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EFFECT OF AGING ON RENAL MEMBRANE-BOUND ENZYME ACTIVITIES*

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SUMMARY

The effects of aging on renal membrane-bound enzymes were studied in 3- and 24-month-old rats of two strains. The specific activities and maximal velocities were 50% lower for maltase, and 35% lower for alkaline phosphatase and phosphodiesterase I in preparations of renal plasma membranes from old rats, as compared to young rats. In a second strain of rats, maltase activity, but not alkaline phosphatase activity, was similarly decreased by aging. Aging did not alter the apparent K_m values or the pH optimum curves of the enzymes. Papain digestion and extraction of the membranes raised the apparent K_m for maltase from 1.3 to 2.0 mM. Papain extracts from old rats showed reduced specific activity and maximal velocity of maltase compared to those of young rats. Thus, the decreases in specific activity of these enzymes during aging probably represent decreases in the concentration of active enzyme molecules on the membrane rather than alterations in the form of the enzyme.

INTRODUCTION

The aging process has been postulated as the accumulation of errors in enzyme synthesis with time [1, 2]. The errors not only result in the loss of enzymatic activity but in alterations in cellular function. In addition, if the enzymes are located on the plasma membrane cellular transport may be affected.

Although generalized renal function decreases with age [3–5], it is not known how aging affects enzymes on the renal membranes. However, decreases in membrane-bound (Na^+–K^+)ATPase activity of renal slices have been found as rats age [6], indicating that membrane-bound enzymes may be altered during aging. Also the specific activity of enzymes located on renal microsomal membranes was noted to decrease during aging [7].

Abbreviations: MES, (2-*N*-morpholino) ethane sulfonic acid; INI, 2-(*p*-iodophenyl)-3-*p*-nitrophenyl-5-phenyltetrazolium chloride.

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This paper examines the effect of aging on three enzymes, maltase, alkaline phosphatase, and phosphodiesterase I, located on the plasma membranes of rat renal proximal tubule cells. The specific activities and kinetics of the enzymes were determined in both membrane and homogenate fractions from old and young rats.

MATERIALS AND METHODS

Reagents

Tris–HCl, Tris base, Triton X-100, dithiothreitol, propylene oxide, OsO_4, (2-*N*-morpholino)ethane sulfonic acid (MES), *p*-nitrophenyl phosphate, *p*-nitrophenyl-5′-thymidylate, glucose 6-phosphate, maltose, *o*-dianisidine, 2-(*p*-iodophenyl)-3-*p*-nitrophenyl-5-phenyltetrazolium chloride (INT), sodium cacodylate, papain, glucose oxidase (crude), and horseradish peroxidase were obtained from Sigma Chemical Company. Sucrose, sodium succinate, and D-glucose were purchased from Mallinkrodt Chemical. Elon and Epon were obtained from Eastman Chemicals and Sephadex G-200 from Pharmacia Fine Chemicals.

Animals

Male albino rats, aged 3–6 months, and 24 months were obtained from two sources: strain No. 1, from the Charles River Breeding Laboratory (Wilmington, Mass.), and strain No. 2 from ARS/Sprague–Dawley Farms (Madison, Wisc.). Old strains of strain No. 1 included some retired breeders, while all of the rats of strain No. 2 were virgins. The rats were held in an isolation room with appropriate precautions, at least one week after arrival.

Assays

Maltase was assayed according to the method of Dahlqvist [8], using 28 mM maltose in 100 mM MES buffer, final volume of 1 ml, and a pH of 6.3 at 37 °C for 10 min. Alkaline phosphatase was measured by the procedure of Ray [9], phosphodiesterase I by the method of Touster et al. [10], succinate dehydrogenase–INT reductase by the method of Pennington [11], and glucose-6-phosphatase by the procedure of Hübscher and West [12] using 30 mM glucose 6-phosphate in 80 mM cacodylate buffer, pH 6.5, at 37 °C for 20 min. Glucose was measured using a glucose oxidase procedure [8] and phosphate by a modification [13] of the Fiske–SubbaRow phosphomolybdate procedure [14] using Elon as the reducing agent. Protein was measured according to Lowry [15] using bovine serum albumin as a standard. Spectrophotometry was performed with a Beckman DU or Coleman 124 spectrophotometer.

