Review article

The age-related increase of D-amino acids and advanced glycation end-products in protein

Noriko Fujii, Takumi Takata

Biochemical Gerontology, Division of Radiation Life Science, Research Reactor Institute, Kyoto University, Osaka, Japan

Abstract

The amino acids that constitute the proteins of life are exclusively L-type. Although both L-type amino acids and their optical isomers, D-type amino acids, exist, the biosynthesis of amino acids *in vivo* is limited to L-type amino acids. Until recently, it was thought that L-amino acids cannot invert to D-amino acids in living organisms. Now, however, it has become clear that D-aspartic acids (D-Asp) are present in proteins of aged tissues from eye lens, retina, brain, skin, teeth, bones, arterial walls, and ligaments; furthermore, the affected proteins are inactive in metabolism and their quantities increase with aging. The appearance of D-Asp is thought to occur nonenzymatically due to long-term racemization. Advanced glycation end-products (AGEs) are also produced in proteins during the process of aging. They have been detected in the proteins that also contain D-Asp residues in the case of cataracts, age-related macular degeneration, and Alzheimer's disease. In this article, a new method to analyze both D-Asp residues and AGEs via a simplified one-shot analysis is described, alongside the commonality of these age-related protein modifications.

1. Introduction: the one-handed structure of amino-acids

It is generally considered that there are between 10,000 and 100,000 kinds of human protein, which carry out a diversity of functions: for example, there are enzymes that accelerate (catalyze) chemical reactions including *in vivo* metabolism/synthesis; structural proteins that function as a support in cells or tissues; proteins with sensory functions; motor proteins related to the movement of cells or tissues; transporter proteins that convey molecules or ions; gene regulatory proteins; proteins that transduce signals between cells; and antibodies for immune functions. To achieve such diverse functions, proteins are highly polymerized compounds (polymers) comprising several hundred amino acids of 20 different varieties united by peptide bonds.

The basic structure of all amino acids is a carbon atom (C) attached to an amino group (-NH2), a carboxyl group (-COOH), a hydrogen atom (-H), and R (which varies depending on the type of amino acid, resulting in different characters including acidity, basicity, hydrophilicity, and hydrophobicity). Due to the tertiary arrangement of the four groups around the C atom, all amino acids are chiral (except for glycine where R is H), meaning that they form optical isomers or "enantiomers" (*i.e.*, mirror images) that cannot be superimposed on one other, as in the case of left and right hands. These mirror image isomers, known as the L-form and the D-form (*Fig. 1*), have exactly the same physical and chemical properties.

During the chemical synthesis of an amino acid (unless asymmetric synthesis occurs), a racemic mixture containing both the L- and the D-enantiomers is obtained. In the same way, it is generally thought that the same mixture of L- and D-amino acids would have been produced on primitive Earth. Although these L-amino acids and D-amino acids would have been subsequently formed into L-polypeptides,
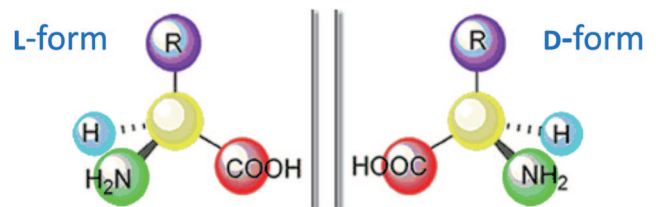

Fig. 1. **L-amino acid and D-amino acid.**

Correspondence to: Prof. Noriko Fujii, PhD
Biochemical Gerontology, Division of Radiation Life Science,
Research Reactor Institute, Kyoto University
2 Asashiro-nishi Kumatori-cho, Sennan-gun, Osaka 590-0494 Japan
Tel: +81-72-451-2496 Fax: +81-72-451-2630
Email: nfujii@rri.kyoto-u.ac.jp
Co-author: Takata T, takumi@rri.kyoto-u.ac.jp

D-polypeptides, and LD-polypeptides by condensation polymerization, only L-polypeptides were formed into proteins during the process of chemical evolution, resulting in the current world of living things.

In terms of an LD-polypeptide, innumerable stereoisomers or "diastereomers" are possible. For example, even in the case of a peptide comprising only three amino acids of a single type, eight kinds of stereoisomer (*i.e.*, LLL, LLD, LDL, LDD, DLL, DLD, DDL, and DDD) can exist. Likewise, in the case of 4 connected amino acids, 2^4 isomers will be formed, while in the case of n connected amino acid, 2^n isomers will be formed. When the complexity extends to 19 different types of amino acid instead of one, it can be seen that the number of isomers will become enormous. Based on these considerations, it is thought that the formation of a specific tertiary structure from such mixtures of D- and L-isomers was not easily attained, which prevented these polypeptides from evolving into proteins, and as a result, they were driven into extinction. By contrast, D-polypeptides are homochiral peptides formed from a single type of enantiomer, just like L-polypeptides, and there seems to be nothing that would prevent them from forming tertiary structures. As a result, it is not clear why D-amino acids were eliminated from primitive Earth during the course of evolution. Indeed, this remains one of the biggest mysteries concerning the origin of life, and it is entirely possible that organisms based on D-polypeptides exist on other planets.

