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Age-dependent deamidation of chicken αA-crystallin

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The major posttranslational modification product of αA-crystallin from chicken eye lenses has one more negative charge than the corresponding primary gene product. These polypeptides were compared by peptide mapping after tryptic digestion and cyanogen bromide cleavage, and the charge difference could be located in a peptide, comprising residues 146–150 of the amino acid sequence of αA-crystallin. Subsequent enzymatic hydrolysis with aminopeptidase showed that asparagine at position 149 of the primary gene product is replaced by aspartic acid. Two-dimensional gel electrophoresis of total lens homogenates from chickens of different ages revealed an age-dependent increase of the deamidated αA-subunit.

Crystallin; Deamidation; Age dependence; (Eye lens)

1. INTRODUCTION

As a result of its unique growth pattern the eye lens retains its proteins throughout the entire life span of the animal, and therefore provides an especially useful system for the study of ageing [1,2]. Posttranslational modifications of lens proteins have most thoroughly been studied in α-crystallin [3–5], which is a major structural protein in almost all vertebrate eye lenses. It has a relative molecular mass ranging between 4×10^5 and 9×10^5, and is composed of two types of polypeptide chains, αA and αB, which are 173 and 175 residues in length, respectively, and show approx. 57% sequence homology [4,6].

Although deamidation has been proposed to contribute to the observed charge heterogeneity of α-crystallin chains [3], it has recently been proved that the major posttranslational products in bovine α-crystallin, αA_1 and αB_1, which have two more negative charges than the corresponding primary gene products αA_2 and αB_2, are in fact derived from αA_2 and αB_2 by cAMP-dependent phosphorylation [7,8]. Yet, in birds and most lower vertebrates, the difference in charge between αA and its major posttranslational product is only −1 [9], which therefore cannot be the result of phosphorylation, and deamidation might indeed be responsible in this case. Here, we analyse the major in vivo posttranslational modification product of αA-crystallin of the chicken, and demonstrate that it differs from the primary gene product by a replacement of aspartic acid for asparagine at position 149. In addition, we describe the age-dependent increase of the deamidation of chicken αA-crystallin.

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2. MATERIALS AND METHODS

Chicken eye lenses of different ages were obtained from the Central Animal Facilities of the University of Nijmegen, School of Medicine. The 5- and 10-year-old chicken eyes were provided by K. Jansen, Ulicoten. The lenses were homogenized in 2 vols buffer (50 mM Tris-HCl, 50 mM NaCl and 1 mM EDTA, pH 7.6) and subsequently used for either isolation of α-crystallin subunits as in [9] or two-dimensional gel electrophoresis according to O'Farrell [10].

The chicken αA subunits were digested with

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trypsin and the resulting peptides were separated by high-voltage paper electrophoresis at pH 6.5, followed by descending chromatography, as described [11]. Cyanogen bromide cleavage of tryptic peptide T18 was carried out according to standard procedures. Digestion of peptides with aminopeptidase M (Boehringer) occurred for 24 h at 37°C in 100 μl of 0.1 N NH_4HCO_3, pH 8.9, containing about 50 nmol peptide and 0.02 mg enzyme. Amino acid analyses after hydrolysis in 6 M HCl or after digestion with aminopeptidase M were carried out on an LKB Alpha Plus or an LC 6001 Biotronik amino acid analyser.

3. RESULTS AND DISCUSSION

α-Crystallin of chicken and other birds contains in addition to the major αA and αB chains a minor acidic subunit, which has only a single charge difference with the major αA band [9]. The major αA chain and the minor acidic subunit were isolated from α-crystallin of 4-month-old chickens, where they occur in a ratio of approx. 5:1. Tryptic peptide maps of the two chains were identical, apart from a change in mobility of a single peptide (fig.1). The amino acid compositions of all corresponding peptides were also found to be identical (not shown), and the difference peptide was identified as T18, comprising residues 146–163 of the αA chain [12]. The difference in mobility [13] indicates that T18 has one more negative charge in the minor chain than in αA. Since chicken αA is encoded by a single copy gene [14] these results confirm that the minor acidic chain is a post-translational derivative of αA. The peptides T4, T9 and T17 were not recovered under the conditions used, but since T18 is quantitatively displaced on the peptide maps of the two chains, this change completely accounts for the charge difference between αA and its isoform, and excludes the presence of additional charge differences between the two chains.

