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LACTATE DEHYDROGENASE IN MUSCLE OF SPOTTED MACKEREL

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ABSTRACT

S. S. Jeng and H. L. Chiang (1974). *Lactate dehydrogenase in muscle of spotted mackerel.* Bull. Inst. Zool., Academia Sinica, 13(2): 61-67. Fish red muscle differs from white muscle in composition of lactate dehydrogenase subunits. In the whole muscle tissue of spotted mackerel, three kinds of electrophoretic bands are distinguished by cellulose acetate electrophoresis. The enzyme from red muscle consists of two bands, that from heart also of two bands, whereas that from white muscle or liver only one band. A study on dissociation and recombination of red muscle lactate dehydrogenase subunits with sodium chloride suggests that the red muscle is composed of two homogenous isoenzymes H_4 and M_4. The constituent isoenzymes of different tissues were separated electrophoretically on cellulose acetate membrane. The separated iosenzymes were subjected to substrate inhibition test, and the relative reaction rates with the reduced form of nicotinamidehypoxanthine dinucleotide and with NADH were measured. It is concluded that the lactate dehydrogenase from the red muscle of spotted mackerel is composed of 27% M_4 and 73% H_4, heart of 10% M_4 and 90% H_4, and white muscle of 100% M_3H.

Fish red muscle differs from white muscle in many aspects. For example, in the mackerel and herring, the red muscle lactate dehydrogenase is very closely related to the heart muscle, but grossly different from the white muscle by comparison of ratios of reaction rates with coenzyme analogues[5]. The red muscle has a lactate dehydrogenase with a high proportion of H (heart) subunits, whereas the white muscle has a greater proportion of M (muscle) subunits[3]. But the situation of combination of these subunits and the proportion of different isoenzymes in red and white fish muscle are not clear. This paper reports that the lactate dehydrogenase in red muscle of spotted mackerel is composed of two isoenzymes H_4 and M_4 in 73% : 27% proportion, and white muscle of 100% M_3H.

MATERIALS AND METHODS

Preparation of tissue extracts

Spotted mackerel, *Scomber australasicus*, about four hr after catch was used as the source. After removing heart and liver, the fish was eviscerated. Three transverse slices of one inch thick muscle were cut: one slice from just back of the pectoral fins, one slice halfway between the first slice and vent, and one slice just back of the vent. Red muscle was separated from the three slices and grouped together. White muscle was obtained in the same manner. The

tissues (heart, liver, red and white muscle) were blended with one volume (v/w) of M/16 phosphate buffer (I=0.05, pH 7.5) by a prechilled homogenizer for 3 min. The homogenate was centrifuged at 8,000 rpm for 15 min, and the supernatant fluid was used for catalytic assay and electrophoretic studies.

Electrophoretic pattern of lactate dehydrogenase

The electrophoretic pattern of lactate dehydrogenase was obtained by using cellulose acetate membrane (Selectron, Schleicher & Schull Co., W. Germany) as the support. Electrophoresis was carried out according to Nerenberg's method[10] using pH 8.6, I=0.05 veronal buffer. After 2 hr of electrophoretic run with a current of 0.8 mA/cm width of strip, lactate dehydrogenase isoenzymes were stained with a solution consisted of 0.025 M Tris buffer pH 7.5, 0.535 M sodium lactate, 0.000376 M NAD (nicotinamide adenine dinucleotide), 0.0031 M nitroblue tetrazolium, and 0.00163 M phenazine methosulfate in final concentrations[2]. Two different methods were used to visualize lactate dehydrogenase:

(a) Usual Method

The cellulose acetate membrane was dipped into the staining solution at 37° for 15–30 min. The lactate dehydrogenase isoenzyme could be localized as a purple band.

(b) Fluorescence Method

The cellulose acetate membrane was dipped into the same staining solution at room temperature for 3–5 min. The membrane was then taken out, fixed in solution of methanol/distilled water/glacial acetic acid, 5/4/1, and viewed under long-wave ultraviolet light. The localization of the isoenzyme of lactate dehydrogenase was indicated by a fluorescent band on the membrane.

Dissociation and recombination of lactate dehydrogenase subunits

Dissociation and recombination of lactate dehydrogenase subunits was after Markert's method[8]. Sodium chloride was added to the red muscle extract. The mixture was frozen overnight and thawed before cellulose acetate electrophoretic resolution. The electrophoretic patterns were visualized by the fluorescence method.