Irrespective, the organisms present on Earth seem to have been produced by an L-amino acid world, the reason for which is not unknown. On this basis, it has long been believed that the one-handed structure of the L-amino acid alone is important in the folding, formation, and/or function of proteins and that it is impossible for L-amino acids to change into the D-form in the body of a living organism. In recent years, however, it has become clear that the D-form of aspartic acid (D-Asp) is present in various tissues, including eye lenses [1-5], retina, conjunctiva, cornea [6], teeth [7,8], bones [9,10], arterial walls [11], ligaments [12], brain [13-15], and skin [16-18], and increases in quantity as an organism gets older, leading to age-related diseases such as cataracts, macular degeneration, arteriosclerosis, and Alzheimer's disease (*Table 1*). D-Asp is thought to be produced nonenzymatically due to chemical racemization during the long-term aging process. Furthermore, it is considered that proteases or other breakdown enzymes will not function on proteins containing D-amino acids. Thus, in the above-mentioned tissues, an increase in D-Asp is thought to cause a change in the superstructure or functions of proteins, which in turn is responsible for disease.

2. Utility of D-amino acid as a new index for aging: Formation of D-amino acid as a function of time

On the one hand, as described in the previous section, it is no exaggeration to say that maintaining the one-handed structure of the proteins comprising organisms is a principle of life. On the other hand, it is possible to make use of this concept in archeology for estimating the time era by measuring the amount of D-amino acid contained in fossils. In other words, while 100% of amino acids in living creatures remain in the L-form, it is often the case that D-amino acids accumulate in fossil samples (without life) due to racemization over a long time span. Thus, because it is possible to measure the D-amino acid content and calculate the time that was needed to produce this amount of D-amino acid, we are able to get an estimate of the era.

Table 1. The presence of D-aspartic acid in protein from various aged tissues.

Tissue	Protein	D-amino acid	Related disease	Specific sites of D-Asp	Ref. No.	Analysis
lens	αA-crystallin	D-Asp	Cataract	Asp58,151,76,84	2,5	CA, LC-MS
lens	αB-crystallin	D-Asp	Cataract	Asp36, 62,96	3,5	CA, LC-MS
lens	βB2-crystallin		Cataract	Asp4	4	CA, LC-MS
retina	?		AMD	?	6	IH
conjunctiva	?		Pinguecula	?	6	IH
cornea	?		CDK	?	6	IH
tooth	phosphophoryn	D-Asp	?	?	7,8	CA
bone	osteocalcin	D-Asp	?	?	9	CA
bone	type I collagen C-terminal tellopeptide	D-Asp	Osteoporosis of Paget's disease	Asp 1211	10	CA
aorta	elastin	D-Asp	Arteriosclerosis	?	11	IH, CA
ligament	elastin	D-Asp	?	?	12	IH, CA
brain	β-amyloid	D-Asp	Alzheimer's disease	Asp1,7, 23	13	CA
brain	myelin	D-Asp	?	Asp23,34,48,145	14	LC-MS
brain	histone H2B	D-Asp	?	Asn25	15	CA
skin	collagen	D-Asp	?	?	UP	LC-MS
skin	elastin	D-Asp	?	?	16,17	IH, CA
skin	keratin	D-Asp	?	?	18	IH, CA

AMD, age related macular degeneration; CDK, climatic droplet keratopathy; CA, conventional analysis by GC or LC; LC-MS, liquid chromaograph mass analysis; IH, immunohistochemistry; UP, Unpulished data by Fujii N, *et al*.; ?, not determined.

