/L ratio of amino acids is determined by analysis of the respective peak areas.

Although technically demanding, this method successfully determined and quantified the sites of D-amino acids in lens protein.



Scheme 1. Comparison of conventional and new methods for analyzing Asp isomers in proteins.

Please cite this article as: N. Fujii, et al., Isomerization of aspartyl residues in crystallins and its influence upon cataract, Biochim. Biophys. Acta (2015), http://dx.doi.org/10.1016/j.bbagen.2015.08.001