Isolation procedure

Plasma membranes were isolated from rat renal cortex by modification of the methods of Kinne [16, 17] and Fitzpatrick [18]. Rats were killed with a blow on the head and exsanguinated. Their kidneys were removed, decapsulated, the cortices dissected, weighed, and placed in iced 0.25 M sucrose. Subsequent operations were performed at 4 °C. The cortex was minced, homogenized gently in 10 vol. of cold 0.25 M sucrose with a Dounce homogenizer. and filtered through nylon mesh. The filtrate was centrifuged at $1000 \times g$ and again at $1500 \times g$ for 10 min to remove nuclei and cell debris, then recentrifuged at $7500 \times g$ for 10 min. A two-layered pellet was

formed; a brown lower layer containing primarily mitochondria, and a pink upper layer containing plasma membranes. The lower layer was saved for the preparation of a mitochondrial fraction and the supernatant was saved for the preparation of a crude microsomal fraction. The upper portion of this pellet was removed and centrifuged for 90 min on a discontinuous sucrose gradient according to the method of Kinne [17]. The purified plasma membrane was found at the 39–41% (w/w) sucrose interface, corresponding to a density of 1.16–1.18. The membranes were removed from the gradient with a Pasteur pipet and washed with 0.25 M sucrose followed by a modified Krebs–Ringer buffer without calcium or phosphate at pH 7.2. The membranes were then suspended in the modified Krebs–Ringer buffer for use in the assays.

Papain digestion and gel filtration

The isolated membrane fractions were incubated with a papain solution according to the method of Stevenson [19]. After a 5-min digestion, the mixture was centrifuged at 20 000 × *g* for 10 min at 4 °C. The supernatant was placed on a Sephadex G-200 column (1 cm × 50 cm) and eluted with 50 mM MES buffer, pH 6.3, at 4 °C. The effluent was monitored at 254 nm with a Pharmacia ultraviolet monitor.

Contamination

The isolated membrane fractions were assayed for mitochondrial contamination by measuring succinate dehydrogenase–INT reductase activity and for microsomal contamination by measuring glucose-6-phosphatase activity.

Electron microscopy

Pellets of isolated membrane were fixed in 2.5% glutaraldehyde for 2 h, post-fixed in 1.3% OsO_4 for 70 min, dehydrated, and embedded in Epon. Sections of the pellet were cut with an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an RCA electron microscope.

RESULTS

The preparation of renal plasma membranes contained both brush border and basal membrane fragments as seen by electron microscopy (Fig. 1). No mitochondria or nuclei were evident. The membrane fractions of both old and young animals were enriched 7-fold in maltase activity, 6-fold in alkaline phosphatase, and 5-fold in phosphodiesterase I activity compared to the activities in the homogenate (Table I). Specific activities for maltase and phosphodiesterase I are similar [20] to those previously reported for rat kidney membrane preparations while alkaline phosphatase values could not be directly compared.

The membrane fraction contained 50% less succinate dehydrogenase activity and the same glucose-6-phosphatase activity as the homogenate. Assuming that mitochondria and microsomes contribute less than 25 and 20% respectively of the cellular protein, contamination of the membrane fraction with mitochondria was less than 10%, and for microsomes less than 20% of the total membrane protein. The enzymatic activities of all the enzymes were the same in the 3- and 6-month-old rats.

The specific activities of the enzymes from the plasma membranes decreased significantly during aging (Table I). The specific activity of maltase decreased 50% in