Racemization is a reversible first-order reaction, as shown in **Scheme 1**; thus, it is possible to calculate, based on formula (2), the time era by measuring the D-amino acid fossil content. Depending on the types of amino acid, the rate of racemization differs. Therefore, it is recommended that the racemization ratio of isoleucine is measured in the case of a comparatively long time span, whereas that of Asp is measured in the case of a short time span. In this way, it is possible to estimate time periods ranging from 100 to 100,000 years ago. Because Asp is the amino acid whose racemization speed is the fastest, occurring even within the lifespan of humans, D-Asp has been detected in teeth [7], eyes [1], skin [16, 17] and ligaments [12] as an effect of aging, as mentioned above. D-Amino acids are produced within a single molecule of protein as a function of racemization even in the case of different tissues. Therefore, racemization occurs in apparently different tissues or in distinct diseases in the same way, demonstrating its universality as an aging index. For example, **Fig. 2** shows how the amount of D-Asp increases with age in homogenate samples of eye lenses, and in the skin and elastin fractions of ligament owing to racemization.
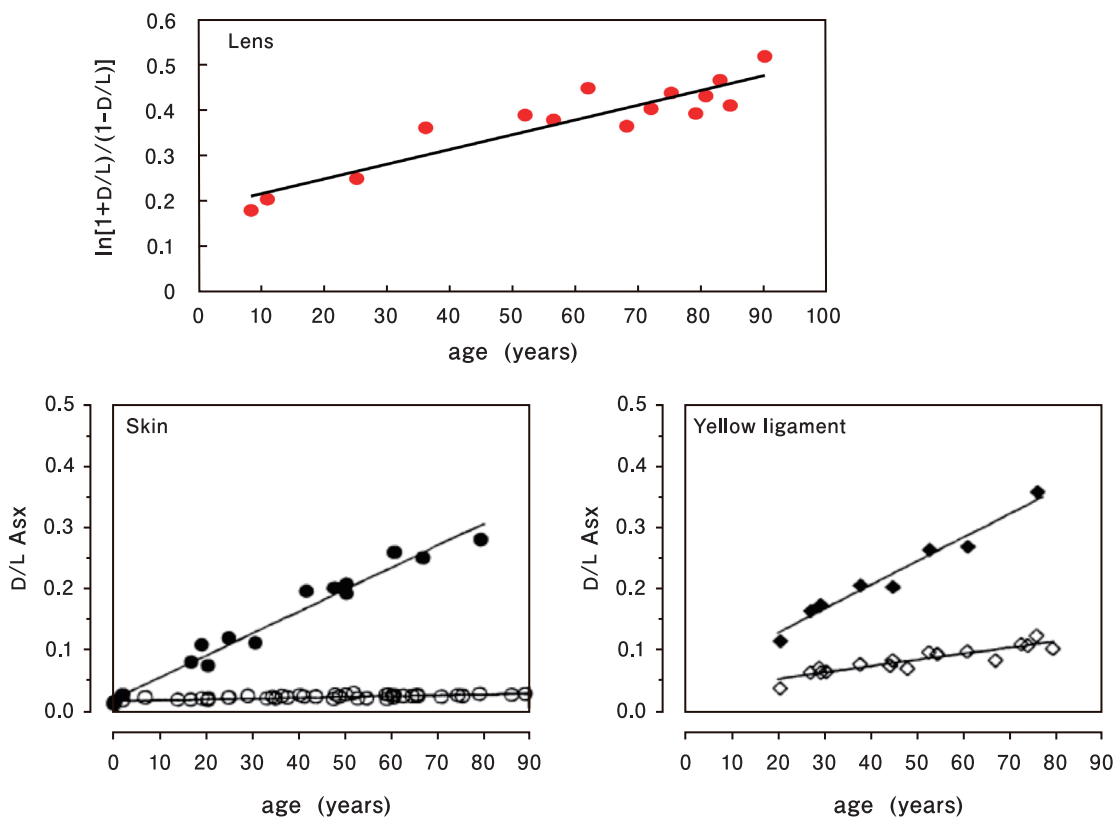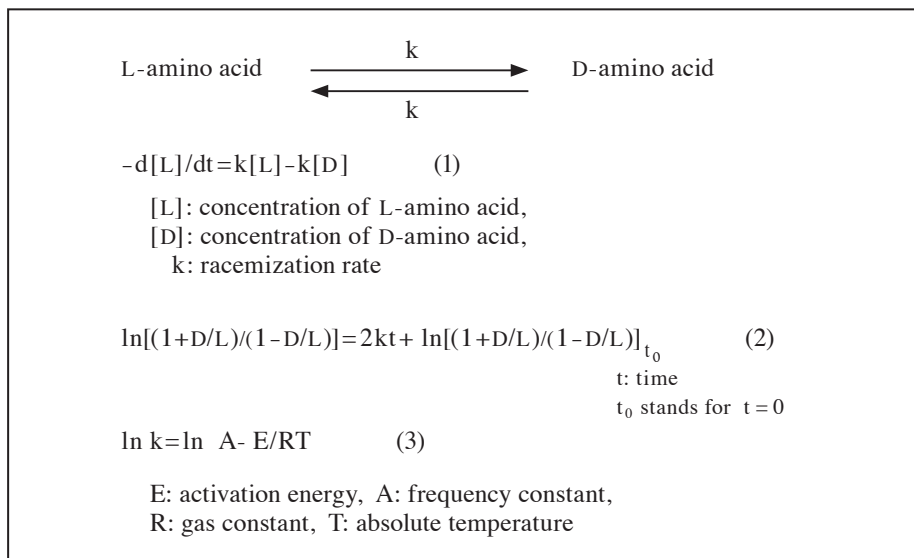
Scheme 1

L-amino acid $\xrightleftharpoons[k]{k}$ D-amino acid

$$-d[L]/dt = k[L] - k[D] \qquad (1)$$

[L]: concentration of L-amino acid,
[D]: concentration of D-amino acid,
k: racemization rate

$$\ln[(1+D/L)/(1-D/L)] = 2kt + \ln[(1+D/L)/(1-D/L)]_{t_0} \qquad (2)$$

t: time
t_0 stands for $t = 0$

$$\ln k = \ln A - E/RT \qquad (3)$$

E: activation energy, A: frequency constant,
R: gas constant, T: absolute temperature

Fig. 2. **Protein-bound D-aspartate increases in various tissues with age.**
● : Homogenate of lens (cited from Reference 1), ○, □ : Total proteins in skin and yellow ligaments,
●, ■ : Elastin fraction in skin and yellow ligaments (cited from Ref. 12).

3. Why Asp residues easily change to the D-form during aging of tissues: Prospective sites of D-Asp formation

For an amino acid to change from the L-form to the D-form through racemization, the H atom connected to the asymmetric C must be eliminated, a reaction that is generally difficult in the mild environment present in living creatures. In the case of Asp residues, however, the R group attached to the C atom is a carboxyl group, and the change to the D-form is relatively easy because racemization can occur through an intermediate comprising a 5-membered ring imide compound. In other words, as shown in *Fig. 3*, L-α-Asp residues can form a 5-membered ring imide through dehydration condensation by nucleophilic attack of a nitrogen atom on the main chain of the amino acid residue adjacent to the C-terminal side. Elimination of the H atom can then occur on the imide; when the H atom is re-added, the configuration may be reversed to a D-imide. Furthermore, when the imide ring re-opens, both α- and β-linkages can occur. Thus, D-α-Asp residues and D-β-Asp residues can be formed from the D-imide body, as well as L-α-Asp residues and L-β-Asp residues from the L-imide body (in total four kinds of isomer can form). Overall, formation of these products is due to synthesis of the intermediate imide, which means that the ease of imide synthesis is linked to the ease of isomerization.

