Iranian Journal of Veterinary Research, University of Shiraz, Vol. 7, No. 1, Ser. No. 14, 2006

Comparison of optic lens proteins among animals at different stages of development

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Summery

The purpose of this investigation was to study and compare the electrophoretic patterns of optic lens proteins of different species of domestic animals at pre- and post-natal ages. Optic lenses were removed from the embryo or adult sheep, cattle, goat, camel and chicken at the slaughter-house then homogenized and subjected to sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). In all animals, except chicken, majority of proteins had molecular weights of less than 33 kDa and their concentrations were not affected by the age of animals at pre- or post-natal stages. A 9 kDa protein which was present in adult sheep optic lens was absent in sheep fetal lenses at different age groups. Prominent differences were observed in camel and chicken. In camel, proteins with molecular weights of 30 and 38 kDa were present, the concentration of which was much lower in other animals. A protein of 57 kDa which constituted the major protein of chicken optic lens was absent in other species of animals. The concentration of proteins in the range of 25-30 kDa increased with the age of chicken embryos. These proteins were remarkably different from those of adult chicken lens proteins except the 57 kDa protein which was also the predominant protein in the embryo. The 38 kDa protein disappeared and a 20 kDa protein appeared in the chicken embryo lens as compared with adult chicken lens. These data indicate extensive differences in the lens proteins of animals and suggest different physiological functions of lens proteins in different animals at different stages of development.

Key words: Domestic animals, Crystallins, Optic lens, SDS-PAGE

Introduction

A third or more of the wet weight and almost all the dry weight of mammalian lens is protein (Weingeist *et al*., 1997). Grinding or stirring of the decapsulated lenses in water or dilute aqueous buffer does not extract all the proteins into solution and the terms water-soluble and water-insoluble have been used to describe the two fractions (Harding and Dilley, 1976). Much of the insoluble proteins isolated from normal or cataratous human lens are produced artifactually during extraction from proteins that may be isolated in a soluble form if oxygen is excluded (Weingeist *et al*., 1997). The water-soluble fraction consists mostly of crystallins which are divided into three groups. Gel filtration separates crystallins into four fractions, which have been labeled alpha-, $beta_H$-, $beta_L$- and gamma-crystallins. Each of these crystallins is made of different subunits with different molecular weights and arrange in complex macromolcular structures (Harding and Dilley, 1976). Avian lens contains yet another crystallin that has been designated delta (Weingeist *et al*., 1997). Chemical properties of lens crystallins from several species of animals have been studied (Mason and Hines, 1966; Zigman, 1969; van Kleef *et al*., 1974; Duhaiman *et al*., 1995; Bera and Ghosh, 1998; Rabbani and Duhaiman, 1998; Denis *et al*., 2003). Calf alpha-, beta- and gamma-crystallins have been widely studied and used as models for investigations on the effect of different chemical, physical and genetic factors on lens proteins (Bjork, 1961; van Kleef *et al*., 1974; Harding and Dilley, 1976; Piatigorsky, 2003). The purpose of this study was to compare the electrophoretic patterns of lens proteins from

different species of domestic animals. The differences between pre- and post-natal proteins were also investigated.

Materials and Methods

Lenses from different domestic animals including: sheep, cattle, camel, goat and chicken were obtained from local slaughter-houses or from the Hospital of Shiraz Veterinary School. Age of sheep fetuses were determined according to the method suggested by Evans and Sack (1973). For collecting lenses from chicken embryos, fertilized eggs were incubated at 37^{o}C and 70% humidity in a multihatch incubator and embryos were collected at specified developmental stages and lenses were removed.

All chemicals were of analytical grade and were obtained from commercial sources. Molecular weight protein markers were from Sigma Chemical Co, St. Louis, MO, USA.

