ACTIVITY OF SARCOPLASMIC RETICULUM OF FAST AND SLOW BEEF SKELETAL MUSCLES.

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INTRODUCTION

Significant difference has been demonstrated to exist between the sarcoplasmic reticulum (SR) of fast and slow twitch mammalian skeletal muscle. Morphometric studies revealed a two to three fold higher volume fraction of sarcoplasmic reticulum in fast than in slow twitch fibers (Tomanek, 1976). In accordance with this, biochemical studies demonstrated that yield of vesicular protein, rate and capacity of Ca^{2+} transport, Ca^{2+}-dependent ATPase activity are considerably lower in sarcoplasmic reticulum from slow twitch muscle (Heilmann et al., 1981).

The present study was undertaken to examin some properties of beef fast muscles SR and compared with the beef slow muscles SR, in view of the importance of the sarcoplasmic reticulum (SR) and Ca^{2+} in the transformation of muscle to meat.

MATERIALS AND METHODS

Cattle of various ages (2-10) years sexes (females, males and castrated males) and types (dairy, beef and their crosses) and 10 in number were used in this study.

Slaughtering was effected by stunning (captive-bolt pistol) and exsanguination. Sampling of muscles began approximately 30 min after slaughter from the Tensor fasciae latae (TFL) as white fast muscle, Psoas major (PS) as red fast muscle and Masseter (M) as red slow muscle. Muscles studied were chosen according to available information on metabolic characteristics (Talmant et al., 1986).

The sarcoplasmic reticulum (SR) vesicles from the three muscles were prepared according to the method described by Lee et al., (1979). However, modification in the process of homogenization was made in this study. The medium contained 20 mM Hepes, PH_4, 15 mM Kcl, 5mM EGTA, 5mM $Mgcl_2$ and 1% bovine serum albumin.
Protein content was determined according to Lowry et al., (1951). Using bovine serum albumin as a standard.
The Ca^{2+}-dependent ATPase activity was determined according to the technique described by Martonosi et al.,(1978), which depended on liberation of inorganic phosphate in the medium. The liberated inorganic phosphate was determined(fiske and subbarow, 1925). ATPase activity was measured in the presence of 5 mM sodium azide as inhibitors of mitochondrial ATPase.

The Ca^{2+}-uptake of SR and the Ca^{2+} accumulation capacity of SR were determined by the method of Martonosi and Ferotos, (1964) modified by Kim et al., (1981). The reaction was started by addition of the SR suspension and stopped by filtration reaction medium on 0.22 um pore size Millipor filter at selected time intervals.
Radioactivity of the filters was measured in a scintillation counter Beckman L5 9800, calcium-45 utilised from Radio Chemical Center Amersham, England, specific activity 10-40 mci/mg calcium. SR proteins were separated by SDS-polyacrylamide gel electrophoresis (Laemmli,1970). The same method was used to examine the purity of SR preparation by comparing the mitochondrial

proteins with SR proteins (Fig.2).

RESULTS

The method utilised in this study for isolating the SR facilitated a good quantity of SR (Table 1).
No contamination was observed with mitochondrial proteins (Fig.2)

TABLE 1
Yield of SR from beef muscles

	TFL	PS	M
SR Yield	0,280 ±0,15	0,271 ±0,10	0,070 ±0,009

Results expressed as mean ± standard error
Yield was expressed as mg protein per g muscle.

The yield of SR from both types of fast-twitch muscles was almost three folds that from slow twitch red muscles (Heilmann et al., 1981). Polyacrylamide-gel electrophoresis profiles (Fig.1) of SR from fast and slow muscles showed a significant difference in the protein pattern between the fast and slow muscle

TABLE 2
Ca^{2+}-dependent ATPase Activities of SR from beef muscles

	TFL ATPase activites		PS ATPase activites	
	(A_1)	(A_2)	(A_1)	(A_2)
Basal ATPase activity	0,342	0,248	0,199	0,509
TOTAL ATPase activity	1,200	1,964	0,861	1,242
Ca^{2+}-dependent ATPase activity	0,812	1,460	0,660	0,732
ATPase Activity + EGTA	0,244	0,138	0,201	0,486

ATPase activities are expressed in u moles pi/mn/mg protein.
A_2, A_1 present ATPase activities in the presence of 10 mM potassium oxalate to that in its absence respectively.