It is thought that imide synthesis occurs easily when the residues adjacent to Asp are those with little steric hindrance such as glycine (Gly), alanine (Ala), and serine (Ser), which have small side chains. Regarding the sites of D-Asp formation observed in human proteins, for example, the residues adjacent to Asp58 and Asp151 in αA-crystallin are Ser59 and Ala152, those adjacent to Asp1 and Asp7 in β-amyloid proteins are Ala2 and Ser8, that adjacent to Asp25 in Histone H2B is Gly26, that adjacent to Asp1211 in Type 1 collagen C-terminal telopeptide is Gly1212, and that adjacent to Asp145 in myelin basic protein is Ala146, showing that D-Asp occurs readily when the side chain of the amino acid adjacent to the D-Asp site is small. Furthermore, the same rule is likely to apply in terms of the tertiary structure around the Asp site on the surface of the protein. Currently, there are not enough examples of specific D-Asp sites in aging proteins; however, by characterizing more D-Asp sites in larger samples of aging tissues, it will be possible to confirm the above ideas. As a result, we will be able to predict which Asp sites of proteins in ailing tissues are apt to become D-Asp residues in the future.

4. Methods to identify D-Asp residues in proteins

4-1. Chemical detection by diastereomer derivatization

In early studies, quantities of D-Asp in tissue samples were measured by hydrolyzing the tissues as a whole, as shown in *Fig. 2*. Even within the same protein, however, there are some sites that easily become the D-form, as outlined above. Therefore, to identify of specific D-Asp sites, it is necessary to conduct a D-amino acid analysis targeting individual residues. However, it is not easy either to detect D-amino acids that are present in only a few positions in a protein whose main component is L-amino acid, or to measure the relative quantity of D-Asp at each position.
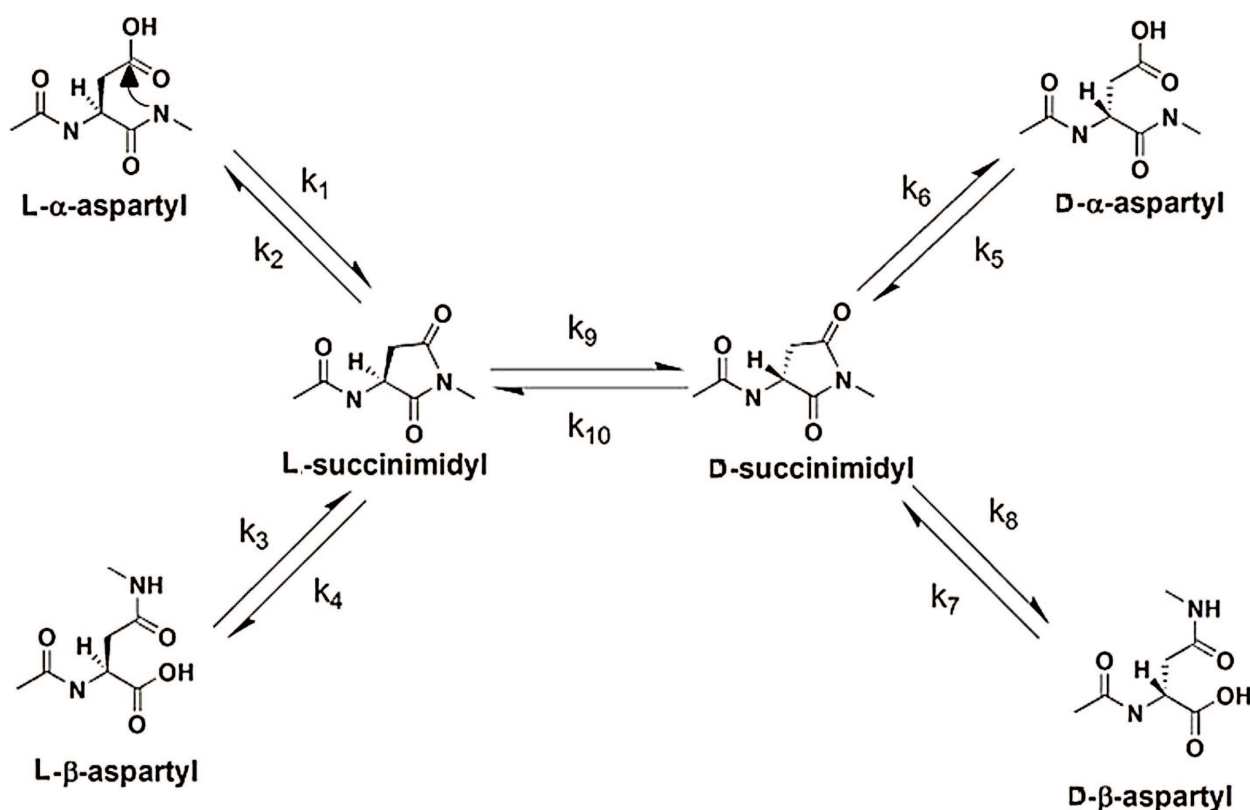

Fig. 3. **Reaction pathways for spontaneous isomerization of aspartyl residues in protein.**

The conventional method of D-Asp identification is as follows. First, the protein is fragmented into peptides by an enzyme called trypsin, and then the peptides are separated and sorted by using reversed-phase liquid chromatography (RP-HPLC). Next, each peptide is identified by a protein sequencer and mass spectrometry, and then subjected to hydrolysis. As mentioned above, L- and D-amino acids have the same physical and chemical properties. If amino acid analysis is carried out after hydrolysis, it is not possible to differentiate between the L-form and the D-form. Therefore, the hydrolyzed amino acids are reacted with a commercially available Boc L-cysteine to obtain a mixture of L-amino acid-L-cysteine and D-amino acid-L-cysteine dipeptides. Owing to the different shapes of the two diastereomers obtained, it is possible to separate them by RP-HPLC. The L-L and D-L dipeptides are eluted at different times; as a result, it is possible to obtain a quantitative ratio of the L- and D-amino acids based on the peak areas.