Lenses were stored frozen at -20^{o}C until use. Protein extracts were prepared by thawing the lenses, homogenizing them in the presence of liquid nitrogen using a hand homogenizer and suspending the homogenate in 0.025 M phosphate buffer (pH = 7.0). In all animals studied, essentially a clear solution was obtained after homogenization, thus no centrifugation was necessary. Protein content of extracts was determined by the method of Lowry *et al.,* (1951) using crystalline bovine serum albumin as standard. Protein extracts were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the discontinuous buffer system of Laemmli (1970). Protein samples were prepared by diluting each sample in a sample buffer to give a final concentration of 1 mg protein /ml in 0.01 M tris-HCl, pH = 6.8, 0.4% SDS, 10% glycerol and 0.04% bromophenol blue. Another series were prepared from each sample except that the protein solution in sample buffer also contained 2-mercaptoethanol. The running gel with dimensions of 120 × 90 × 1 mm was made of 7.5% acrylamide in 1.2 M tris-HCl, pH = 8.8 and 0.3% SDS. In order to study the effect of gel concentration on resolution of protein bands, a gel with 10% or 12.5% acrylamide concentration and gel gradient systems containing 5-20% or 7.5-15% acrylamide were also used. The stacking gel contained 3% acrylamide in 0.25 M tris-HCl, pH = 6.8 and 0.2% SDS. Samples were heated in boiling water for 10 min and 40 µl was applied to each slot. The electrode buffer contained 0.025 M tris-HCl, 0.192 M glycine and 0.1% SDS at pH = 8.16. Electrophoresis was performed at constant 25 mA and gels were fixed in a solution containing 45.4% methanol and 9.2% acetic acid. Gels were then stained with 0.25% Coomasie brilliant blue in 25% isoprepanol, 10% acetic acid and destained with a 5% acetic acid/7% methanol solution. Molecular weight protein markers were myosin heavy chain (Mr=205,000), β-galactosidase (Mr = 116,000), phospho-rylase-b (Mr=97,000), bovine serum albumin (Mr = 66,000), ovalbumin (Mr = 45,000), carbonic anhydrase (Mr = 29,000), soybean trypsin inhibitor (Mr = 21,000), α-lactalbumin (Mr = 14,200) and aprotinin (Mr = 6,800).

Results

Electrophoretic pattern of lens proteins of different animals is showed in Figs. 1 to 4. In Fig. 1 the results of SDS-PAGE on a 7.5% acrylamide gel is showed. Extensive differences were observed in the number of protein bands and the corresponding molecular weights in different animals. In all species except chicken, the majority of proteins had molecular weights of less than 33 kDa. A protein band with molecular weight of approximately 9 kDa was present in the lens of adult sheep but was absent from sheep fetal lenses at different stages of development. Instead, a protein of approximately 12 kDa was present in sheep fetus and was not found in adult sheep (compare sample No. 1 with 6-11 in Fig. 1). A 38 kDa protein was present in the lens of all species studied, except that its concentration was very low in sheep and cattle but very high in camel. A similar protein with a slightly lower molecular weight was seen in chicken lens, which was also present in high concentrations. A distinctive feature of camel lens was presence of a 30 kDa protein and slightly different distribution of proteins in

the

Fig. 1: SDS-PAGE pattern of lens proteins of animals on a 7.5% acrylamide gel. M: molecular weight markers; 1: adult sheep; 2: adult cattle; 3: adult camel; 4: 9-month-old calf fetus; 5: adult chicken and 6-11: sheep fetus at 2, 2.5, 3, 3.5, 4 and 4.5-month-old

Fig. 2: SDS-PAGE pattern of lens proteins of animals on a 5-20% acrylamide gel gradient. 1-4: goat fetus at 2, 3, 4 and 4.5-month-old; 5-8: sheep fetus at 2, 3, 4 and 4.5-month-old; 9: adult sheep; 10: adult camel; 11: 9-month-old calf fetus; 12, 13: adult cattle and 14, 15: adult chicken

ranges of 6.8 to 29 kDa, as compared with sheep and cattle.

