ANALYSIS OF cDNA SEQUENCE OF YAK MYOGLOBIN AND ITS OXIDATION IN MUSCLES

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Introduction

Yak is the only type of bovine which can live and reproduce at the altitude above 3,500 m with strong resistance to high altitude, coldness and hypoxia as a result of a long-term natural selection. Many animals living in the plateau of high altitude, including humans, cobayas, rats, yaks, and zokers, increase the amounts of red blood cell (RBC), hemoglobin (Hb) and myoglobin (Mb) in order to improve oxygen transportation to tissues so that they can maintain their normal metabolism even at the hypoxia environment, however besides increasing the amount of oxygen-carrying proteins, whether any other mechanisms evolved in animal's hypoxia adaptation was unclear.

Therefore, the objective of this study was, with comparison with common bovine, to analyze yak myoglobin cDNA sequence in order to foresee the possibility of its structural change that may affect yak's oxygen transportation capability, and determine the changed susceptibility of yak's myoglobin and cardiac muscles to oxidation caused by the higher oxidation stress in hypoxia condition.

Materials and Methods

Skeletal muscles (longissimus dorsi) and cardiac muscles were obtained from three adult male Maiwa yaks from Ruoergai Prairie, and three adult male bovines from Chengdu Plain of Sichuan (n = 3). The muscles were removed quickly from slaughtered animals within 1 hour and kept frozen at -20°C until use.

cDNA sequence of yak Mb. Total RNA was extracted from yak cardiac muscle with Trizol reagent. The primers were designed based on Mb cDNA of other species from Genbank for amplifying yak Mb, with the upstream primer 5'-ATGGGGCTCAGCGACG-3', and downstream 5'-TTAGCCATGGAAGCCCAG-3'. The first-strand complementary DNA synthesis reactions were performed with a Superscript RT kit (Tiangen, China). The PCR was conducted at 94°C for 5 min, followed by 34 cycles consisting of 30 s of denaturation at 94°C, 30 s of annealing at 57°C, and 1 min extension at 72°C. The PCR product was purified and extracted band was ligated into TA vector and then transformed into *E. coli* (Top10) for incubation. The positive colonies were selected for further sequencing.

Determination of myoglobin amount was determined as described by Chen et al. (1998). Muscle sample of 1 g was homogenized with 10 ml buffer (3 mM $MgCl_2$, 5 mM EDTA, 75 mM Tris, pH 7.2) before centrifuged at 10,000 g at 4°C for 10 min. The supernatant was collected and absorbance was measured at 576 nm. Mb concentration was calculated according to the formula: $E^{1mmol1cm576nm} = 12.8$ (Li *et al*., 2005).

Measurement of Mb oxidation in muscles. The muscle sample were cut into 1 cm slices and the meat cores with a diameter of 2 cm were obtained, wrapped with oxygen-permeable PVC film and stored at 4℃ for 70 hr. Reflectance absorbance at both 525 nm and 572 nm were recorded with an integrating sphere during storage. The amount of metmyoglobin（MetMb） was calculated (Stewart et al., 1965).

Measurement of lipid oxidation (TBARS procedure) *in skeletal muscle* was modified from Witte (1970). During storage at 4°C for 6 days, 10 g skeletal muscle was homogenated with 25 ml 20% trichloroacetic acid (TCA) and 20 ml distilled water for 1 min in a blender before mixture was filtrated. One equal volume of filtrate and 20 mM TCA were mixed and incubated at 25°C for 20 hr, and absorbance of TBARS was read at 532 nm.

Statistical analysis. Data were analyzed with T test by Statistica 6.10, and the results were expressed as means ± S.E.M. Significant difference ($P < 0.05$) among means of variables were determined by the least significance difference test.

Results and Discussion

The cDNA sequence of yak Mb. The Mb cDNA sequences between yak and bovine were compared. Our data indicated that the sequence amplified was an open reading frame coding 153 amino acids, which agreed with our expectation. The cDNA sequence of yak myoglobin had two bases different (cac→cat and gcc →gct, at the 89th and 91st amino acids, respectively) from that of common bovine from BC102986(NCBI). All amino acid sequences of myoglobin from both yak and bovine, however, were the same.