Separation of lactate dehydrogenase isoenzyme on cellulose acetate membrane

The crude tissue extract was separated electrophoretically on a cellulose acetate membrane using the same electrophoretic method described above. The tissue extract was applied with a drawing pen to the membrane in a narrow band on the entire starting line. After electrophoretic run, two marginal strips of 0.3–0.5 cm width were cut along the direction of the current, and visualized by the fluorescence method. The location of the lactate dehydrogenase on the main strip could be identified by these two marginal strips. Eighteen strips of 5×6.6 cm cellulose acetate membrane were applied to separate adequate amount of lactate dehydrogenase isoenzymes from red and white muscle for assays of activity and substrate inhibition, and $NHXDH_L/NADH_H$ activity ratio test.

Enzyme assay method

(a) Specific Activity

Levels of lactate dehydrogenase in the extracts and in the eluates from the cellulose acetate membrane were determined by measuring the oxidation of NADH[4]. The assay solution consisted of 0.1 M phosphate buffer pH 7.5, 1.5×10^{-4} M NADH, and 3×10^{-4} M pyruvate in final concentrations.

(b) Substrate Inhibition

The optimal pyruvate concentration of the isoenzyme eluted from cellulose acetate membrane was measured by mixing eluates with assay solutions of different pyruvate levels.

(c) $NHXDH_L/NADH_H$ activity ratio

The $NHXDH_L/NADH_H$ activity ratio[6] signifies the rate of reaction with NHXDH as coenzyme at a low level of pyruvate 3.0×10^{-4} M ($NHXDH_L$), divided by the rate with the reduced nature coenzyme NADH at a high pyruvate 1.0×10^{-2} M ($NADH_H$).

RESULTS

Electrophoretic patterns of lactate dehydrogenase of different mackerel tissues

After visualization by the usual and fluo-

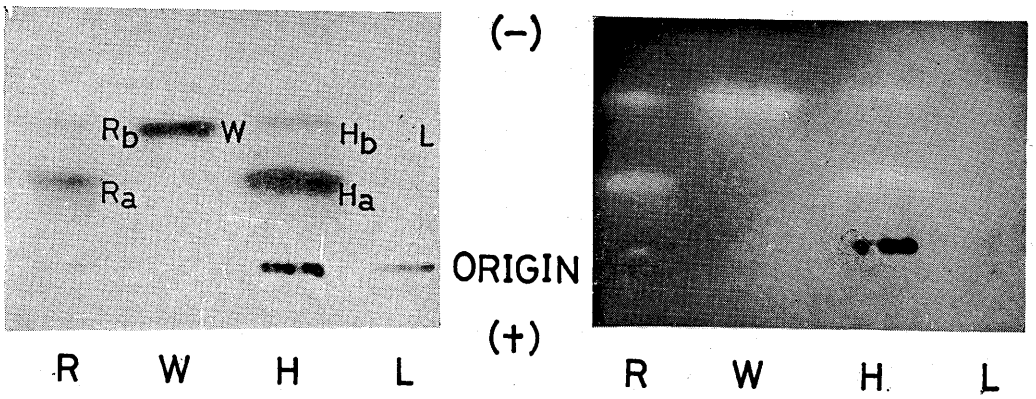


Fig. 1. Cellulose acetate electrophoregram of lactate dehydrogenase of spotted mackerel (R, red muscle; W, white muscle: H, heart and L, liver). *Left*, the patterns visualized by the usual staining method; *Right*, visualized as fluorescent bands under ultraviolet light.

rescence methods, the electrophoretic patterns of different mackerel tissues are shown in Fig. 1. In the mackerel, only three kinds of electrophoretic bands could be distinguished. Red muscle lactate dehydrogenase consisted of two bands, designated as Ra and Rb. Heart lactate dehydrogenase also consisted of two bands, represented as Ha and Hb. White muscle and liver had only one band, represented as W and L.

The assay of lactate dehydrogenase of each tissue extract showed that the red muscle had specific activity of 180 units/g tissue, white muscle of 700 units/g tissue, heart and liver of 130 and 5 units/g tissue, respectively. In order to know the activity ratio of Ra and Rb in red muscle, Ra and Rb were separated by cellulose acetate electrophoresis and assayed. It was found that the ratio of activity of Ra/Rb was 3/1.