Proteins of chicken lens were remarkably different from other species. A protein with molecular weight of 57 kDa constituted the major component of lens protein in chicken. This protein was either absent or present in very low concentrations in other species. Also in chicken, the pattern

Fig. 3: Same as Fig. 2 except that a 7.5-15% acrylamide gel gradient was used

Fig. 4: SDS-PAGE pattern of chicken embryo lens proteins on a 12.5% gel. 1: 12-day-old embryo; 2: 14-day-old embryo; 3: 16-day-old embryo; 4: 18-day-old embryo; 5: 20-day-old embryo; 6: 21-day-old embryo and 7, 8: same as 6 except that the concentration of protein in each slot was 20 or 10 μg, respectively, instead of 40 μg (see methods section)

of distribution and the size of proteins in the range of 6.8-29 kDa were completely different from other species. Figs. 2 and 3 are the results of SDS-PAGE on 5-20% and 7.5-15% acrylamide gel gradient, respectively. In these pictures, the distinctive differences among different species are also evident. It is interesting to note that essentially no difference was observed in the lens proteins of sheep and goat. Age of fetus

did not appear to have significant effect on lens proteins. Figs. 1 to 3 show that different gel concentrations can be used to resolve proteins with molecular weights of more than 33 kDa and less than 14.2 kDa.

In Fig. 4, the SDS-PAGE results of lens proteins of chicken embryo at different stages of development are showed. Although the intensity of bands in the range of 25-28 kDa increased with age, significant differences were not seen in the number of protein bands with the stage of embryo developement. However, these proteins were remarkably different from those of adult chicken lens proteins (compare Figs. 1 and 4), except the 57 kDa protein which was also the predominant protein in the embryo. Absence of the 40 kDa and the presence of the 20 kDa proteins in the chicken embryo lens is noticeable.

Finally, when 2-mercaptoethanol was present in the sample buffer, the results of SDS-PAGE were essentially identical to those without 2-mercaptoethanol (results not showed).

Discussion

Many investigations on the lens proteins have involved lenses from different species of animals, such as cattle (Stauffer *et al*., 1973; Morgan *et al*., 1989; Walker and Borkman, 1989), cat (Buchen *et al*., 1989), rat (Bonavolenta *et al*., 1989; Dillon *et al*., 1989; Bettelheim *et al*., 1995), mouse (Russel *et al*., 1979; Zwaan, 1983), rabbit (Mason and Hines, 1966; Lien-The and Hoenders, 1974; Basu *et al*., 1983), pig, horse, dog, cat and rhesus monkey (de Jong *et al.*, 1975), camel (Duhaiman *et al*., 1995), goat (Bera and Ghosh, 1998) and chicken (Linser and Irvin, 1987). These studies include different aspects of lens functions, many of which deal with properties of lens proteins. Several reviews are available which present chemical properties of mammalian lens proteins (Harding and Dilley, 1976; Weingeist *et al*., 1997). Investigation on lens proteins of different animals is not only important in elucidation of the evolutionary biochemistry of these proteins and to establish inter-species relationship of proteins of this important tissue but also to provide model systems for studying the effects of different physical and chemical agents as related to a particular lens protein. Such information can then be extended to humans. Extensive studies in tissue culture and in animal models have documented that lens proteins undergo fundamental alterations due to aging and exposure to different agents (Harding and Dilley, 1976; McFall-Ngai *et al*., 1985; Srivastava, 1988; Srivastava *et al*., 1994; Bettelheim *et al.,* 1995; Sandilands *et al*., 2002). Changes in protein properties include cross linking, aggregation, insolubilization, degradation and etc.

Data presented in this study show that extensive variation exists in lens proteins of animals. In all species studied, the main protein bands are localized in the region of 13 to 33 kDa. The soluble fraction of mammalian lens proteins is made of three major groups: alpha-, beta- and gamma-crystallins. The alpha-crystallin from a number of mammalian lenses has been characterized. The alpha-crystallin of bovine (van Kleef *et al.,* 1974), human (Stauffer *et al*., 1973), rabbit (Lien-The and Hoenders, 1974), pig, horse, dog, cat and rhesus monkey (de Jong *et al.*, 1975) lenses are very similar and are made of four major subunits, each having a molecular weight of approximately 20 kDa. The amino acid composition of two of these subunits (called A_1 and A_2) are very similar to each other but differ extensively from those of the other two subunits (B_1 and B_2) which are also similar to each other (Stauffer *et al*., 1973). The amino acid sequence of the A_2 chain from different species is very similar and the number of replacements between the different sequences is relatively small and most of the changes alter the net charge of the polypeptide (de Jong *et al*., 1975). These subunits are held together by hydrogen bonds and hydrophobic interactions to make macromolecular structures with molecular weights of 600 to 4000 kDa, depending on the degree of subunit aggregation (Harding and Dilley, 1976; Weingeist *et al*., 1997). In this study, during the preparation of samples for SDS-PAGE, the alpha-crystallin was probably disintegrated into its subunits and a protein with a molecular weight in the range of 20 kDa, present in the lens of all species,

