Enzyme Activity of the Mitochondrial Respiratory Chain in Meishan Pigs

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Abstract The enzyme activity of hepatic mitochondria from Meishan was compared with those of Landrace, Large White and Duroc of 90 kg in weight. Although there were no significant breed differences in succinate-cytochrome c reductase (complexes II and III), cytochrome c oxidase (complex IV) and NADH oxidase, which measures the entire respiratory chain from NADH to O₂, Meishan showed the activity of NADH-ferricyanide reductase (complex I) to be 17-24% less than that in the other breeds. Meishan required a longer time period to reach a weight of 90 kg than the other breeds, but changes in NADH-ferricyanide reductase with ageing were not observed. Since seven subunits of complex I were encoded by mitochondrial DNA and Meishan and European breeds have different restriction enzyme cleavage patterns of mitochondrial DNA, the differences in the activities of Meishan and other breeds may possibly be associated with variation in mitochondrial DNA. The inheritance mode of NADH-ferricyanide reductase activity is now being investigated.

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Key words : mitochondrial enzyme, breed difference, Meishan, pig

The Meishan is a native breed, originating from the Taihu Lake region in the lower Changjiang River valley¹⁾. The restriction enzyme cleavage patterns of mitochondrial DNA (mtDNA) of this breed differ from those of European breeds²⁾. MtDNA in mammalian cells encodes 13 polypeptides known to be components of the enzyme complexes of the mitochondrial membrane³⁻⁸⁾. Thus, variation in mtDNA may likely have effect on the energy metabolism of animal cells. Recently, we found that part of the activity of cytochrome c oxidase, complex IV of the respiratory chain, is maternally inherited in mice⁹⁾.

In this paper, we compared several enzyme activities of the mitochondrial respiratory chains of Meishan, Landrace, Large White and Duroc to examine the possibility of maternal inheritance of mitochondrial energy metabolism.

Materials and Methods

Three boars and seven gilts of Meishan were supplied to Ibaraki Branchi, Shirakawa Livestock Breeding Station from China in 1986. We obtained their progenies in 1987 and bred them in the Swine Management Section of the National

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Institute of Animal Industry. Liver samples of Meishan were obtained from their next generation and those of Landrace, Large White and Duroc pigs, from herds in the Swine Management Section and Ibaraki Prefectural Experiment Station of Swine.

Twelve pigs of each breed, weighing about 90 kg, were examined for breed differences in enzyme activity. Ageing effects were also studied using 36 Meishan pigs ageing from 7 to 38 weeks. Essentially the same numbers of females and castrated males were used. Since sex differences in enzyme activity were not significant (Table 1), data of both sexes were analyzed together.

Mitochondria were isolated from fresh livers. A 10% tissue homogenate was prepared with a Potter-Elvehjem homogenizer (50 ml) in a buffer containing 0.25 M sucrose, 3 mM HEPS, 0.5 mM EGTA (pH 7.4). After removal of nuclei and cell debris by centrifugation at $700 \times g$ for 10 min, the mitochondria were isolated by centrifugation at $7,000 \times g$ for 10 min and washed twice. The final pellets were resuspended in a homogenizing buffer at a protein concentration of 10–20 mg/ml and stored -80°C .

Cytochrome c oxidase and NADH oxidase activities were measured in freeze-thawed mitochondria. On the day of assay, the suspension was thawed and adjusted to a protein concentration of 1 mg/ml in the homogenizing buffer. NADH-ferricyanide reductase and succinate-cytochrome c reductase activities were measured in submitochondrial particles. For preparation of mitochondrial particles, the suspension was thawed and sonicated by a sonifer (Tosho Denki Co., Model UCD-130) for 5 min at one-minute intervals. The unbroken mitochondria were removed by centrifugation at $18,000 \times g$ for 10 min and submitochondrial particles sedimented at $150,000 \times g$ for 45 min. The particles were resuspended in the homogenizing buffer to a protein concentration of 1 mg/ml. Protein was determined by the method of Bradford¹⁰ using the reagent of Bio-Rad Protein Assay with bovine serum albumin as the standard after diluting the specimens with 0.1 N NaOH solution.