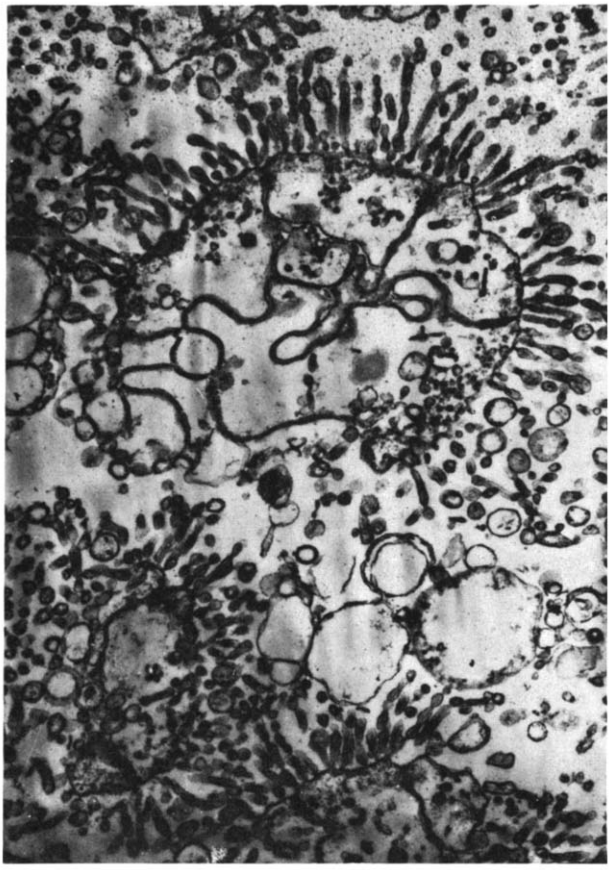

Fig. 1. Electron micrograph of a membrane pellet isolated from rat renal cortex. The preparation was fixed with glutaraldehyde and post-fixed in OsO_4. Magnification $\times 15\,000$.

strain No. 1 and 40% in strain No. 2. The specific activity of alkaline phosphatase decreased 35% in strain No. 2 and was unchanged in strain No. 1 with aging. The specific activity of phosphodiesterase I also decreased 35% in strain No. 2 with aging. The activities of both the homogenate and the membrane fractions were decreased to the same extent.

Kinetic analysis of the enzymatic activity were performed on membrane fractions from young and old kidneys (Fig. 2, Table I). Decreases in the V of maltase were seen in both strains of rats during aging (Fig. 2). The degree of decrease was similar to the decrease in specific activity. The apparent K_m of the enzyme (1.3 μM) was not affected.

Decreases in the V of alkaline phosphatase were also seen in membrane fractions of rats of strain No. 2 during aging.

The decrease in V was similar to the decrease in specific activity. However, in strain No. 1, both old and young rats had a similar V for alkaline phosphatase activity. The apparent K_m, which was the same (30 μM) for both strains, was not altered by aging.

TABLE I

EFFECT OF AGING ON RENAL MEMBRANE ENZYME ACTIVITIES

Specific activities are expressed as μmoles glucose released/h/mg protein at 37 °C (maltase) and μmoles p-nitrophenol released/h/mg protein at 24 °C* and 37 °C** (alkaline phosphatase*, and phosphodiesterase I**). Each value represents the mean and standard error of 8 experiments for strain no. 1 and 5 experiments for strain no. 2. Statistical significance is defined as $P < 0.05$.

	Strain No. 1			Strain No. 2		
	24 months	3 months	old/young	24 months	3 months	old/young
Maltase						
Fractionated extract				500. ±70	790 ±60	0.63
Membrane	50 ±5	100 ±9	0.50	50 ±10	83 ±5	0.60
Homogenate	7.2±1.0	15.0±1.0	0.48	9.4±0.5	13.9±0.5	0.68
			$P < 0.001$			$P < 0.002$
Alkaline phosphatase						
Membrane	36 ±5	44 ±4	0.82	23 ±4	36 ±2	0.64
Homogenate	6.0±0.6	6.8±0.5	0.88	3.7±0.3	5.5±0.5	0.67
			N.S.			$P < 0.02$
Phosphodiesterase I						
Membrane	–	–	–	18 ±2	27 ±3	0.67
Homogenate	–	–	–	3.6±0.5	5.2±0.3	0.69
						$P < 0.05$

A decrease in the V of phosphodiesterase I was seen in the membrane fraction of strain No. 2 rats during aging. The decrease was again similar to the decrease in specific activity; no change in the apparent K_m of the enzyme (67 μM) was observed.

After papain digestion and chromatography, the membrane extracts from young and old animals gave identical elution patterns (Fig. 3). A major peak at the void volume was seen, along with several subsequent minor peaks, one of which was papain. All of the maltase activity of the extracts was present in the peak at the void volume (Fig. 3). This peak represented 90% of the maltase activity originally present in the isolated membrane fraction. The other 10% was found in the pellet remaining after papain digestion. Maltase specific activity was 50 times greater in the fractionated extract than in the homogenate (Table I). The 35% decrease in activity seen during aging was still present after extraction. No alkaline phosphatase or phosphodiesterase I activity was present in the extract.

Kinetic analysis of the maltase activity was performed on the fractionated extract from young and old rats (Fig. 4). A decrease in the V of maltase was seen with aging. The apparent K_m was altered from 1.3 to 2.0 mM in both young and old animals.