likely belongs to the subunits of alpha-

crystallin. Inter-conversion of the A_2 to A_1 and B_2 to B_1 does occur during embryonic development as well as due to aging. Also the pattern of distribution of subunits differs in different layers of lens (Harding and Dilley, 1976). Furthermore, the subunits are subject to limited proteolysis resulting in degradation of these polypeptides to smaller fragments with molecular weights of 13-17 kDa (van Kamp *et al*., 1973). As the SDS-PAGE pictures reveal, several proteins are present in the range of 9-17 kDa which are probably produced during embryonic as well as post-natal development. In particular, as can be seen in Fig. 1, a protein with a molecular weight of 9 kDa absent in the lens of sheep fetus appears in adult sheep as well as adult cattle and camel. In chicken, a protein with a molecular weight of approximately 20 kDa is present in all stages of embryonic and post-natal life.

Beta-crystallin constitutes a larger proportion of the water-soluble protein of the mammalian lens than either alpha- or gamma-crystallin (Harding and Dilley, 1976). Beta-crystallins are heterogenous and can be separated into two fractions by gel filtration chromatography (Weingeist *et al*., 1997). These are called B_L (50 to 80 kDa) and B_H (100-550 kDa). B_L is a macromolecular complex with multiple subunits of 23 to 30 kDa, while B_H is made of subunits of 25 to 27.5 kDa (Harding and Dilley, 1976). In the SDS-PAGE analysis of lenses from all species, many protein bands are located in the region of 20 to 30 kDa and probably belong to different subunits of beta-crystallin. These proteins are very similar in sheep, goat and cattle but are different in camel. The pattern of protein bands does not change considerably during aging of sheep and goat fetus and are not very different from those of adult animals. Our results show considerable differences between chicken and other species with respect to beta-crystallin subunits. The concentration of beta-crystallin in chicken embryo is much lower than adult chicken and slight increase in this protein with advancement of age of embryo occurs. The nature of these crystallins and the mechanism(s) involved in appearance of high concentration of these proteins after hatching is not known at present.

Mammalian gamma-crystallins are the smallest of crystallins. It is a mixture of proteins of similar molecular weights displaying slight difference in the net charge (Harding and Dilley, 1976). Calf gamma-crystallin can be fractionated into four fractions with isoelectric point of 7-8 and molecular weight of 19.1 to 20.8 kDa. Molecular weight and amino acid composition of calf (Mason and Hines, 1966), rat (Zigman, 1969) and human (Clark *et al*., 1969) are similar. Gamma-crystallins make up about 1.5% of lens proteins of adult mammals, but constitute as much as 60% of the soluble proteins in weaning animals (Weingeist *et al*., 1997). The SDS-PAGE method used in this research fails to resolve proteins with molecular weight about 20 kDa and it is not possible to differentiate different gamma-crystallins and observe the variation due to species or age.

The results of this study distinctively show presence of a 38 kDa protein in camel lens and two proteins of approximately 38 and 57 kDa in adult chicken which are absent in other animals. In chicken embryo the latter constitute the major protein of the lens. The true identity and their significance in the structure of the lens of these animals are not known at present.

Linser and Irvin (1987) identified a population of neuralgia-like cells in the embryonic chick retina, which expressed high levels of delta-crystallin as a function of normal development. Whether the 38 kDa protein is the result of proteolysis of the 57 kDa protein after hatching (as the concentration of the latter decreases and that of the former increases in adult chicken, see Figs. 1-4) remains to be investigated.

Acknowledgement

This research was financially supported by the grant 75-VE-930-547 from Shiraz University Research Council.

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