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Comparative Study on Cytochrome Oxidase of Rat Muscle Tissues

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쥐 근조직의 Cytochrome Oxidase에 대한 비교 연구

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요 약

쥐 골격근의 크루드 미토콘드리아에 있는 시토크롬 옥시다제의 활성을 비교하였다. 붉은색의 빠른 트윗치 근육은 가장 높은 효소 활성을 나타냈고, 흰색의 빠른 트윗치 근육은 가장 낮았으며, 붉은 색이며 느린 트윗치 근육은 그 중간이었다. 위 세가지 타입의 근육에서 힘 염색한 결과 시토크롬 옥시다제의 전기영동상의 이동성이 다르게 나타났다. 이동성의 순서는 타입 I > 타입 II_A > 타입 II_B이었다. 면역학적 전기영동의 결과는 위의 결과를 뒷받침 하였다.

INTRODUCTION

Rodent skeletal muscles have been divided into three different fiber types and are classified as follows (Baldwin *et al.*, 1972; Brooke and Kaiser, 1970; Peter *et al.*, 1972): (i) fast-twitch white fibers (type II_B), which have a low respiratory capacity, a high myosin ATPase activity; (ii) the fast-twitch red fibers (type II_A), which have a high respiratory capacity, a high glycogenolytic capacity, and a high myosin ATPase activity; and (iii) the slow-twitch red fibers (type I), which have a high respiratory capacity, a low glycogenolytic capacity, and a low myosin ATPase activity.

In studies on rodents, the soleus muscle, which consists predominantly of slow-twitch fibers (85% type I) (Ariano *et al.*, 1973), has been used for biochemical studies on slow-twitch red fibers. For biochemical studies on fast-twitch white fibers, the white portion of the vastus lateralis of the rat has been used as this consists of white fibers (100% type II_B) (Baldwin *et al.*, 1972). The deepest red portion of the vastus lateralis in the rat, closest to the femur, which consists of fast-twitch red fibers (75% type II_A) (Baldwin *et*

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al., 1972), was used for studies on fast-twitch red fibers. Since cytochrome oxidase activity is closely related to oxygen uptake in animals (Booth and Narahara, 1974; Mela *et al.*, 1976), it may be possible to find differentiated tissues with dissimilar metabolic functions and roles.

METHODS AND MATERIALS

(1) Crude Cytochrome Oxidase Preparation from Muscle

Routinely, 6 to 12 animals (Sprague-Dawley rat) were sacrificed to prepare muscle mitochondria. Leg muscles such as soleus (slow-red muscle type), the red portion of the vastus lateralis (fast-red muscle type), and the white superficial layer of the vastus lateralis (fast-white muscle type) were prepared by dissection and suspended in 20 volumes of a solution containing 20 mM sodium pyrophosphate, 50 mM imidazole pH 6.8, 0.25 M sucrose, and 5 mM EGTA. The muscles were homogenized. After centrifugation at $800 \times g$ for 20 minutes, supernatants were obtained to prepare mitochondrial fraction. This fraction was resuspended in 0.1 M KCl and 0.1 M potassium phosphate pH 8.0 and washed once. For a crude prepartion of muscle cytochrome oxidase, the mitochondrial pellets were solubilized in 50 mM Na-glycine buffer pH 9.5 containing 1% NP-40. The supernatants were collected after centrifugation at $15,000 \times g$ for 20 minutes. Cytochrome oxidase activity was measured by monitoring the rate of decrease in the absorbance of ferrocytochrome c at 550 nm upon addition of the enzyme according to Phan and Mahler (1976).

(2) Gel Electrophoresis

Gel electrophoresis under nondissociating conditions was performed as described below. The 4% polyacrylamide gel was prepared with Fairbanks' polyacrylamide stock solution. The gel buffer contained 50mM Na-glycine pH 9.5 and 0.02% NP-40. The running buffer was 50 mM Na-glycine pH 9.5. The electrophoresis was run at a constant current of 2 mA per gel at 4°C and bromophenol blue was used as a marker in a separate gel. The gels were stained for heme according to the procedure of Thomas *et al.* (1976) except for a slight modification. The gel was fixed in 3 mM 3, 3′, 5, 5′-tetramethylbenzidine (TMBZ) solution in methanol: 0.25 M Na-acetate buffer (1 : 2), pH 5.0 for one to two hours. After fixation, a drop of 30% H_2O_2 was added to each reaction tube containing 5 ml TMBZ solution and blue-green color was developed in 30 minutes.

(3) Immunoelectrophoresis

1% agarose in 0.09 M barbital buffer pH 8.6 containing 0.02% NP-40 was prepared on an 8×10 cm glass plate. Electrophoresis was performed at 10 mA per plate (300 volts at room temperature) with bromophenol blue as marker (Axelsen *et al.*, 1975). After electrophoresis, a trough was cut in the gel between the sample wells and 50~100 μl of antibody was applied. The gel was incubated in a humid chamber to allow the formation of precipitin lines. Rabbit antibody prepared against purified rat heart cytochrome oxidase was used for immunoelectrophoresis.

