DETERMINATION OF METMYOGLOBIN REDUCING ENZYME SYSTEM COMPONENTS IN BOVINE SKELETAL MUSCLE BY THE COMBINATION OF SDS-PAGE AND IMMUNOBLOTTING TECHNIQUE

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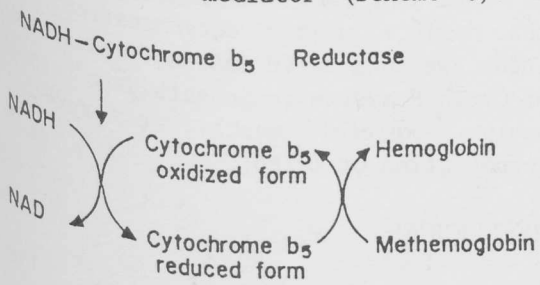
INTRODUCTION

The accumulation of brown metmyoglobin (metMb) results in an undesirable appearance of meat surfaces. MetMb reducing enzyme system is believed to prevent the accumulation of metMb in muscle or meat. Recently, we have shown the presence of NADH-cytochrome b_5 reductase system in bovine skeletal muscle as a metMb reducing enzyme system (Arihara et al. 1989a,b,c). NADH-cytochrome b_5 reductase system is composed of NADH-cytochrome b_5 reductase(Cyt.b_5R) and cytochrome b_5 (Cyt.b_5). MetMb was well reduced by Cyt.b_5R in the presence of Cyt.b_5 in vitro. In erythrocytes, it is well known that methemoglobin(meHb) is reduced by Cyt.b_5R system (Hultquist et al. 1984). MetHb is reduced by Cyt.b_5R using Cyt.b_5 as an electron-transfer mediator (Scheme 1). We think that metMb is also reduced by this mechanism in muscle or meat, although there is no strong proof in vivo.



Scheme 1

We have been studying the presence of bovine skeletal muscle Cyt.b_5R system, using specific antibodies as tools for the detection of the components. In this study, with the help of these antibodies, we have attempted the determination of Cyt.b_5R and Cyt.b_5 in bovine skeletal muscle by the combination of SDS-PAGE and immunoblotting technique to gain more information on the metMb reduction system.

MATERIALS AND METHODS

Cyt.b_5R and Cyt.b_5 used for antigens or standard solutions were prepared from bovine erythrocytes via the procedures described in the previous paper (Arihara et al. 1989a). Antibodies raised in rabbits were purified via affinity chromatography as previously described (Arihara et al. 1989a,b).

Determination of Cyt.b_5R system components was carried out as follows.

SDS-PAGE was performed according to the method of Laemmli(1970), using 12.5% gel(separating part). Samples were dissolved in Laemmli's sample buffer by heating for a minute in boiling water. Bovine skeletal muscles were sliced with scissors and homogenized with sample buffer in Potter's homogenizer before heating. After SDS-PAGE, the proteins were transferred from the gel to Clearblot P-Membrane(Atto Co., Tokyo) via a procedure described previously (Arihara et al.1989b). After electro-blotting, blots on the membrane were immuno-stained using the Bio-Rad Immun-Blot assay kit. Rabbit anti-Cyt.b_5R or anti-Cyt.b_5 antibodies(first antibody), goat anti-rabbit IgG-horseradish peroxidase conjugate(second antibody) and 4-chloro-1-naphtol(horseradish peroxidase development reagent) were used. Absorbance of immunostained bands on the membrane were measured by reflection mode at 570nm with a shimadzu chromato-scanner CS-930.

RESULTS AND DISCUSSION

The immunostainability in this study was highly sensitive and specific. Purified erythrocyte Cyt.b_5R and Cyt.b_5 were well stained. Both components in bovine liver homogenate were immunostained as a single band, respectively. Cyt.b_5R and Cyt.b_5 in bovine skeletal muscle homogenate also had a single band, respectively. Based on the position of molecular weight protein standards, the apparent molecular weight values of bovine skeletal muscle Cyt.b_5R and Cyt.b_5 were 35,000 and 18,500, respectively. Although Cyt.b_5R in fresh muscle sample migrated as a single immunostained band, that in stored meat sample sometimes had another minor band(Data not shown, Arihara 1989c).

Standard curves for purified Cyt.b_5R and Cyt.b_5 were shown in Figure 1. The assays for both components were quantitative enough over the entire range examined. The

recoveries of both components added to bovine skeletal muscle are shown in Table 1. From these results, this method is convenience for determination of Cyt.b_5R system components in muscle.