All spectrophotometric assays were carried out at 30°C at a final volume of

Table 1. Enzyme activities of mitochondrial respiratory chain of male and female Meishan and Landrace pigs

Breed	Enzyme	Specific activity*	
		Male	Female
Meishan	NADH-ferricyanide reductase	$5.371 \pm 0.419^{**}$	5.246 ± 0.466
	Succinate-cytochrome c reductase	0.385 ± 0.180	0.318 ± 0.128
	Cytochrome c oxidase	1.078 ± 0.086	1.006 ± 0.110
	Rotenone-sensitive NADH oxidase	0.046 ± 0.010	0.044 ± 0.009
Landrace	NADH-ferricyanide reductase	6.629 ± 0.765	6.903 ± 0.974
	Succinate-cytochrome c reductase	0.332 ± 0.099	0.300 ± 0.110
	Cytochrome c oxidase	1.030 ± 0.073	1.041 ± 0.181
	Rotenone-sensitive NADH oxidase	0.045 ± 0.008	0.043 ± 0.010

*Enzyme activities are calculated as μmoles of substrate reduced or oxidized per min per mg of protein.

**Mean $\pm$ S. D. for 7 pigs.

2.0 ml. NADH-ferricyanide reductase was assayed by the method of MALCZEWSKI and WHITFIELD¹¹⁾ except that the final concentration of $K_3Fe(CN)_6$ was 0.5 mM. The reaction was started by adding 25 μ l of submitochondrial particle suspension to the reaction mixture. Succinate-cytochrome c reductase was measured as described by TISDALE¹²⁾. The reaction mixture contained 50 μ l of a submitochondrial particle suspension. Cytochrome c oxidase was assayed by a modification of the method of WHARTON and TZAGOLOFF¹³⁾. The reaction was initiated by adding 20 μ l of a freeze-thawed mitochondria suspension to the reaction mixture containing 1.0 mg ferrocytochrome c.

NADH oxidase was analyzed by the method of GREEN and ZIEGLER¹⁴⁾ with modification. 150 μ l of a freeze-thawed mitochondria suspension were incubated with the assay mixture for 4 min at 30°C. The reaction was initiated by adding 100 μ l of 2 mM NADH and 20 μ l of 1.0% cytochrome c.

The same number of samples was used each day for each breed and two readings were taken for each sample.

Results and Discussion

Table 2 shows the activities of NADH-ferricyanide reductase, succinate-cytochrome c reductase, cytochrome c oxidase and NADH oxidase for Meishan and the other three breeds. NADH-ferricyanide measured the first part of the respiratory

Table 2. Enzyme activities of mitochondrial respiratory chain of four pig breeds

Enzyme	Breed	Specific activity*
NADH-ferricyanide reductase	Meishan	5.233 $\pm$ 0.586 ^{**} , ^a
	Landrace	6.883 $\pm$ 0.779 ^b
	Large White	6.800 $\pm$ 1.258 ^b
	Duroc	6.260 $\pm$ 0.688 ^b
Succinate-cytochrome c reductase	Meishan	0.366 $\pm$ 0.113 ^a
	Landrace	0.357 $\pm$ 0.071 ^a
	Large White	0.308 $\pm$ 0.057 ^a
	Duroc	0.343 $\pm$ 0.060 ^a
Cytochrome c oxidase	Meishan	1.046 $\pm$ 0.120 ^a
	Landrace	1.037 $\pm$ 0.124 ^a
	Large White	1.091 $\pm$ 0.091 ^a
	Duroc	0.999 $\pm$ 0.125 ^a
Rotenone-sensitive NADH oxidase	Meishan	0.046 $\pm$ 0.008 ^a
	Landrace	0.043 $\pm$ 0.009 ^a
	Large White	0.043 $\pm$ 0.006 ^a
	Duroc	0.048 $\pm$ 0.008 ^a

*Enzyme activities are calculated as μ moles of substrate reduced or oxidized per min per mg of protein.

**Mean $\pm$ S.D. for 12 pigs.

^a, ^b Column values with different subscripts differ significantly ($p < 0.001$).

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chain (complex I) using ferricyanide as an artificial acceptor. Meishan showed lower activity of NADH-ferricyanide reductase than did Landrace, Large White or Duroc. All differences in Meishan and the other breeds were significant ($p < 0.001$). No significant differences were observed among Landrace, Large White and Duroc.

Succinate-cytochrome c reductase measured complex II and III together, and cytochrome c oxidase, the terminal portion (complex IV) of the respiratory chain. There were no significant breed differences in these enzyme activities.