RESULTS

(1) Cytochrome Oxidase Activity

Crude submitochondrial fractions have been prepared under identical conditon from various rat muscles and cytochrome oxidase activities have been compared (Table 1). In general, crude submitochondrial preparation from fast red muscle has the highest enzyme activity, slow red muscle next and fast white muscle the lowest. Occasionally, preparations from slow red muscle and fast red muscle have shown similar enzyme activities. General tendency of enzyme activity in muscle preparation is fast red≥slow red>fast white in decreasing order.

Table 1. Comparison of cytochrome oxidase activities from different muscle fiber types. Enzyme activity is in k/min/mg.

No. Expt.	Muscle type		
	Slow red	Fast red	Fast white
1	84.4	153.5	37.1
2	150.9	251.4	67.0
3	52.1	89.7	39.3
4	67.8	136.2	54.9
5	64.7	169.5	59.5
6	82.2	102.7	40.3

(2) Electphoretic Mobility

It has been possible to detect electrophoretic mobility variation of cytochrome oxidase under nondissociating condition by use of heme staining technique (9). The electrophoretic

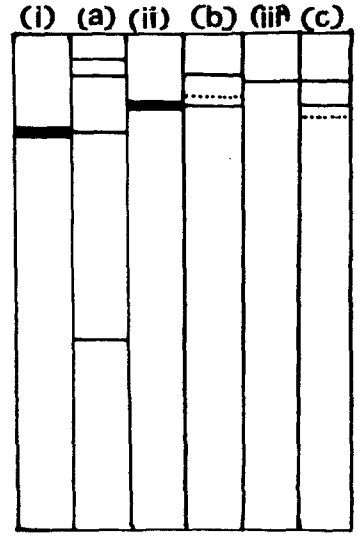

Fig. 1. (i), (ii), (iii): Heme staining of submitochondrial preparations extracted with 1% NP-40 of slow red, fast red, and fast white muscle tissues. (a), (b), (c): protein staining of (i), (ii), (iii) by Coomassie blue.

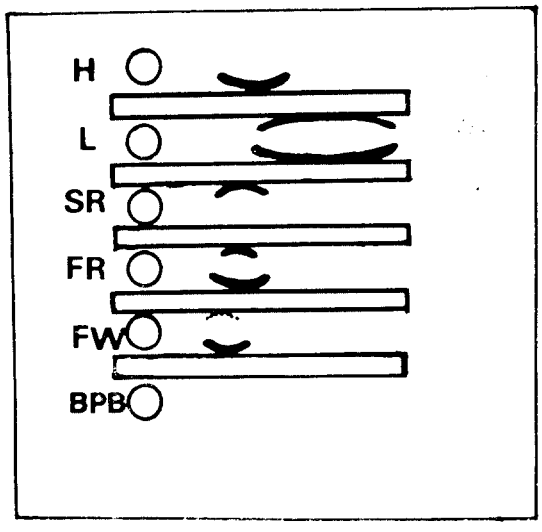

Fig. 2. Immunoelectrophoresis of cytochrome oxidase from various rat tissues. Submitochondrial preparations solubilized in 50 mM α-glycerophosphate, 50 mM KCl pH 8.0, 1% NP-40. The agar plate consisted of 1% agarose, 0.09 M barbital buffer pH 8.5, 0.02% NP-40. Following electrophoresis, a trough was made for the antiserum. H: heart, L: liver, SR: slow red, FR: fast red, FW: fast white, BPB: bromophenol blue.

mobility of the heme bands in the order fastest to slowest migration is slow red>fast red> fast white. The different band patterns correspond with protein bands stained with Coomassie blue, indicating that the heme band is associated with a protein component (Fig. 1).

(3) Immunoelectrophoresis

Results of immunoelectrophoresis have been confirmed the presence of different cytochrome oxidase of different tissues (Fig. 2).

DISCUSSION

(1) Cytochrome Oxidase Activity

The variation from sample to sample in enzyme activities are large (up to 3 fold) even among the same muscle types. For example, fast red muscle preparation of one experiment has cytochrome oxidase activity of 251.4 compared to 89.7 of another experiment (Table 1). This kind of variation is conspicuous especially in fast red and slow red muscle preparations. It seems that this phenomenon is intrinsic and the enzyme activities are not identical depending on the organisms. It would be cautious to say the tendency rather than the actual value.

(2) Electrophoretic Mobility: Is It Real That Electrophoretic Mobility of Cytochrome Oxidase Is Different Depending on Tissue Type?

When comparing electrophoretic mobilities, cytochrome oxidase preparation should be used fresh to give nondiffusible clear heme band. Occasionally heme band split to show more than 1 blue-green band. The reason for this is not known. Crude submitochondrial preparations from different muscle tissues did not show differences in electrophoretic mobility on rare occasions. The results from immunoelectrophoresis always substantiated the difference

in migration. However, the nature of the difference should be identified.

SUMMARY

Cytochrome oxidase activities have been compared from crude sumbitochondrial preparations of rat skeletal muscle tissues. Fast red (type II_A) preparation has highest cytochrome oxidase activity, slow red (type I) next, and fast white (type II_B) the lowest. Differences of electrophoretic mobilities have been detected by heme staining. Migration of heme band is in the order of slow red>fast red>fast white from fastest to slowest. Results of immunoelectrophoresis have substantiated the above finding.

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