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Enzyme Distributions in the Nephrons of Marine Teleosts

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Marine teleostean nephrons are classified into three types (Edwards, 1929, 1935; Ogawa, 1962). The nephron of most marine teleosts is composed of a glomerulus, neck segment, first and second proximal tubules, and collecting tubule (Edwards, 1929; Ogawa, 1957). The nephrons of a few species of marine teleosts are similar to freshwater teleostean nephrons, consisting of a glomerulus, neck segment, first and second proximal tubules, distal tubule, and collecting tubule (Ogawa, 1959). The nephrons of some other marine species are aglomerular, and are composed of only two regions, second proximal and collecting tubules (Marshall, 1934; Ogawa, 1958).

In a previous paper (Endo and Kimura, 1982), we reported the enzyme distributions in freshwater teleostean nephrons. The structures and functions of marine teleostean nephrons are different from freshwater teleosts, and little is known about enzyme distributions in the seawater teleostean nephrons. This research deals with the enzyme distributions in three types of marine teleostean nephrons.

Materials and methods

Eight specimens of the red seabream *Pagrus major* (mean body weight 104.5 g), nine specimens of the yellowtail *Seriola quinqueradiata* (mean body weight 1062.5 g), 10 specimens of the marine catfish *Plotosus lineatus* (mean body weight 63.3 g), and six specimens of the frogfish *Phrynelox tridens* (mean body weight 110.3 g) were used in this study. The red seabream and yellowtail have typical marine teleostean nephrons. Nephrons of the marine catfish resemble freshwater teleostean nephrons. Nephrons of the frogfish are aglomerular. Trunk kidneys of these specimens were removed and frozen by precooled dry-ice acetone. Fresh frozen sections (6~10 μm thick) were cut in a cryostat and mounted on cover slips. They were immersed in appropriate incubating media at 32°C for 30 min. After incubation, the sections were rinsed with distilled water and mounted in glycerol-gelatine.

The enzyme histochemical techniques included: 1. Demonstration of the oxidative enzymes, using the following substrates: malate, NADH, and isocitrate. The incubation mixtures were prepared according to Hess et al. (1958) or Nachlas et al. (1958). 2. Demonstration of hexokinase with the azo dye method of Meijer (1967). 3. Demonstration of glucose-6-phosphatase activity with the metal precipitaion method of Wachstein and Meisel (1956).

Control tests of all stainings were carried out by immersion in the incubating media which lacked specific substrates.

Results

We did not observe enzyme distributions in the neck segment of the nephrons of all species, because this region was short or absent.

Highest activity of glucose-6-phosphatase occurred in second proximal tubules of the yellowtail (Fig. 1A). A moderate reaction was located in the second proximal tubules of the red seabream and marine catfish. The first proximal tubules of glomerular nephrons of the above-mentioned three species showed weak activity. Glucose-6-phosphatase activity disappeared in the second proximal tubules of aglomerular nephrons of the frogfish, the distal tubules of the marine catfish, and the collecting tubules of all species.

Malate dehydrogenase exhibited the highest activity in the collecting tubules of all species and the distal tubules of the marine catfish (Fig. 1B). Weak and sometimes moderate reactions were displayed in the first and second proximal tubules of the glomerular nephrons. The second proximal tubules of the aglomerular nephrons showed a moderate reaction (Fig. 1C). The distributions of NADH and isocitrate dehydrogenases paralleled the distribution of malate dehydrogenase, but the intensities of NADH and isocitrate dehydrogenase activities were slightly weaker than malate dehydrogenase.

Moderate hexokinase activity occurred in the first and second proximal tubules of the glomerular nephrons and the second proximal tubules of aglomerular nephrons (Fig. 1D).