No age-related differences in the character of the activity curves of each enzyme were seen when the pH of the assay medium was varied from a pH of 3 to 9 (maltase) or from a pH of 7 to 11 (alkaline phosphatase and phosphodiesterase I).

Differences in the membranes between old and young rats were not due to differences in the purity of the membrane preparation, since (1) the total protein concentration of the initial homogenates from old and young rats were the same, (2) the protein to wet-weight ratios of the preparations from the old and young rats

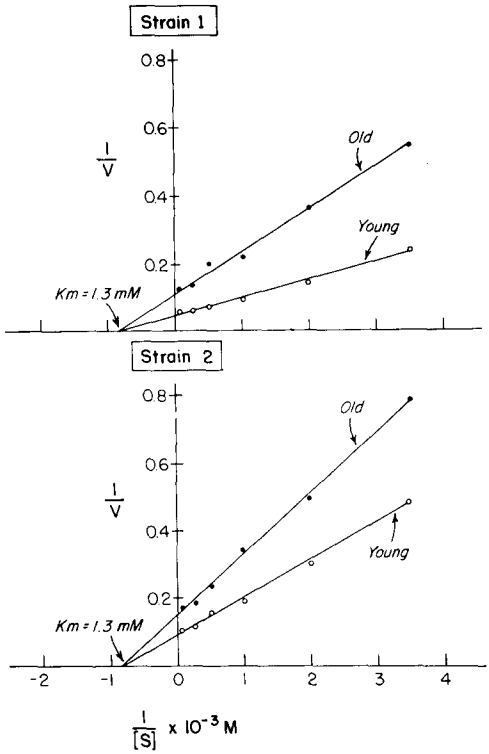

Fig. 2. Lineweaver–Burk plots of maltase activity of renal plasma membranes in young (3 month) and old (24 month) rats from two strains (Strain No. 1 from the Charles River Breeding Laboratory and strain No. 2 from ARS–Sprague–Dawley Farms). The velocity is given as mg glucose released per h per mg protein. The substrate used was maltose.

were the same throughout the purification process, and (3) the degree of enrichment of each enzymatic activity was similar in preparations from the old and young rats, even after 50-fold purification.

DISCUSSION

The specific activities of maltase, alkaline phosphatase, and phosphodiesterase I of rat renal plasma membranes were significantly decreased during aging. The decreases in specific activity were accompanied by decreases of the maximal velocity but not of the apparent K_m of the enzymes. Decreases in maltase activity remained after papain extraction of the membrane fractions. The pH optima of the enzymes were not affected by aging.

This finding is consistent with several hypotheses. Firstly, errors in the machinery for protein synthesis may accrue with time, resulting in a decreased production of enzyme molecules. This would lower the specific activity and V but not the K_m of the enzyme, as was found in old rats in the present study. Secondly, errors in protein synthesis might result in an increase of inactive or ineffective enzyme molecules, but not in the total number of enzyme molecules. This would also lead to results found in

