Degradation of crystallins from a psoriatic patient undergoing PUVA therapy*

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Rceived 25 May 1990

Comparative physico-chemical and spectroscopic analyses were carried out in human lens proteins obtained from extracts of normal, senile and PUVA cataracts. Mass recovery analysis reveals a large protein concentration loss in the PUVA cataract relative to the normal lens and senile cataract. This protein loss parallels an increase in the degraded polypeptide chains. However, the tryptophan content (2.1 mol/mol of 20 kDa protein subunit) and the apparent fluorescence quantum yield ($\varphi_f = 0.056$) of the tryptophan residues which are believed to be involved in the development of UV-induced cataracts are unchanged after age-related alterations and/or in vivo photochemistry associated with psoralen (8MOP) photosensitized reactions.

Photosensitizer; 8-Methoxypsoralen; PUVA cataract; Lens protein; Lens fluorescence; Tryptophan

1. INTRODUCTION

The conjugated effect of UV and aging leads to the oxidation of human lens proteins which become yellow, fluorescent and undergo polymerization [1,2]. The study of the modifications induced by endogenous [3–5] or exogenous photosensitizers such as drugs has been stimulated by the discovery that 8MOP photoproducts can be found in cataracts developed by patients undergoing PUVA therapy with this psoralen derivative [6,7]. It is presently believed that tryptophan, the only UVB-absorbing amino acid residue in proteins is responsible for the primary photochemical processes leading to the formation of fluorescent pigments and *N*-formylkynurenine, a photooxidation product of Trp which is a good photosensitizer [2,4]. However, it has been shown that although Trp in vitro is very sensitive to UV photolysis in model systems, Trp residues are rather stable to photooxidation in human lens aging processes leading to cataract formation [8,9]. This surprisingly different behavior has been called the lens paradox [10].

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* This manuscript is dedicated to the memory of J. Conte who became deceased during this work

Abbreviations: 8MOP, 8-methoxypsoralen; PUVA, psoralen + UVA; Trp, tryptophan

The present work deals with the physicochemical and spectroscopic modifications of the ocular lens during the normal lens aging (senile cataract formation) and during the cataractogenesis induced in the lens of a psoriatic patient undergoing PUVA therapy with 8MOP. Using physicochemical, biochemical and spectroscopic techniques, the molecular size, the variation of the concentration, the content in Trp residues and the fluorescence quantum yield of human α, β_H, β_L, LM and γ-crystallins from a normal completely transparent lens, from a senile cataract and from a PUVA-induced cataract have been determined.

2. EXPERIMENTAL

2.1. *Crystallins*

Fresh crystallins weighing about 175 mg (expressed in fresh tissues) were obtained from human donors, 28 years old (normal lens), 65 years old (senile cataract) and 57 years old (PUVA cataract) at the Departamento de Oftalmologia e de Dermatologia do Hospital de Santa Maria, Lisboa. Crystallins were solubilized after homogenization (nucleus and cortex) in a pH 7.5 Tris buffer containing 50 mM NaCl. The solutions were extensively dialyzed at 4°C to remove salts and then lyophilized and stored at −20°C until used.

The clinical aspects regarding the psoriatic patient are described elsewhere [11].

2.2. *Low pressure gel permeation liquid chromatography (FPLC)*

The various protein fractions of the crystallins were separated according to their molecular weight with a Pharmacia FPLC equipment using Superose 6 or 12 columns provided by Pharmacia Fine Chemicals. The eluent was the solution used for the homogenization.

Superose columns were calibrated with proteins of known molecular mass purchased from Sigma. The following standards were

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

used: thyroglobulin (670 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (68 kDa), carbonic anhydrase (31 kDa) and cytochrome *c* (12.5 kDa). For each chromatogram, 300 μl of a buffered solution containing 6 mg/ml of lyophilized crystallins were injected and 400 μl fractions were collected. The protein concentration in each pooled fraction of the five crystallin proteins was determined by the Lowry method [12].