Rotenone-sensitive NADH oxidase activity was calculated from the activity differences in the absence and presence of rotenone. Rotenone is a specific inhibitor of the respiratory chain that interrupts electron flow between NADH dehydrogenase and ubiquinone, and rotenone-sensitive NADH oxidase was used to measure the entire respiratory chain from NADH to O_2 . There were no significant differences in the activity among the breeds.

In this study, we found NADH-ferricyanide reductase activity of Meishan to be lower than those of Landrace, Large White and Duroc. Measurement for each breed was made with the live weight being 90 kg in each case. Since the growth rate of Meishan is clearly less than that of European breeds, we investigated whether differences in NADH-ferricyanide reductase activity between Meishan and other breeds would be due to differences in the age at the time of slaughtering. Fig. 1 shows ageing changes in NADH-ferricyanide reductase activity of Meishan from 7 to 38 weeks of age. The regression coefficient of the activity for weeks of age was not significant and age-related changes could be ignored.

MtDNA in animals is inherited maternally and encodes 13 polypeptides known to be components of enzyme complexes of the mitochondrial membrane³⁻⁸). Studies on Chinese hamster mutant cells have demonstrated that decrease in the activities of complex I and cytochrome c oxidase in the mutant are due to reduction in the levels of mitochondrially coded subunits^{11,15}). We found that the RR mouse strain, having unique restriction enzyme cleavage patterns of mtDNA, showed lower activity of

Fig. 1. NADH-ferricyanide reductase activity in mitochondria from Meishan pigs at different ages. Each point represents one individual. Activity is expressed as μ moles of ferricyanide reduced per min per mg of protein.

cytochrome c oxidase of mitochondria than C 57 BL/6 and BALB/C strains, and that this was maternally inherited to some degree⁹⁾. Although variation in mitochondrial respiratory activity associated with performance traits have been reported in sheep¹⁶⁾, swine¹⁷⁾ and cattle¹⁸⁾, to our knowledge, no study has been conducted on phenotypic variation in mitochondrial enzyme activity associated with mtDNA polymorphism in livestock.

WATANABE *et al.*^{19,20)} found the restriction enzyme cleavage patterns of Japanese wild pigs and Taiwan native breeds to differ from those of European breeds and consider the pigs to possibly have derived from two different maternal origins, European and Asian wild pigs. Our previous paper²⁾ indicated differences in restriction enzyme cleavage patterns of Chinese three breeds, Meishan, Jinhua and Minzu, and European breeds. The patterns of these breeds are probably the same as those of the Asian type described by Watanabe *et al.*²⁰⁾. Watanabe *et al.*¹⁹⁾ found Large White to have the patterns of both Asian and European types, but we have yet to find Asian-type patterns in our Large White specimens.

Meishan showed 17-24% lower activity than Landrace, Large White and Duroc. Complex I is comprised of more than 20 different polypeptide subunits²¹⁾, seven of which are encoded by mtDNA. Since mtDNA encodes 13 polypeptides, almost 60% of the polypeptide coding capacity of mtDNA is utilized for the assembly of complex I. Differences in NADH-ferricyanide reductase activity observed for Meishan and other breeds may be possibly associated with mtDNA variation. A study is now being conducted on the inheritance mode of activity by the reciprocal crossing of Meishan and Landrace.

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梅山豚のミトコンドリア呼吸鎖酵素活性

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梅山豚の肝臓から得たミトコンドリアの呼吸鎖酵素活性をランドレース，大ヨークシャー，デュロックのものと 90 kg 時に比較した。コハク酸チトクロム c 還元酵素（複合体 II，III），チトクロム c 酸化酵素（複合体 IV），NADH から O₂ までの呼吸鎖全体を測定する NADH 酸化酵素に品種差は見られなかったが，NADH-フェリシアナイド還元酵素（複合体 I）で梅山豚は他の品種より 17-24% 低い活性を示した。梅山豚は他の品種より明らかに 90 kg に達する日齢が遅いが，NADH-フェリ

シアナイド還元酵素の活性に加齢に伴う変化は観察されなかった。複合体 I はその 7 個のサブユニットがミトコンドリア DNA によりコードされ，梅山豚のミトコンドリア DNA の制限酵素切断型がヨーロッパの品種と異なるため，梅山豚と他の品種間で観察された NADH-フェリシアナイド還元酵素の差がミトコンドリア DNA の変異と関連している可能性がある。我々は現在，この酵素活性の遺伝様式を調査中である。

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