To further localize the charge difference in T18, this peptide was cleaved with cyanogen bromide, resulting in two fragments, T18a and T18b, which were separated by high-voltage paper electrophoresis and chromatography (fig.2). This revealed that the change in charge is located in T18a, comprising residues 146–150: Val-Pro-Ser-Asn-Met. The fact that the amino acid compositions after acid hydrolysis of the different peptides were identical suggests that an Asn/Asp difference is involved. Subsequent hydrolysis of total T18 with aminopeptidase M indeed revealed the presence of asparagine in T18 from αA, but not in that from the minor αA chain. Since the enzymatic hydrolysis was incomplete, no integral values were obtained. However, 50 nmol T18 yielded 23 nmol Asn and 1.6 nmol Asp in the case

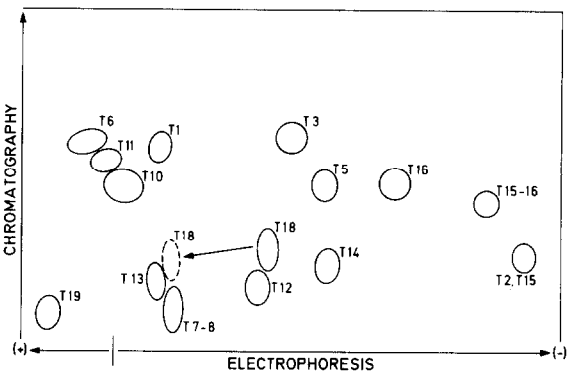

Fig.1. Peptide map of tryptic digest of chicken αA-crystallin. The arrow indicates the displacement of T18 (dashed) derived from the minor acidic chain.

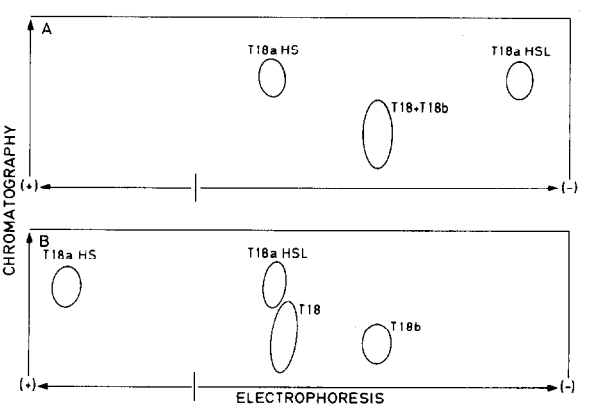

Fig.2. Separation of the cyanogen bromide fragments of tryptic peptide T18 from αA (A) and from the minor acidic chain (B), using high-voltage paper electrophoresis (pH 6.5) followed by descending chromatography. Peptide T18a comprised residues 146–150 of the αA chain, and occurred in the homoserine (HS) and the homoserine lactone (HSL) form; peptide T18b contained residues 151–163, while some unfragmented T18 (residues 146–163) remained.