Via this procedure, we previously clarified that in, crystallin lens samples obtained from patients suffering from age-related cataract, Asp58 and Asp151[2] in αA-crystallin, Asp36 and Asp62[3] in αB-crystallin, and Asp4[4] in βB2-crystallin were highly susceptible to becoming the D-form (*Table 1*). Although this method is accurate, it is relatively laborious. First, it is necessary to extract proteins that are abundant in a number of tissues, and to isolate and purify each protein. Second, it is necessary to fragment all proteins into peptides via enzymatic treatment, to separate and/or sort them, and then to identify them. Last, it is

necessary to hydrolyze the peptides, and then to conduct the D-amino acid analysis described above. The whole procedure takes a vast amount of time, which is considered a weak point. In section **4.2**, we describe a novel method that we developed to solve these problems.

4-2. Chemical detection by LC-MS

The new method to easily and rapidly identify D-amino acid sites in proteins is based on liquid chromatography–mass spectrometry (LC-MS). Below we describe the process that we developed to analyze D-Asp residues in crystallin-lens. Notably, this method avoids the complicated isolation and purification of lens proteins. First, the lens is fractured by using ultrasonic waves and homogenized in neutral buffer solution to separate the water-soluble (WS) fraction from the water-insoluble (WI) fraction. Trypsin is added to each fraction to fragment the proteins into peptides, which are then analyzed in one step by using LC-MS (*Fig. 4-A*).

Note that the WI fraction is first solubilized by adding 8M urea to a final urea concentration of 2M, at which point it is possible to add protease. Trypsin cuts the proteins at the C terminus of lysine and arginine residues; therefore, a mixture of multiple peptides, whose C terminus is either lysine or arginine, is obtained. This mixture of peptide fragments from multiple proteins processed by multiple proteases is subjected to LC-MS, which separates the peptides depending on their relative solubility, resulting in several peaks (*Fig. 4-B*). Next, tandem MS analysis is
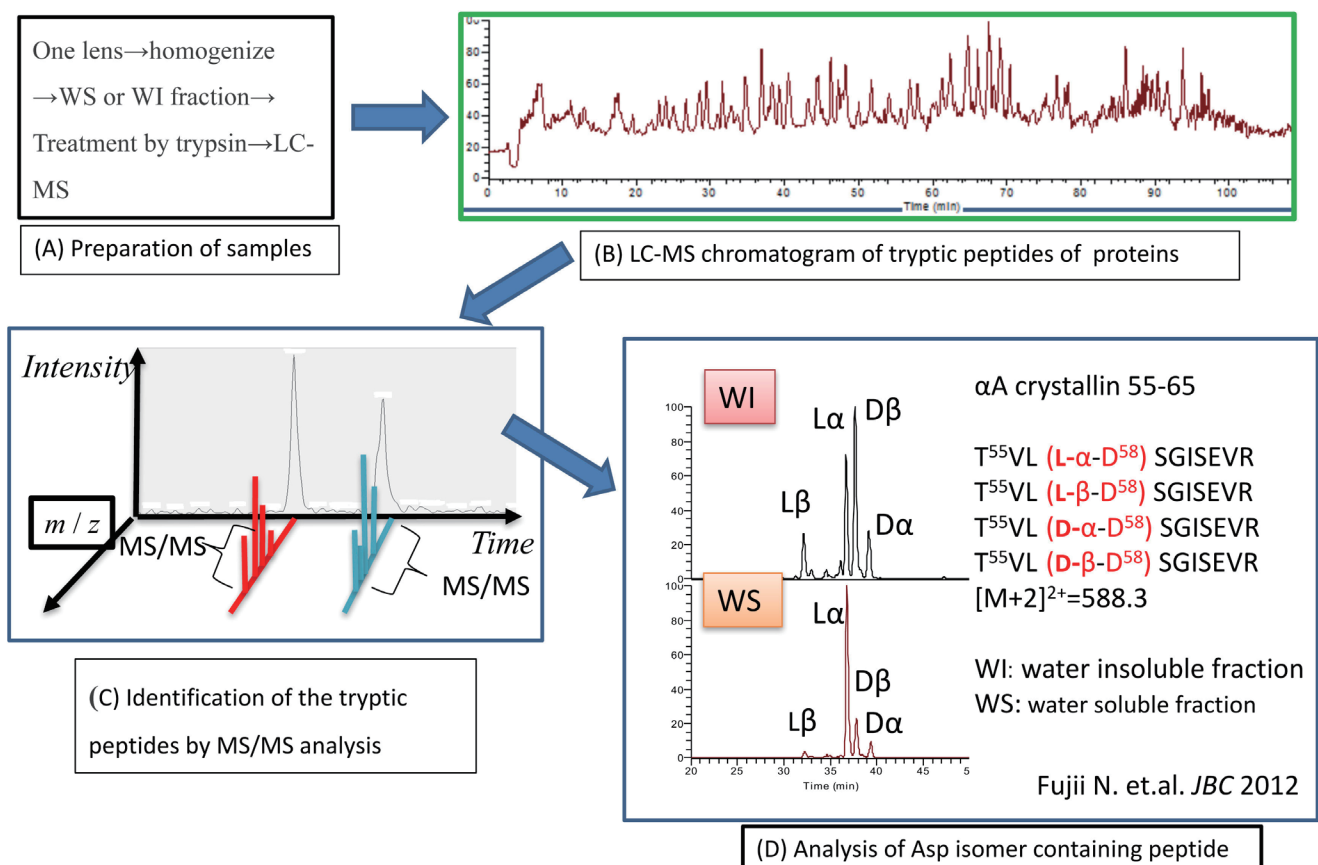

