POST-SYNTHETIC MODIFICATIONS OF ALDOLASE ISOZYMES IN RABBIT LENS DURING AGING

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1. Introduction

Enzyme alteration during aging has been recently demonstrated; post-translational modifications have been distinguished from biosynthetic errors [1]. Lens is a very interesting material for the study of these processes, because protein synthesis stops soon after differentiation into fiber cells; the latter are laid down, layer upon layer, throughtout the life. The fiber cells do not synthesize DNA and RNA [2,3]. Consequently, the protein modifications of the central nucleus obligatorily result from post-translational events. From this point of view, the most studied protein is α-crystallin, the chains of which seem to be deamidated and degraded during aging [4–6].

Deamidation, as a processus of aging was described for several other proteins, especially aldolase A [7,8]. This deamidation occurs in vivo [9], resulting in five subunits (α^4), ($\alpha^3\alpha'$) ($\alpha^2\alpha'^2$) ($\alpha\alpha'^3$) (α'^4) in muscle, and occurs also in red cells during their life span [10].

We recall that three types of aldolase are known in higher animals, A, B and C, which are tetrameric molecules. The three types have different activities towards two substrates. Fructose-1-6-diphosphate (FDP) and fructose-1-phosphate (F1P); the aldolase activity ratio of aldolase A is 50, while that of C is about 5. Aldolase C is more negatively charged than aldolase A. In brain, A and C are present, and their hybridization gives five isozymes (A4, A3C1, A2C2, A1C3 and C4). Kochman et al. [11] have described the presence of aldolase C, hybridized with aldolase A, in rabbit lens; but the isozymic distribution in the different zones was not studied.

In this paper, we demonstrate the occurrence of a 'Cross Reacting Material' (CRM) in the intermediary zone (cortex fiber cells) and in the central zone (nucleus fiber cells) where no protein biosynthesis occurs. The most important fact is the presence in both zones of two supplementary aldolase isozymes resulting from aging.

2. Materials and methods

Several rabbit lenses were removed and each zone was immediately dissected and aldolase extracted. Aldolase activities towards FDP and F1P were measured on fresh extracts by the method of Sibley and Lehninger [11] adapted to the measure of F1P aldolase activity [12] and expressed in International Units (IU) per g wet weight. Electrophoresis, followed by specific staining of aldolase isozymes, was performed according to [13] adapted to starch gel.

Aldolase A was purified from rabbit muscle [14], aldolase C from rabbit brain [15,16]. Antisera against both types were prepared in chicken; they were monospecific, as proved by the technique of double diffusion on agar gel. In some experiments, extracts at suitable dilutions were mixed with an equal volume of antiserum anti A or anti C, incubated 1 h at 37°C, 1 h at 4°C and then centrifuged. Supernatants were then submitted to electrophoresis on starch gel. Controls were incubated with normal chicken serum.

Isoelectric focusing on acrylamide gel was performed according to [17] with ampholines pH 3.5–10. After electrofocusing, bands of aldolase activity were revealed by pouring on the gel a gelose containing the specific staining solution [14].

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Amount of protein antigen was estimated by radial immunodiffusion in plates according to Mancini et al. [18]. Surfaces were proportional to the antigen amount; the latter was expressed in Arbitrary Units (AU), one unit corresponding to a precipitated area of 100 cm^2 per g of fresh tissue.

We chose to measure the activity of extracts in the wells towards F1P because this substrate is cleaved to some extent by aldolase C and not (or hardly) by aldolase A. In these conditions it was possible to estimate the activity of aldolase C, although it was partly hybridized with aldolase A, because F1P aldolase activity of hybrids is proportional to the number of C subunits [19]. Finally the ratio $\frac{\text{F1P aldolase activity (in IU per g)}}{\text{Antigen amount (in AU per g)}}$ was determined for each zone.