TABLE 3
The $^{45}Ca^{2+}$ accumulation of SR from beef muscles

Ca^{2+} uptake kinetics		TFL	PS
Initial velocity 10mM oxalate		1,88	0,888
Initial velocity oxalate absence		0,368	0,200
Initial velocity 10mM oxalate + EGTA		1,408	0,308
$^{45}Ca^{2+}$ accumulation Capacity	^{45}Ca[1mM]	11,20	7,33
	^{45}Ca[0,4mM]	5,77	2,37

Intial velocity was expressed as u mole Ca^{2+}/mg protein/min
$^{45}Ca^{2+}$ accumulation capacity was expressed as u mole Ca^{2+}/mg protein
The free calcium concentration was calculated using an apparent binding constant of 1mM EGTA

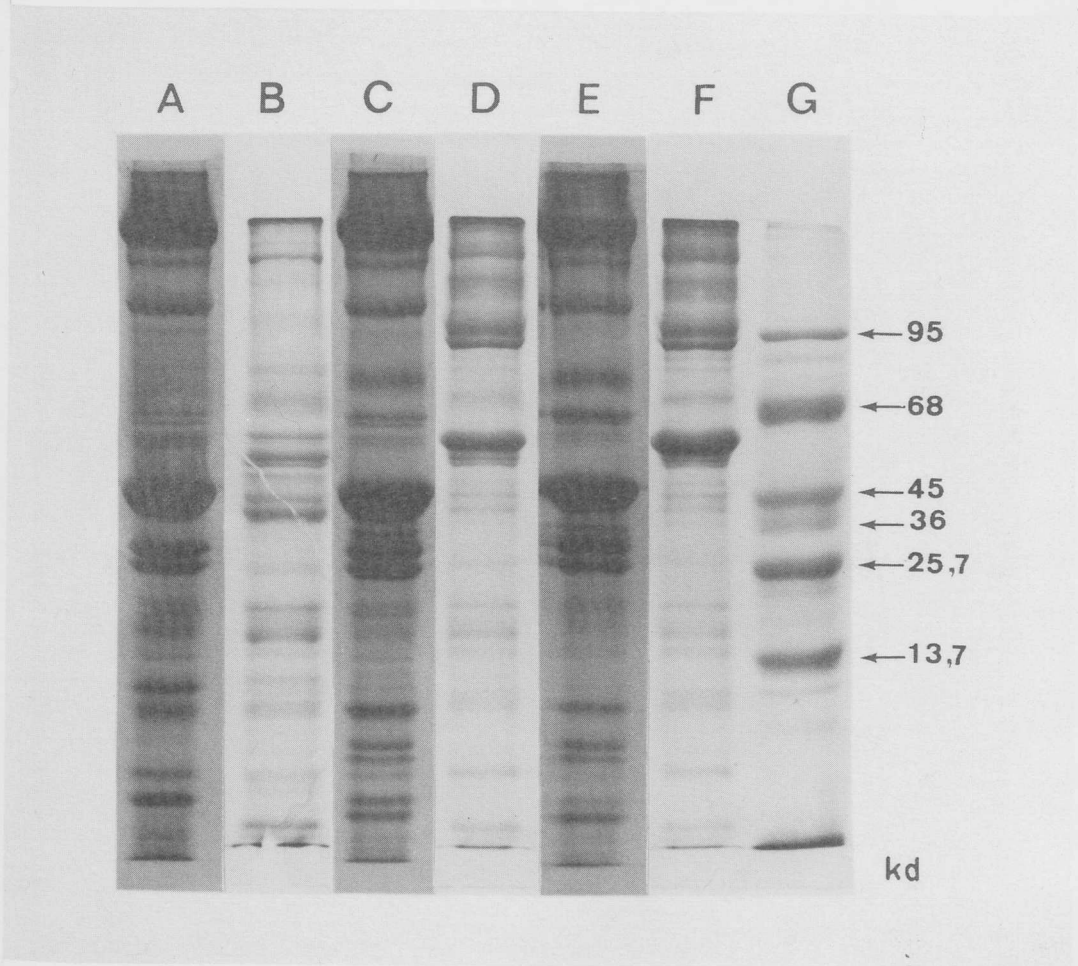

Fig.2: Polyacrylamide-gel electrophoretic profiles of SR and mitochondrial proteins of beef muscles.