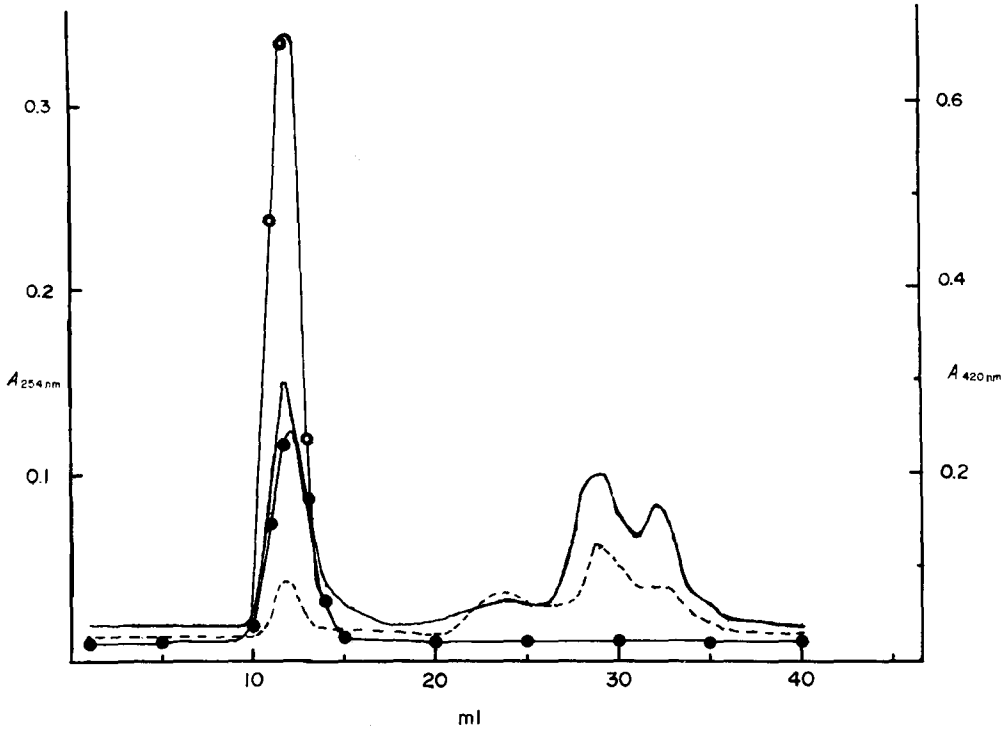

Fig. 3. Sephadex G-200 gel filtration pattern of a papain extract of rat renal plasma membranes. Maltase activity from young (○–○) and old (●–●) rats is given in absorbance units produced (at 420 nm) by a 0.1-ml aliquot of fractionated papain extract. Absorbance at 254 nm is also shown; ———, young and - - -, old.

the present study. The two hypotheses have been differentiated by ingenious experiments of Zeelon et al. [21]. They reported that a 50% decrease in the specific activity of aldolase occurred during aging in nematodes, without any loss of the protein which cross-reacted with the antibody to aldolase.

Thirdly, the data in the present study could be explained by binding to renal membranes of non-competitive inhibitors of the enzymes that accumulate with aging and this would alter the maximal velocity but not the K_m of the enzymes. Although no specific examples of this phenomenon are known, the data are consistent with this hypothesis. Fourthly, the enzymes may be structurally altered in the membranes from old rats. Conformational changes in renal microsomal membranes during aging have been proposed by Grinna and Barber [7] who found a rise in the K_m of glucose-6-phosphatase with age. However, we found no change in the apparent K_m for the enzymes studies in either strain during aging. This suggests that the enzymes were not structurally altered.

Papain extraction of the membrane fraction raised the apparent K_m for maltase from 1.3 to 2.0 mM in both young and old rats. The change in the apparent K_m probably represents a change in the maltase environment produced by the extraction procedure. This change was not affected by aging.

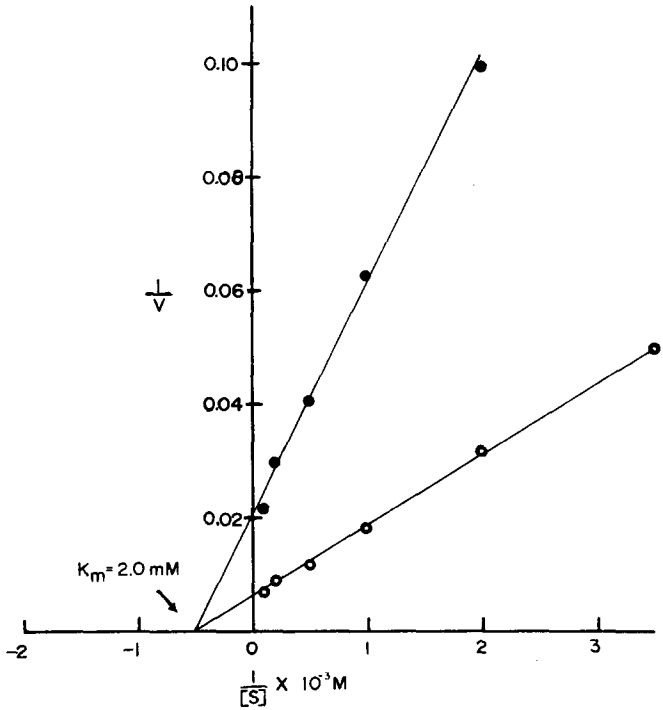

Fig. 4. Lineweaver–Burk plot of maltase activity of fractionated papain extracts from young (○–○) and old (●–●) rats of strain No. 2. The velocity is given in mg released per h per mg protein. The substrate used was maltose.

No decrease in the specific activity or V of alkaline phosphatase was seen in rats of strain No. 1 during aging. This observation focuses on the difficulties of extrapolating data on aging across a wide number of species or strains within the species.

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