2.3. *Spectroscopic analysis*

Absorption spectra were recorded with a Perkin-Elmer Lambda 9 spectrophotometer whereas the fluorescence was studied with a SPEX Fluorolog fluorometer.

2.4. *Alkaline denaturation*

The Trp content of the five crystallin fractions was determined after alkaline denaturation with 2 M KOH by measuring the Trp fluorescence at 350 nm according to the method described in [13].

3. RESULTS AND DISCUSSION

3.1. *Biochemical and physicochemical analysis*

The separation by FPLC of *a constant mass* of lyophilized proteins extracted from a normal lens, from a senile and from a PUVA cataract is shown in Fig. 1. The molecular masses found for the α, β_H, β_L, LM and γ-proteins are 800, 150, 90, 45 and 20 kDa, respectively. They are in good agreement with previously reported values [14]. Furthermore, they are identical for the three studied lenses.

The most important differences between the three chromatograms reside in their absorbance at 280 nm and the presence of low molecular weight peptides (Fig. 1). The decrease in the absorbance of the protein fractions of the PUVA cataract at 280 nm with respect to the same fractions obtained with the normal or senile lens may suggest either the photodegradation of the indole ring or a protein concentration loss.

The determination of the protein concentration by the Lowry method on the chromatographed fractions leads to a protein mass from the PUVA cataract which represents only a quarter of the mass obtained with the normal and senile lenses (Table I). Moreover, a larger proportion of proteins eluting at the same time as the α-crystallin is found in the cataractous lens as compared to the normal lens. These heavy fractions amount to about 23, 47 and 74% in the normal, senile and PUVA crystallins, respectively.

Despite the experimental error (about 20%) inherent in the determination of the protein concentration by the Lowry method, the protein loss observed with the PUVA cataract is highly significant. This loss is particularly important for the low molecular weight fractions (Table I). The PUVA lens chromatogram (Fig. 1) shows that the protein loss parallels the appearance of fractions with a molecular mass lower than 10 kDa which probably correspond to degraded polypeptidic chains [15]. The relatively small amounts that are obtained with chromatography preclude their analysis. It should be emphasized that shorter peptides may not have been detected under the present experimental conditions.

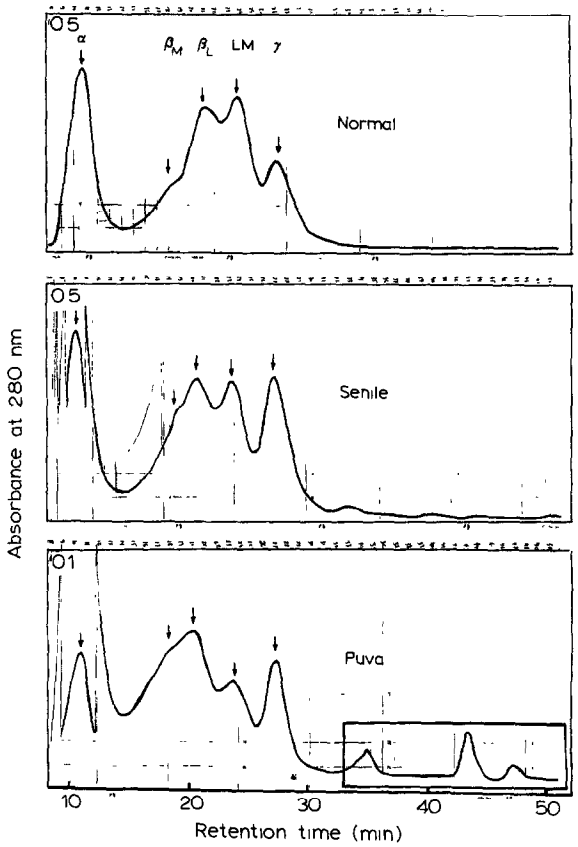

Fig. 1. FPLC chromatogram on Superose 12 of the various protein fractions of the three human lenses. Absorbance was measured at 280 nm as a function of the retention time (min). The rectangle on the bottom chromatogram emphasizes the increased production of small peptides (see text).