(A) Preparation of samples

(B) LC-MS chromatogram of tryptic peptides of proteins

(C) Identification of the tryptic peptides by MS/MS analysis

(D) Analysis of Asp isomer containing peptide

αA crystallin 55-65

T[55]VL (**L-α-D[58]**) SGISEVR
T[55]VL (**L-β-D[58]**) SGISEVR
T[55]VL (**D-α-D[58]**) SGISEVR
T[55]VL (**D-β-D[58]**) SGISEVR
$[M+2]^{2+}=588.3$

WI: water insoluble fraction
WS: water soluble fraction

Fujii N. et.al. *JBC* 2012

Fig. 4. **A new rapid analysis of D-amino acids in proteins by LC-MS/MS.**

LC-MS, liquid chromatography–mass spectrometry; WI, water-insoluble fraction; WS, water-soluble fraction.
The panels are reproduced from Ref. 5.

conducted on each of these peaks, which detects a variety of ions corresponding to peptides cut at their peptide bonds (**Fig. 4-C**). By checking them against an MS database, the amino acid sequences can be determined, which enables us to identify the peptides. This process is automatically carried out by the LC-MS/MS equipment. Furthermore, peptides in which amino acid deamidation or oxidation has occurred during aging or oxidative stress can be identified because the mass of these modified peptides differs from that of the original peptides. Therefore, a search will easily clarify which residues have been modified. This method has been widely applied in conventional proteomic analysis.

On the other hand, peptides that contain different isomers of Asp residues do not show any mass difference. For this reason, analysis of amino acid isomers by MS has been considered impossible. During the course of our research, however, we found that peptides with different Asp isomers are separated by LC [19]. On this basis, it follows that a peptide with the same mass and sequence that elutes in multiple peaks must represent a peptide containing Asp isomers. Because Asp58 in αA-crystallin obtained from the lens of elderly patients was found to be highly isomerized via the existing diastereomer derivative method (**Table 1**), we tested our method on this protein. **Fig 4-D** shows LC-MS chromatograms of the tryptic peptide of αA-crystallin including the Asp58 residue, *i.e.*, T^{55}VLD^{58}SGISEVR^{65} ($[M+2]^{2+}$ = 588.3. In such a mass chromatogram, a peak is observed depending on the mass of the peptide detected. Essentially, therefore, it is the case that only one peak is obtained for a peptide with a certain mass. Concerning the T^{55}VLD^{58}SGISEVR^{65} peptide, however, four peaks were observed because Asp58 was isomerized to four kinds of

isomer (**Fig. 4-D**). Thus, it became clear that a peptide with multiple peaks on the mass chromatogram is a peptide that contains Asp isomers. By using this mass chromatogram method, we were able to determine the isomeric sites and the ratios of Asp isomers in proteins. To identify the specific Asp isomers, four variants of the peptide containing each type of Asp isomer were synthesized and their elution times on LC were compared with those measured for the sample peptide. As is clear in **Fig. 4-D**, there was far more isomerization of Asp58 in αA-crystallin in WI fraction as compared with WS fraction. Among the isomers, Dβ-Asp was the most abundant, followed by Lβ-Asp and Dα-Asp. Overall, the total amount of these three unusual isomers was greater than that of the original Lα-form [5]. The results were consistent with the existing analysis [2,3].

At present, it is possible to easily identify proteins including isomers and to determine the isomerization sites by using this analysis based on LC-MS (one-shot analysis), instead of purifying proteins. Asp58, Asp76, Asp84, and Asp151 in αA-crystallin, and Asp36, Asp62, and Asp96 in αB-crystallin were identified as the sites of isomerization. For all these sites, isomerization of Asp was found to be more abundant in the WI fraction than in the WS fraction (**Fig. 5**), showing that isomerization of Asp is linked to the insolubilization of proteins [5]. Furthermore, the problems of small quantities of Asp isomers in a sample and the long processing time are improved markedly by the LC-MS analysis; in addition, it is possible to use this method to identify and determine isomerized sites in samples of cataract at different stages, or to identify abnormally aggregated proteins deposited in tissue samples from age-related eye diseases and determine the isomerization sites.
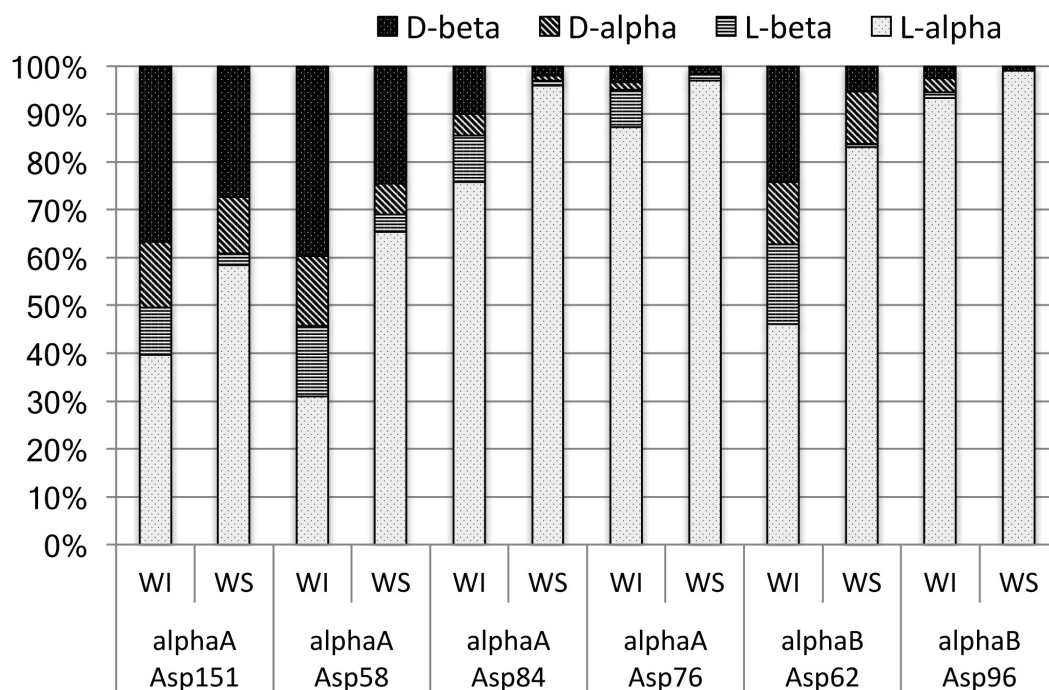

Fig. 5. Aspartic acid isomers in lens protein.