The amount of myoglobin in yak skeletal and cardiac muscles. Mb contents in yak skeletal and cardiac muscles were 488.3±11.6 nmol/g and 823.4±45.4 nmol/g , which were higher by 18.8% ($P > 0.05$)and 61.2% ($P < 0.05$), respectively, than those of bovine, although the difference in skeletal muscle was not significant.

Mb oxidation in yak cardiac and skeletal muscles. Myoglobin was oxidized faster in cardiac muscle than in skeletal muscles during storage at 4°C (Figure 1). In cardiac muscles, although the contents of MetMb in yak and bovine cardiac muscle were almost the same at the starting point, they increased fast and steadily with storage time and reached their maximum values at about 20 hr, but MetMb content in yak was about 10% lower than that in bovine. For skeletal muscles, myoglobin was oxidized much slower than that in cardiac muscles and the oxidation reached the maximum values almost after 65 hr. MetMb content in bovine increased from 7.5% to 45.8% at an increasing rate of 0.55% per hour, while in yak from 2.9% to 15.4% at the rate of 0.18% per hour. The dramatically difference of oxidizing rate between bovine and yak suggested that myoglobin be oxidized much slower than that in yak, even in the same storage condition and there was no difference of amino acid sequence of myoglobin.

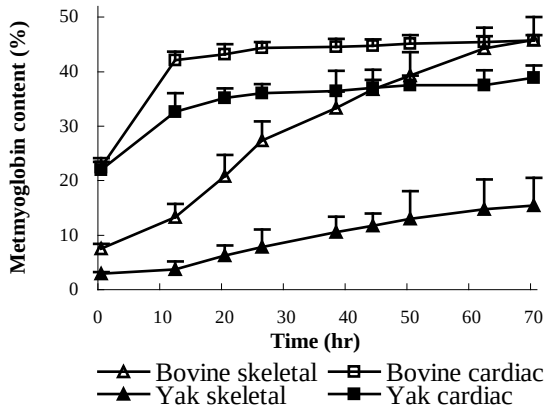

Figure 1. Mb oxidation of yak and bovine cardiac and skeletal muscles during storage at 4°C

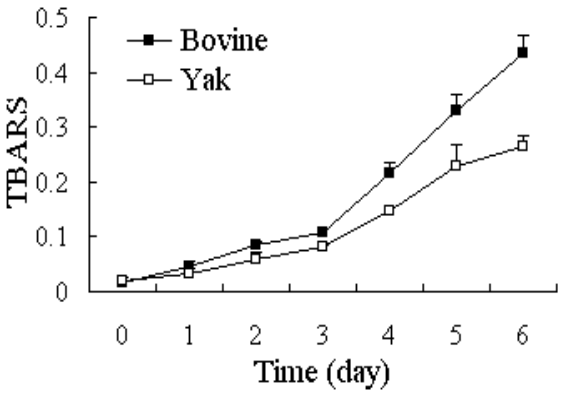

Figure 2. Lipid oxidation comparison from yak and bovine skeletal muscle at 25°C

Lipid oxidation in yak skeletal muscle. To further understand the mechanism by which yaks protect their myoglobin from oxidation, lipid oxidation in both yak and bovine muscles were determined during storage. It has long been believed that myoglobin oxidation is highly affected by lipid oxidation via a variety of primary and secondary lipid oxidation products (Yin & Faustman, 1993). At the same time, MetMb, the oxidized form of myoglobin, could be the catalyst for lipid oxidation in both in vivo and in vitro systems.

After stored for 6 days, TBARS values of both yak and bovine muscles increased steadily with storage time, with this value in yak increasing from 0.021 on day 0 to 0.266 on day 6 at the increasing rate of 0.04 per day, while in bovine TBARS increased from 0.017 to 0.435 at the increasing rate of 0.07 per day, almost doubled to the value in yak (Figure 2). Our data clearly showed that lipid oxidation in yak skeletal muscles was significantly slower than that in bovine.

Conclusions

From our study, yak Mb that has been adaptive to the hypoxia environment was not due to the change of its gene and thus the structure caused by the evolution, instead, the increased Mb content and slower rate of both Mb and lipid oxidation may partially be responsible for yak's resistance to oxidation stress. Whether and how lipids in yak tissues protect yak myoglobin need further study for better understanding of animals' adaptation to hypoxia conditions.

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