Dissociation and recombination of red muscle lactate dehydrogenase subunits

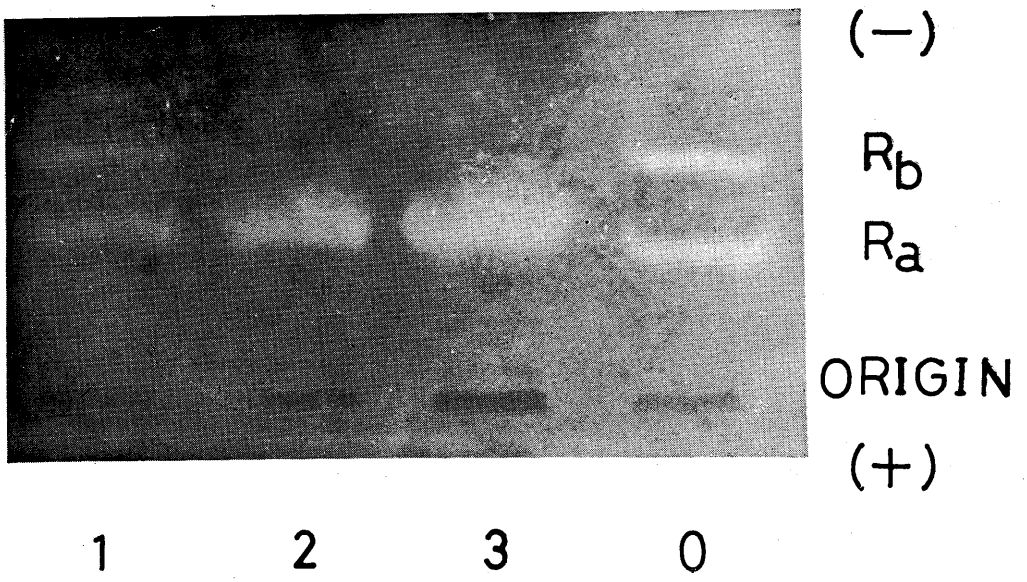
When different amounts of sodium chloride were added to red muscle extracts, frozen overnight and thawed, the lactate dehydrogenase subunits were dissociated and recombined as shown in Fig. 2. Recombination of the subunits rendered the fluorescence of Rb weaker, that of Ra stronger, and resulted in the appearance of one to two band between Ra and Rb. It is noted that the new band(s) existed within the region between Ra and Rb along Ra side, but not outside Ra and Rb.

Pyruvate inhibition of lactate dehydrogenase of different mackerel tissues

Isoenzymes of different mackerel tissues, i.e., Ra, Rb, Ha, Hb and W, separated by cellulose acetate electrophoresis were subjected to substrate inhibition test. As shown in Fig. 3, both Ra and Ha had maximum activities at a pyruvate concentration of 3.0×10^{-4} M, Rb and Hb both the same optimal pyruvate at a pyruvate concentration of 5.0×10^{-3} M, while the optimal pyruvate concentration for W was 3.0×10^{-3} M. Comparison of the optimal pyruvate concentrations indicated that the optimal pyruvate for W was situated at a 3/4 position between Ra(Ha) and Rb(Hb). The liver extract was not subjected to pyruvate inhibition test because its lactate dehydrogenase activity was very low.

Lactate dehydrogenase of mackerel red muscle

It is well known that lactate dehydrogenase exists as five isoenzymes of differing subunit compositions in a variety of animals. Since there were present only three electrophoretic bands in mackerel tissue (Fig. 1), the analysis of the subunit compositions of the isoenzymes seemed worthwhile. Ra and Ha moved toward anode, therefore, their isoenzyme subunits should contain more H (heart) type subunits. On the



Fig, 2. Dissociation and recombination of lactate dehydrogenase subunits from the red muscule of spotted mackerel. 0, 1, 2, and 3 represent isoenzyme patterns of red muscle extracts added 0, 1.25, 1.50 and 2.0 M NaCl respectively, frozen overnignt and thawed before cellulose acetate electrophoretic resolution. Enzyme activity was not reduced significantly by treatment with NaCl.