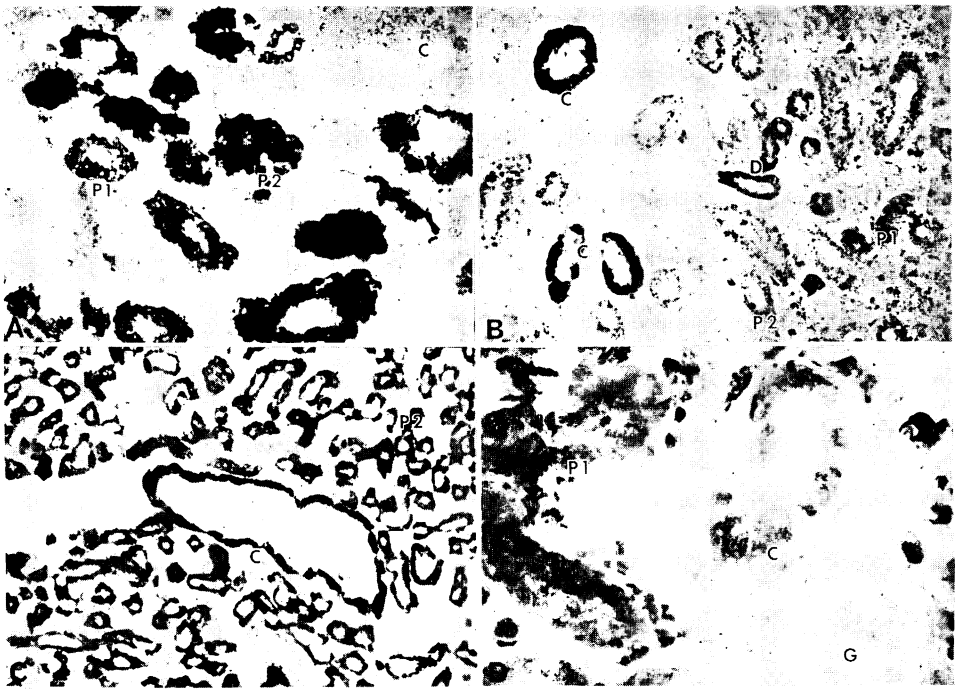

Fig. 1. A: Reaction of glucose-6-phosphatase in yellowtail (*Seriola quinqueradiata*) nephrons. Second proximal tubules react intensely and first proximal tubules react weakly for this reaction. However, the activity of glucose-6-phosphatase disappears in collecting tubule. ×230. B: Reaction of malate dehydrogenase in marine catfish (*Plotosus lineatus*) nephrons. Distal and collecting tubules exhibit intense activity of the reaction, but first and second proximal tubules show weak activity. ×130. C: Reaction of malate dehydrogenase in frogfish (*Phrynelox tridens*) nephrons. Intense activity of the reaction is seen in collecting tubule, while second proximal tubules react moderately for the reaction. ×85. D: Reaction of hexokinase in sea bream (*Pagrus major*) nephrons. Although moderate activity of the reaction is sited in first proximal tubule, collecting tubule shows weak activity. ×300.
P1, first proximal tubule; P2, second proximal tubule; D, distal tubule, C, collecting tubule, G, glomerulus.

There was weak activity in the distal and collecting tubules of all nephron types. No reactions showed any activity in the glomeruli.

Discussion

In mammalian nephrons, gluconeogenesis takes place in both the proximal and distal tubules (Nagai, 1979). The gluconeogenesis in the glomerular nephrons of marine teleosts seems to be performed only in the proximal tubules; the glucose-6-phosphatase activity was observed in only the proximal tubules of the glomerular nephrons of the species presently examined. Aglomerular nephrons which did not exhibit the glucose-6-phosphatase activity probably do not correspond to gluconeogenesis.

Endo and Kimura (1982) already reported that the proximal portions (first and second proximal tubules) are anaerobic and the distal portions (distal and collecting tubules) carry out aerobic metabolism in freshwater teleosts. In the marine teleostean nephrons, anaerobic enzymes are located primarily in the proximal portions and intense activities of aerobic enzymes are seen in the distal portions. The marine as well as freshwater teleostean nephrons have a metabolic difference between the proximal portions and distal portions. In the development of a mesonephric nephron, a dual origin of the mesonephric duct and the mesonephric blastema has been reported (Gray, 1930; Maschkowzeff, 1934). The metabolic difference

between the proximal and distal portions in teleostean nephrons may be related to the development of the nephrons.

In rat nephrons, intense activities of aerobic and anaerobic enzymes are located in both the proximal and distal portions (Walker, 1963; Kazimierczak, 1963; Abe and Shimizu, 1964). Enzyme distributions are similar in many mammalian species (Sternburg et al., 1956). However, the muscle type of lactate dehydrogenase, which is an anaerobic isozyme, is localized in the proximal portions, and the heart type of lactate dehydrogenase, which is an aerobic isozyme, is observed in the distal portions of mammalian nephrons (McMillan, 1967). Thus, the metabolic difference between the proximal and distal portions of mammalian nephrons is found by examining the isozyme distributions. Therefore, the teleostean nephrons are characterized by the difference of metabolism at the level of enzyme distributions. Metabolic difference at the level of enzymes is probably due to a mesonephric origin.

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海産硬骨魚のネフロンの酵素組織化学的研究

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海産硬骨魚のネフロンの各部位における酵素分布について検討した．グルコース-6-フォスファターゼ反応は糸球体尿細管の近位尿細管のみに局在していたが，無糸球体尿細管ではその酵素活性が見られなかった．用いた全ての海産硬骨魚の腎において，解糖系酵素（ヘキソキナーゼ）は主に近位尿細管に，好気的代謝酵素（リンゴ酸，NADH およびイソクエン酸脱水素酵素）は主に遠位尿細管と集合管に分布していた．なお，糸球体では行われた全ての酵素反応はほとんど活性を示さなかった．

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