Shown are the relative amounts of aspartic acid (Asp) isomers present at Asp151, Asp58, Asp84 and Asp76 in αA crystallin, and Asp62 and Asp96 in αB crystallin in the water-insoluble (WI) and water-soluble (WS) fractions of lenses from elderly donors. Figure is reproduced from Ref. 5.

5. Structural changes, aggregation, and functional changes caused by D-amino acid production in proteins

When the D-form of an amino acid is produced, even if it is only in one position in a protein, the amino acid side chain will be reversed, and simultaneous β-formation induced the extension of the main chain. For this reason, the structures of proteins will be changed and protein aggregation will occur.

Indeed, a significantly large amount of abnormally aggregated and heterogenous α-crystallin was found in lens samples from elderly individuals (>80 years), while the chaperone activity (which functions to suppress the aggregation of other crystallins) was only 40 % of that of normal α-crystallin [20]. Moreover, it was clarified recently that isomerization of Asp in αA-crystallin and αB-crystallin is responsible for the aggregation and the separation of α-crystallin [21,22]. Based on this finding, it has been proposed that Asp isomerization leads to the aggregation and insolubilization of α-crystallin, making the protein unstable and insoluble, and ultimately resulting in cataracts (**Fig. 6**).

6. Commonality of isomerization and synthesis of AGEs involving Asp residues

Similar to the isomerization of Asp residues, in aging tissues an Amadori rearrangement reaction can take place in which an N terminal amino group, ε-amino group of a lysine or arginine side chain, or aldehyde group of saccharide in proteins reacts to form a Schiff base. As a result, nonenzymatic glycation is observed in aged tissues, which produces a variety of advanced glycation end-products (AGEs) [23] through oxidation, dehydration and condensation. This reaction has been called the Maillard reaction or so-called browning reaction of soy sauces in the field of food sciences. It has become clear since the 1970s that intermediates or bridging structures of the Maillard reaction accumulate in various tissues in living organisms [24].

It has been observed that the amounts of these substances increase with aging, and may be associated with lifestyle diseases including diabetic complications, arteriosclerosis, and hyperlipidemia. Furthermore, they are produced and accumulated in proteins in eyes [25,26] and skin [27] by ultraviolet light. Both isomerization of Asp in proteins and AGE synthesis have been observed in common sites of various types of tissue. Specifically, AGEs have been detected in abnormal condensation deposits in tissue samples from age-related eye diseases, such as crystallin lens [28], age-related macular degeneration [29], pinguecula [30], and corneal degeneration [31]. It has also been clarified that samples of lesioned tissue [6], including those from age-related disease of eyes and skins [18], show an accumulation of proteins containing both AGEs and D-β-Asp.

On the other hand, when we irradiated the skin of mice with UVB at 100 mJ/cm^2 and analyzed tissue samples for proteins that were positively stained with antibodies specific for proteins containing D-β-Asp and N^ε-carboxymethyl
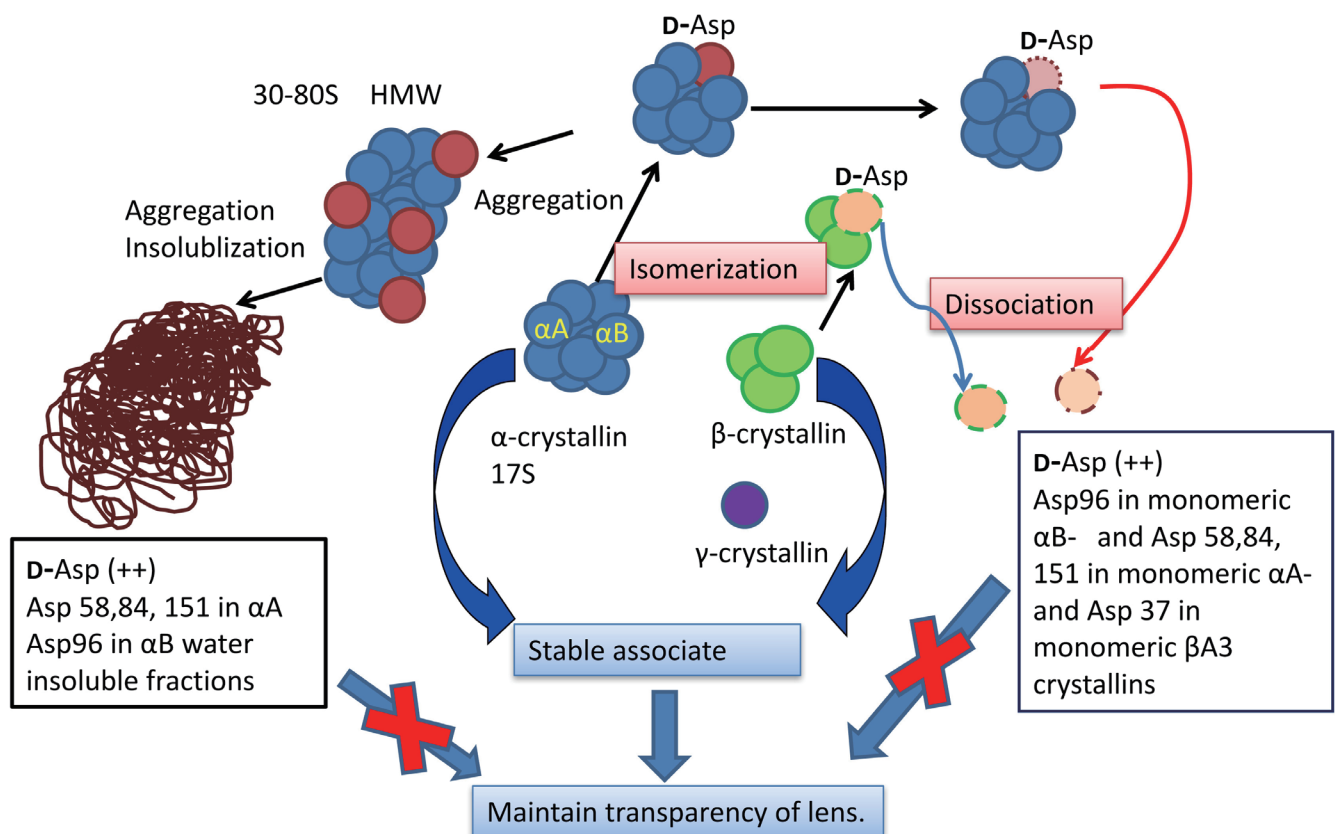