Cyt.b_5R and Cyt.b_5 contents of bovine skeletal muscle purchased from local supermarkets were shown in Table 2.

In erythrocytes, it is known that sufficient amounts of Cyt.b_5R system components are present to prevent the accumulation of metHb(Yubisui 1982). The weight ratio, Cyt.b_5R : Cyt.b_5 : Hb is approximately 1 : 3.3 : 75,000 calculated from these contents in human erythrocytes(Yubisui 1982). On the other hands, the ratio in bovine skeletal muscle, Cyt.b_5R : Cyt.b_5 : Mb was approximately 1 : 4.3 : 290-720 calculated from the results of this study and Mb contents of bovine skeletal muscle. From the comparison of these values, sufficient amounts of Cyt.b_5R system components exist to prevent metMb accumulation at least in living bovine skeletal muscle.

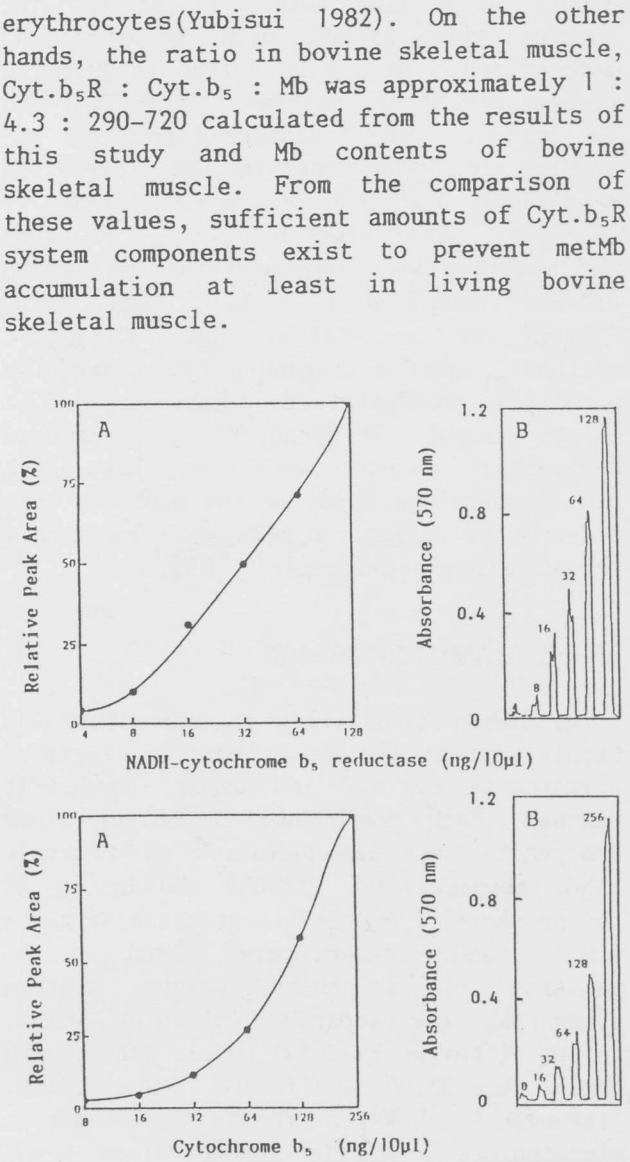


Figure 1. Standard curves for purified NADH-cytochrome b_5 reductase and cytochrome b_5.

A: Standard curves; B: Densitogram.

Table 1. Recoveries of NADH-cytochrome b_5 reductase and cytochrome b_5 added to bovine skeletal muscle.

	Added (ng)	Found (ng)	Recovery (%)
NADH-Cytochrome b_5 Reductase	0	5.2	
	8	12.4	90.0
	16	20.3	94.4
	32	34.6	91.9
Cytochrome b_5	0	24.5	
	8	31.8	91.3
	16	39.7	95.0
	32	54.5	93.8

Table 2. Contents of NADH-cytochrome b_5 reductase and cytochrome b_5 in bovine skeletal muscle.

	Contents (µg / g tissue)	
	Mean	Range
NADH-Cytochrome b_5 Reductase[1]	13.8[3] ± 2.6[4]	9.0-18.2
Cytochrome b_5[2]	59.0 ±20.9	22.8-96.0

1:n=19
2:n=12
3:Average of three trials
4:Standard deviation

CONCLUSION

Determination method of metMb reducing enzyme system(Cyt.b_5R system) components in muscle was established by the combination of SDS-PAGE and immunoblotting technique. From the results of the determination in this study, we considered that sufficient amounts of Cyt.b_5R system components were present in bovine skeletal muscle to prevent the accumulation of metMb.

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