3. Results

Table 1 gives the mean aldolase activities, the mean antigenic amount of aldolase C, and the mean ratio $\frac{\text{F1P activity}}{\text{Antigen amount}}$ in the epithelial cells, the cortex fiber cells (intermediary zone) and the nucleus fiber cells (central zone). It is seen that in both these latter zones (without protein biosynthesis, and with a notable FDP aldolase activity), there is a 'CRM' for aldolase C: the ratio $\frac{\text{Activity}}{\text{Antigen}}$ which is 3.4 ± 0.085 in the epithelial cells, is reduced to 1.7 ± 0.078 in the intermediary zone, and to 1.4 ± 0.061 in the central zone. The difference is very significant ($P < 0.01$).

Fig.1 shows the electrophoretic pattern of aldolase isozymes in adult lens. 3 or 4 bands are seen in the epithelial zone: their migration corresponds to the hybrids A_2C_2 and AC_3, and to the pure tetramer C_4 (as shown by the comparison with the brain isozymic pattern). The same isozymes are seen in the intermediary and in the central zone. But, in addition, two supplementary bands appear: one between A_2C_2 and AC_3, and another stronger one, between AC_3 and C_4.

In contrast, no supplementary isozymes appear in

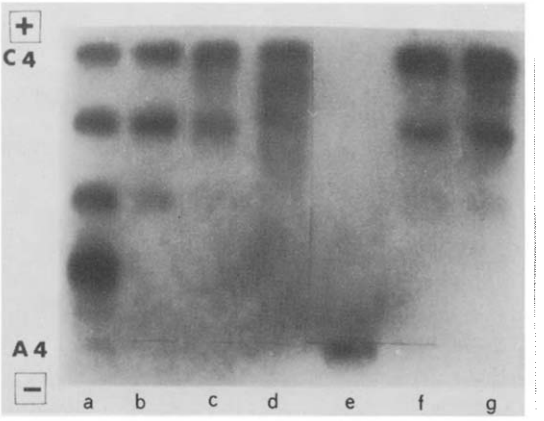

Fig.1. Aldolase isozymes of rabbit tissues. (a) Brain; (b) epithelial cells of lens; (c) intermediary zone of lens; (d) central zone of lens; (e) muscle; (f) 6-day old rabbit lens; (g) 45-day-old rabbit lens.

Table 1
Aldolase activities and amounts of antigen in the three zones of the lens

	Aldolase activities[a] (in IU per g) FDP	F1P	Antigen amount (in AU per g)	Ratio $\frac{\text{Activity}}{\text{Antigen}}$
Epithelial cells	11.5	0.395	0.116	3.4 ± 0.085
Intermediary zone	32.8	1.07	0.635	1.7 ± 0.078
Central zone	18.3	0.53	0.375	1.4 ± 0.06