contrary, Rb and Hb moved toward cathode, therefore, the isoenzyme subunits should contain more M (muscle) type subunits. The dissociation and recombination of red muscle lactate dehydrogenase indicated that Ra and Rb were probably H_4 and M_4. If Ra and Rb were not composed of homogenous subunits, there should be some bands outside the region between Ra and Rb after recombination of subunits. Figure 2 indicated that this was not the case. As mentioned above, the activity ratio Ra/Rb was 3/1, therefore, if Ra and Rb are H_4 and M_4 respectively, after recombination the predicted ratio of H_4, H_3M, H_2M_2, HM_3, and M_4 will be 495:220:66:12:1. The new band(s) found above Ra indicated that the supposition of Ra and Rb to be H_4 and M_4 was very probable.

It is seen from Fig. 3 that Ra and Ha have the same optimal pyruvate concentration at 3.0×10^{-4} M; Rb and Hb also have the same optimal pyruvate concentration at 5.0×10^{-3} M. These facts strongly supported the possibility that Ra and Ha had the same subunit composition, Rb and Hb being identical. Since the electrophoretic band of W (white muscle) move more slowly toward anode than Rb(Hb) (Fig. 1), and its optimal pyruvate concentration situated at a 3/4 position between Ra(Ha) and Rb(Hb)

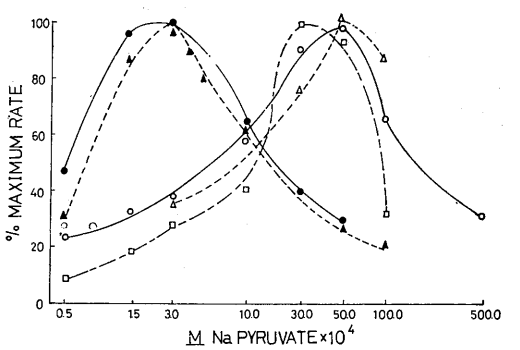


Fig. 3. Pyruvate inhibition of lactate dehydrogenase isoenzymes from spotted mackerel (Ra ●, Rb ○; Ha ▲, Hb △; W □; see Fig. 1 for isoenzyme composition).

(Fig. 3), it is presumed that the composition of white muscle is M_3H. The electrophoretic band of the liver enzyme had the same mobility as the white muscle, therefore, it is quite likely that the subunit composition of the liver enzyme is also M_3H.

$NHXDH_L/NADH_H$ activity ratio of lactate dehydrogenase of different mackerel tissues

Kaplan and Cahn[6] used the relative reaction rate of lactate dehydrogenase with NHXDH and with NADH to calculate the percentage of H (heart) subunit in the enzyme. The $NHXDH_L/NADH_H$ activity ratios of the isoenzymes separated from cellulose acetate electrophoresis (Ra, Rb, Ha, Hb and W), and tissue extracts (red muscle, heart and white muscle) are shown in Table I. Table I indicates that the values for Ra and Ha, and those for Rb and Hb are almost identical. Presuming that Ra (Ha) is H_4 (H%=100) and Rb (Hb) M_4 (H%=0), a line could be drawn to connect Ra (Ha) and Rb (Hb) as shown in Fig 4. If W (white muscle) is M_3H, then it should fall on the curve at the position corresponding to 25% H, and actually this was the case.

This experiment not only proved that the composition of W was M_3H, but also indicated that Ra (Ha) and Rb (Hb) were really H_4 and M_4. The percentage of H subunits in the whole red muscle could be calculated from the curve to be 73%, and that of heart to be 90%.

TABLE I

Comparison of lactate dehydrogenase $NHXDH_L/NADH_H$ activity ratio of various separated isoenzymes and tissue extracts of spotted mackerel.

Sample		$NHXDH_L/NADH_H$
Isoenzyme	Ra	1.40
	Rb	0.05
	W	0.33
	Ha	1.41
	Hb	0.10
Tissue extract	Red muscle	1.05
	Heart	1.25
	White muscle	0.35

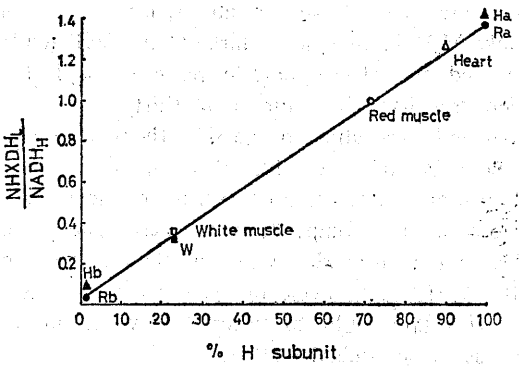


Fig. 4. Relationship of ratio $NHXDH_L/NADH_H$, for spotted mackerel lactate dehydrogenase, to the percentage of H subunit (Ra, Ha, Rb, Hb and W represent separated isoenzymes; Red muscle, Heart, and White muscle represent whole tissue extracts).