Fig. 6. Structural alterations of lens protein.

Aspartic acid (Asp) isomers accelerate not only the abnormal aggregation [5], but also dissociation to the monomer [21,22] of lens proteins.

lysine, we noted that the proteins that were co-stained at the same tissue sites were keratin-1, keratin-6B, keratin-10, and keratin-14 [18]. These results indicated that isomerization of Asp and synthesis of AGE had occurred simultaneously in the same proteins.

Frequently, AGEs are detected by using AGE-specific antibodies. Now, however, it is possible to analyze AGEs with greater precision and less difficulty as compared with the existing analysis. As mentioned in section **4.2**, proteins can be fragmented by using enzymes and then analyzed directly by LC-MS/MS. The amino acids that are susceptible to AGE synthesis are Arg, Lys, and His. If we analyze fragmented proteins containing these residues, identification of those that have become AGEs will be easily attained because, in this case, the mass of the peptide will have increased as compared with its theoretical value. For example, in the case of Lys, the mass increases by +58 for the AGE of carboxymethylation, and by +72 for the AGE of carboxyethylation, while the mass of the AGE of Arg increases by +55, and that of His increases by +28.

Table 2 summarizes data on isomerized sites of Asp residues in crystallin lens measured by our LC-MS analysis, as well as data from an analysis of modifications of AGEs conducted by Wilmarth *et al*.[32]. The fact that the sites that are modified to AGEs are located near the Asp isomers indicates that they are likely to be linked to one other. It might be said that clarifying a chemically linkage between AGE synthesis and Asp isomerization will open new avenues to the solution and the prevention of disease.

Conclusion

It has been a common belief of life science that the amino acids of biological origins consist of only L-amino acids. As has been shown in this paper, however, L-amino acids in proteins are nonenzymatically isomerized during the process of aging in nature. Owing to this, their structure changes, causing abnormal aggregation and malfunctions that lead to disease. It is thought that when a D-amino acid is produced in a peptide bond, the protein will not function sufficiently because there will be a change in the structure around the residue, as well as an alteration of interactions with other proteins. Such phenomena are thought to prevail not only in cataracts but also in other diseases of aging on the whole. Truscott *et al*. recently reported the detection of Asp isomers in myelin basic protein in brain samples from multiple sclerosis patients, based on the LC-MS method described in the present paper [14].

Hereafter, this kind of analysis can be applied to a variety of aging diseases. Furthermore, we recently succeeded in synthesizing variants of RNase in which the Asp residues in the active site were replaced with the four types of isomer (*i.e.*, Lα-Asp, Lβ-Asp, Dα-Asp, and Dβ-Asp)and measured their activity. In all cases where an RNAase variant containing isomers was obtained except for the Lα-Asp form, no protein activity was observed. Based on this, it was concluded that isomerization of Asp markedly alters protein function [33]. For a long time, it was not known why heteropycnosis, which changes the structure and/or function of proteins, occurs with aging. Now, however, it has become clear that such changes are due to the isomerization of Asp residues. At the same time, another astonishing fact is that the homochirality of the amino acid that is the most basic property of proteins disappears during the aging process of living organisms. The D-amino acid is simply a mirror image isomer of the L-amino acid, but it can also be said to be a ubiquitous and effective molecular index of aging in the L-amino acid world of life.

Declaration of Conflict of Interest

There is nothing that corresponds to the conflict of interest in achieving this research.

Table 2. *Aspartic acid isomerization sites are located near AGE-modified sites in lens crystallins.*

Crystallin	Isomerization	AGE modification	Other modification
Alpha A	Asp58	Arg55 (+55)	
Alpha A	Asp76	His79 (+28)	
Alpha A	Asp84		Gln90 (+1)
Alpha A	Asp151		His154 (+42)
Alpha B	Asp62		Ser59 (+80), Met67 (+16)
Alpha B	Asp96	Lys90 (+58), Lys92 (+58), Lys103 (+58)	
Beta B2	Asp3	Lys10 (+72)	

AGE, advanced glycation end product: +1, deamidation; +14, methylation; +16, oxidation; +28, modification of S, R (unknown modification); +42, acetylation; +55, arginine modification; +58, carboxymethyl lysine; +72, carboxyethyl lysine; and +80, phosphorylation.

Reference

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