The Trp content of the α, β_H, β_L, LM and γ-crystallins of the three lenses, measured after alkaline denaturation, is constant and equal to 2.1 mol/mol of Trp per 20 kDa subunit. These values are also in good agreement with the literature [16].

The linear relationship between the absorbance at 280 nm and the protein content of the α-, β-, γ-proteins of the three lenses (data not shown) suggests that they

Table I

Determination of the protein concentration in the various crystallin fractions by the Lowry method

Fraction	Normal lens	Senile cataract	PUVA cataract
α	69	141	52
β_H	10	20	5
β_L	48	38	3
LM	83	64	4.5
γ	50	76	2.9
Total	260	339	67.4

Results are expressed as μg of fraction/mg of lyophilized crystallin

all have the same specific absorbance, i.e. $E^{1\%}_{280nm} = 30$. Moreover, the comparison of the specific absorbance of the individual proteins demonstrates that the cataractogenesis does not change this specific absorbance. It has to be mentioned that the theoretical specific absorbance at 280 nm due to 2 Trp residues and 5 or 6 Tyr residues [16] is always smaller than the experimental value [17]. In the present study it corresponds to 30% of the observed specific absorbance. The presence of other chromophores could explain, at least in part, the additional absorbance.

3.2. *Emission spectroscopy*

In agreement with the constant value of the number of Trp residues in the studied crystallins, the fluorescence maximum wavelength and the fluorescence quantum yield ($\phi_f = 0.056 \pm 0.01$) are not modified in the various protein fractions of the three lenses. In fact, this is consistent with the conservation of the secondary structure of the proteins of the human lenses during the cataractogenesis [18].

In addition, the increased exposure of Trp residues to a hydrophilic environment in going from γ- to α-crystallins, which corresponds to a 12 nm red-shift of the fluorescence maximum, should lead to enhanced photochemical susceptibility of these residues in the high molecular weight proteins [18]. However, the more important degradation of low molecular weight proteins in the PUVA cataract, may suggest that indole rings are not involved in the cataractogenesis induced by 8MOP.

Phosphorescence and fluorescence spectroscopies do not make it possible to detect the presence of 8MOP photoproducts in the various protein fractions of the PUVA lens. Thus, excitation at 330 nm leads to the typical fluorescence of the eye pigments with bands centered at 420 and 465 nm. It corresponds to the so-called NT (non-tryptophan) fluorescence [19]. At low temperature, a phosphorescence emission is observed at 450 nm which has been attributed to 8MOP photoproducts by Lerman [6]. However, this phosphorescence is present in all the fractions whatever the observed lens.

4. CONCLUSIONS

In a normal cataractogenic process induced by aging of the eye lens (senile cataract), the photooxidation of proteins leads to an increased aggregation of α-crystallins. As a consequence, the solubility of the proteins decreases. The present results show that the presence of a photosensitizer such as 8MOP (PUVA cataract) tends to aggravate this process characterized by the relative increase in the percentage of α-crystallins. On the other hand, it can be seen that the PUVA lens was more easily solubilized than the senile proteins. Furthermore the relative increase in the heavy fractions observed with the PUVA cataract is accompanied by an increased formation of small peptides which are not found in the senile cataract. These results suggest that the primary chemical mechanisms of the cataractogenic damage in the senile and PUVA cataracts are not the same.

As far as the protein fragmentation is concerned, one may suggest two possible mechanisms. The first one could involve a post-oxidative proteolytic activity as already proposed for the radical-induced degradation of mitochondrial proteins [20]. The second possibility is the occurrence of secondary chemical reactions due to the propagation of damage induced at primary photochemical targets.

Despite the large mass variations observed with the various proteins of the PUVA lens, the biochemical and spectroscopic analyses do not demonstrate any modification in the number of their Trp residues as compared to similar fractions of the senile and normal lenses.

Acknowledgements: This work was supported by the Research Project INIC/JNICT no. 906.86.185 from Portugal. We gratefully thank Dr L. Simoneau for his advice for the separation of lens proteins and Mrs Josiane Haigle for the preparation of the figure.

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