DISCUSSION

Based on all experimental data, the conclusion is that the lactate dehydrogenase from red muscle of spotted mackerel is composed of 27% M_4 and 73% H_4, heart of 10% M_4 and 90% H_4, and white muscle of 100% M_3H. The similarity of red muscle and heart is demonstrated by the present study showing that both red muscle and heart lactate dehydrogenase consist of M_4 and H_4 in very close proportions.

It is of great interest to note that the red muscle of spotted mackerel lactate dehydrogenase comprises two distinct homogenous isoenzymes M_4 and H_4. It may be speculated that the metabolism of fish red muscle is intermediate between that of heart and muscle, though closer to heart.

In a variety of animals, M_4 is the major constituent of skeletal muscle. For example, in human beings, distribution of M_4 isoenzyme of lactate dehydrogenase in the muscle tissue is over 45% of the total enzyme[7]. It is not clear why in spotted mackerel the lactate dehydrogenase

of white muscle contains no M_4 isoenzyme, but only M_3H isoenzyme. Markert and Faulhaber[9] studied the electrophoretic patterns of lactate dehydrogenase in muscles of thirty species of fish and were able to classify them into four groups based on whether the fish muscle contained one, two, three or five major isoenzymes. However, the composition of these isoenzymes was not mentioned. Whether the presence of 100% M_3H isoenzyme in mackerel white muscle is also applicable to other fish white muscle needs to be studied further.

Cellulose acetate electrophoresis was used to study the isoenzyme pattern of lactate dehydrogenase by several other workers[1,11]. The advantages of cellulose acetate electrophoresis over starch gel as the media are that it is faster (1-2 hr) and requires only a small amount of staining solution. Separation of isoenzyme preparatively with cellulose acetate electrophoresis could also be achieved if the sample was applied to the membrane along the entire starting line.

It can be seen from Fig. 1 that there is no difference in the patterns of lactate dehydrogenase isoenzymes whether they are visualized by the usual method or the fluorescence method. But the time required for staining isoenzyme by the fluorescence method is only about 1/10 of the usual method. Besides, the staining solution does not become turbid so easily as by the usual method, thus rendering the staining solution repeatedly usable.

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花腹鯖肌肉乳酸脫氫酵素特性之研究

鄭森雄　蔣宏隆

魚類之血合肉與白肉之乳酸脫氫酵素 (LDH) 具有不同之生理功能。爲知此功能與同功酵素 (isoenzyme) 有何關係，乃以花腹鯖爲原料，詳細研究其同功酵素之特性，並與其他組織比較。實驗後發現：花腹鯖各組織內 LDH 之酵素活性（單位／克組織）分別爲白肉 700，血合肉180，心臟 130，及肝臟 5。各組織經醋酸纖維素電泳分離後，以組織化學染色，可知血合肉及心臟皆由二種同功酵素組成，但白肉及肝臟僅由一種同功酵素組成。將各組織之同功酵素利用醋酸纖維素電泳分離以後，以其受不同濃度丙酮酸鹽之抑制，輔酶 NHXDH 及 NADH 對其作用之關係，以及血合肉抽出液行 LDH 組成單元之分離與重合等方法研究各個同功酵素之特性後；得知花腹鯖血合肉乃由 27% 之 M_4 及 73% 之 H_4 組成；白肉由 100%之 M_3H 組成；心臟由 10% M_4 及 90% H_4 所組成。由此實驗可知，就 LDH 同功酵素之組成來講，花腹鯖血合肉確實類似於其心臟。花腹鯖白肉之 LDH 由 M_3H 組成，而血合肉由 M_4 及 H_4 組成，似乎意味着，生物組織中 LDH 之性質，除了和 M 與 H 之百分比有關外，與其同功酵素之組成應亦有關係。