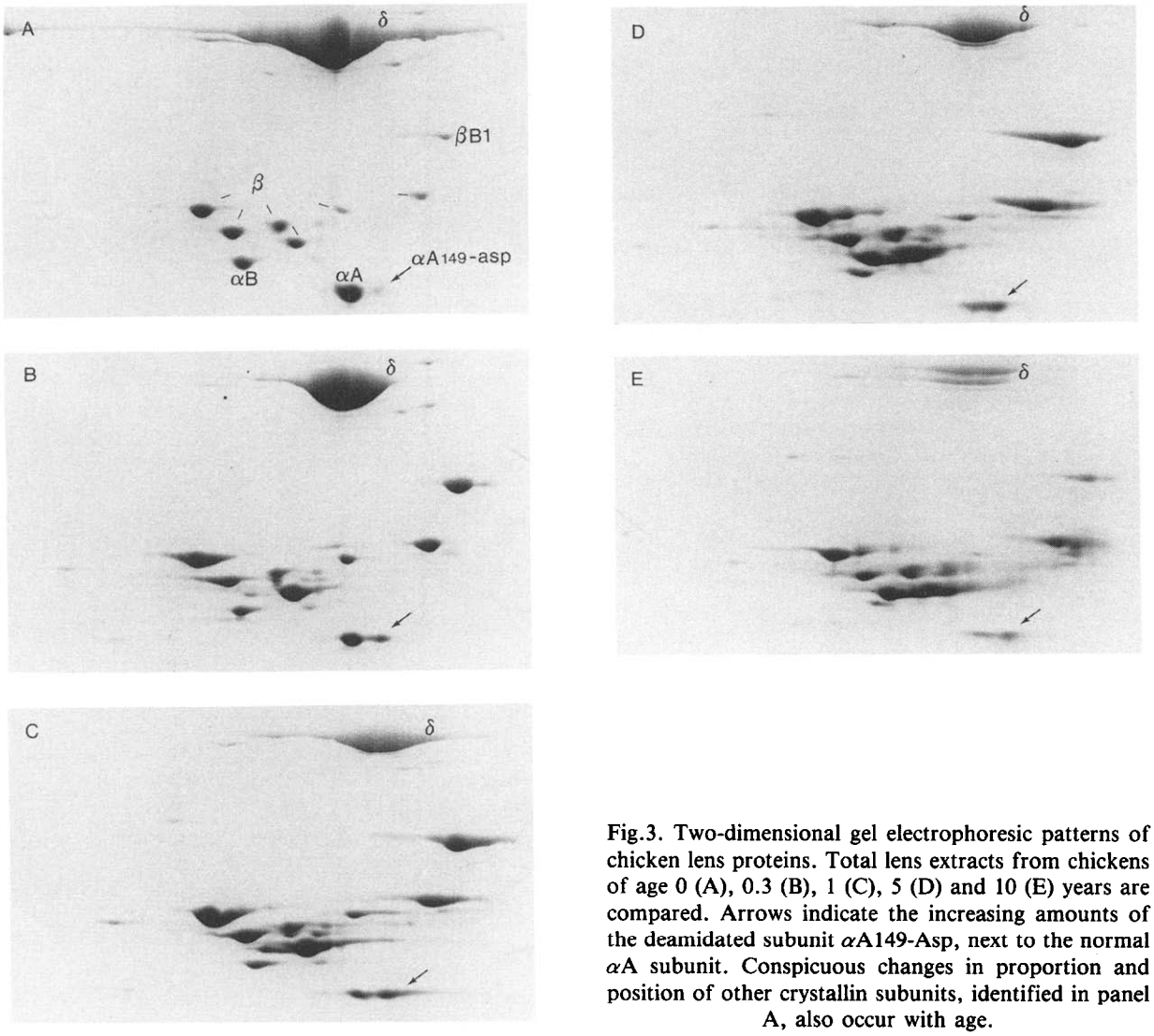

Fig.3. Two-dimensional gel electrophoresic patterns of chicken lens proteins. Total lens extracts from chickens of age 0 (A), 0.3 (B), 1 (C), 5 (D) and 10 (E) years are compared. Arrows indicate the increasing amounts of the deamidated subunit αA149-Asp, next to the normal αA subunit. Conspicuous changes in proportion and position of other crystallin subunits, identified in panel A, also occur with age.

of αA, while from its acidic isoform 10.6 nmol Asp and no Asn at all could be recovered. From these results it is clear that the charge difference between the two αA chains of the chicken is due to deamidation of asparagine at position 149. The minor αA subunit of chicken can thus be designated as αA149-Asp.

All birds, reptiles and amphibians investigated have asparagine at position 149 of the αA chain [12,15,16], while an isoform of αA with one additional negative charge is often present in these species [9]. It therefore seems likely that deamidation of 149-Asn is widespread in these lower vertebrate classes, while phosphorylation of αA is presumably restricted to mammals [8]. Among the mammals investigated only the opossum has asparagine at position 149 [11], and strikingly enough this is also the only mammalian species with pronounced amounts of a possible one-step deamidation form of αA.

Lens proteins from chickens of different ages were compared to establish whether deamidation of 149-Asn in αA increases with age. To avoid handling artefacts whole lens homogenates were subjected directly to two-dimensional gel electrophoresis (fig.3). The steady increase of

αA149-Asp as compared to normal αA, from being almost absent at hatching to the predominant form at 10 years, clearly demonstrates that this deamidation is an age-dependent process in chicken lenses. Increasing deamidation in ageing proteins has been documented for cytochrome *c* [17] and aldolase A [18], and has been suggested for other proteins [19].

Deamidation of proteins has been proposed to provide molecular timers for development, turnover and ageing of proteins and cells [19]. In a few cases the deamidation of specific asparagine residues has been associated with changes in protein function [20] and it has been proved that in vitro deamidated peptides and proteins are substrates for the enzyme carboxymethyltransferase [21]. It is therefore of interest to explore further the structural and functional implications of deamidation of α-crystallin.

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