Fluorescent and compositional changes in crystallin supramolecular structures in pig lens during development

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Abstract

Water soluble proteins (WSPs) in Sus scrofa lenses from pigs in different developmental stages: (young (G1), young adult (GII), and middle-aged (GIII)) were separated using GF-HPLC, yielding fractions of different molecular weights. Non-tryptophan (345/420 nm) and tryptophan (280/345 nm) fluorescence was measured in these fractions. Relative non-tryptophan fluorescence increased with age at a rate directly correlated to the molecular weight of aggregates forming the different chromatographic fractions, while tryptophan fluorescence tended to decrease. The crystallins constituting each fraction were separated using 2D-electrophoresis and after development with Coomassie blue they were identified using MS-TOF. Also, the protein content of each spot was quantified by subsequent scanning and integration. The proportions of unchanged crystallins characteristically changed with age in chromatographic fractions of different molecular weights. Thus it was possible to relate these changes with those occurring in the fluorescent properties and molecular weight of supramolecular structures. © 2005 Elsevier Inc. All rights reserved.

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1. Introduction

Changes experienced by crystallin levels throughout ontogenesis result from the interaction between protein synthesis and post-translational changes. In this way lens transparency depends on the properties of its component proteins, most of them organized in supramolecular structures in which the proportions and interactions between monomers are essential for this function (Ajaz et al., 1997; Fu and Liang, 2002; Bateman et al., 2003), as well as the adequate three-dimensional structure of the component proteins maintained by the α-crystallins having a chaperone-like function (Raman et al., 1995; Takemoto and Boyle, 1998). The loss of the adequate three-dimensional structure leads to pathological changes of lens function such as cataract, which is part of the group of conformational diseases (Harding, 2002).

Structural changes in crystallins and their aggregates are related to post-translational changes that modify their properties (Schuaerte and Gafni, 1995; Weinreb et al., 2000); they decrease light transmission through the lens, with an associated increase in light scatter (Suarez et al., 1993), and increase color and fluorescence mainly due to binding of 3-hydroxy kynurenine derivatives (Garner et al., 2000; Aquilina and Truscott, 2000) and to glycation processes (Scalbert and Birlouez-Aragon, 1993). When crystallins are separated by 2D-electrophoresis, an increase in anionic and lower molecular weight forms is seen with age (Shih et al., 1998; Ueda et al., 2002; Lampi et al., 2002).

Vertebrate lenses have marked taxon-dependent differences in crystallin composition; a further uniqueness is accumulation of post-translationally modified forms due to the avascular nature of the lens and its mandatory exposure to light throughout the life of the individual. This makes them common targets of comparative studies on the action of environmental agents on proteins in different life stages...