[a]Mean of 4 experiments

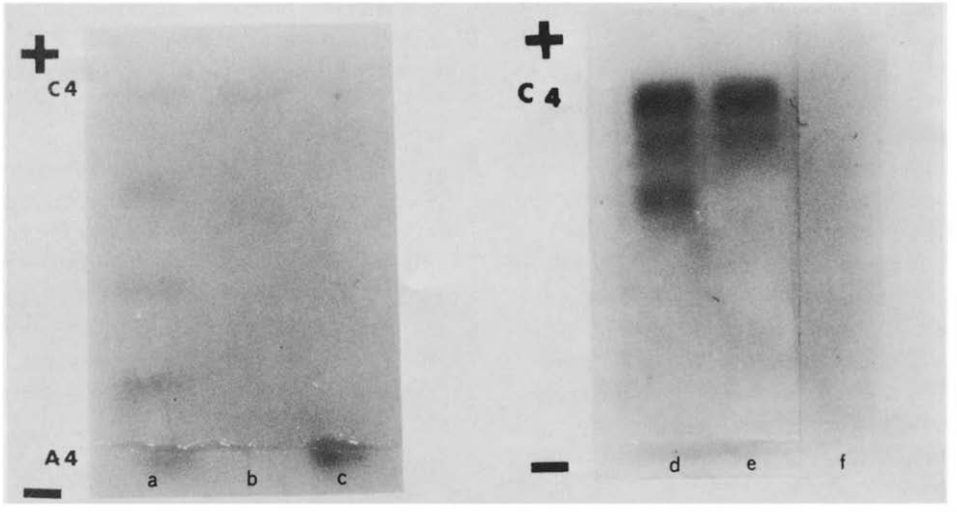

Fig.2. Action of antisera on brain and lens aldolases. (a) Brain; (b) brain + anti A; (c) brain + anti C; (d) central zone; (e) central zone + anti A; (f) central zone + anti C.

the electrophoretic pattern of lens of a 6 day-old rabbit. But these isozymes were seen on the 45th day of life.

Fig.2 gives the electrophoretic pattern after action of antiserum anti aldolase A and anti aldolase C. After action of anti A, the most anodic new hybrid and the pure tetramer C_4 persist, but the other hybrid disappears. After action of Anti C, all isozymes disappear. Fig.2 gives also, for comparison, the electrophoretic pattern of brain aldolases, with and without anti A and anti C.

Fig.3 shows the results of electrofocusing experiments. Because of its high resolution power, a great number of bands can be seen. The micro-heterogeneity of C_4 is well visible [16]. Principally in the central zone, multiple supplementary bands are seen, the migration of which corresponds to hybrids C_2A_2 and CA_3 and to their subunits.

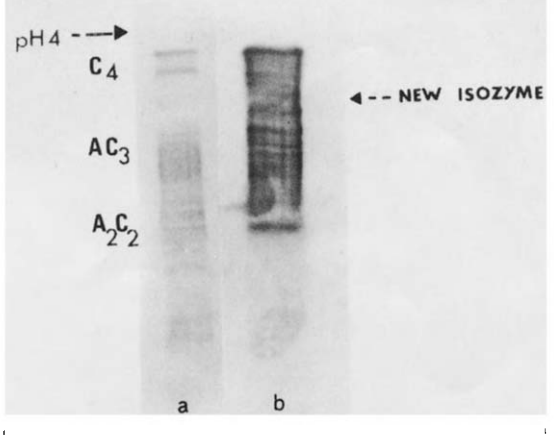

Fig.3. Iso-electrofocusing in acrylamide gel of rabbit brain and lens – with ampholines, pH 3.5–10. (a) Brain; (b) central zone.

4. Discussion

We have found, in the intermediary and in the central zone, a 'CRM' for aldolase C giving evidence for the inactivation of the enzyme with aging. But the most interesting result consists of the appearance of two new supplementary isozymes (which were never found in any tissue).

Immunological experiments indicate that they correspond probably to a pure tetramer C_4 and to an hybrid C_3A with modified charges. They do not exist in the lens of a 6-day-old rabbit, but are already seen on the 45th day of life. This fact and the fact that they appear in zones where no protein biosynthesis occurs, show that they are post-synthetic modifications, a consequence of aging.

How to explain the modification of the charge of aldolase C and perhaps of aldolase A, resulting in these new isozymes? Anodisation of several enzymes have been described in red cells [20,21]. Deamidation was proved for α-crystallin [4–6]; multiple deamidations possibly would occur, resulting in a great modification of charge. Cathodisation of aldolase C possibly would result from the loss of several amino acid residues (negatively charged): shortening of chains was proven for α-crystallin [5]. Perhaps the oxidation of 'exposed' thiols would also occur.

Finally, the complex electrophoretic and electrofocusing patterns seems to represent different degrees of aging of aldolases A and C (with perhaps preferential hybridization of some), the normally charged hybrids being the youngest. The appearance of a 'CRM' represents the final step of aging, before the complete degradation of the molecule. These patterns offer a striking example of post-synthetic modification of enzymes. These modifications possibly play an important role in the process of aging.

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