A- Masseter mitochondria.
B- Masseter SR.
C- Psoas major mitochondria.
D- Psoas major SR.
E- Tensor fasciae latae mitochondria
F- Tensor fasciae latae SR.
G- Standard proteins: phsphorylase b (95 Kd), BSA (68 Kd), ovalbumine (45 Kd), glyceraldehyde -3-P- deshydrogenase (36 Kd), Chymotrypsinogene (25, 7Kd), cytochrome C (13,7Kd)
Kd: Kilo dalton

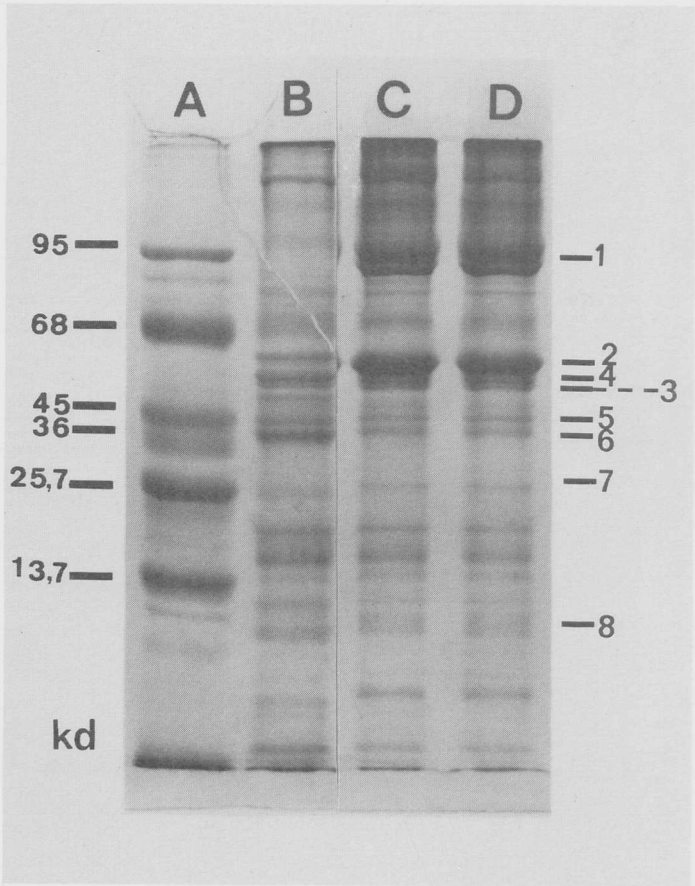

Fig.1: Polyacrylamide-gel electrophoretic profiles of SR from beef muscles.

A- Standard proteins: phosphorylase b (95 Kd), BSA (68 Kd), ovalbumine (45 kd), glyceraldehyde -3-P- deshydrogenase (36 Kd), chymotrypsinogene (25, 7 Kd), Cytochrome c (13, 7 Kd).

B- Masseter SR

C- Psoas major SR

D- Tensor fasciae latae SR

1- Ca^{2+} dependent ATPase of SR

2- Calsequestrin

3- M^{55}

4- 57 Kd

5- 47 Kd

6- 44 Kd

7- 25 Kd

8- 12 Kd

Kd: Kilo dalton

vesicles concern not only the presence of additional proteins, but also their relative amounts. Two major bands were distinguishable (100, 63 Kd)as well as 15 minor bands (57,55,47,44,25, and 12 Kd) The major band of 100 Kd represent the Ca^{2+}-dependent ATPase of SR (Sarzala et al.,1981) while the 63 Kd band probably represent the calsequestrin (Zubrzycka-Gaarn et al., 1982). The minor band of 55Kd represent probably the polypeptide namely M_{55} observed by Michalak et al. (1980) and Ikemoto (1982).
Fig.1 (B) presents the profile of the SR from masseter muscle (M) as slow red muscle; the number of protein components here is higher than in those isolated from fast twitch muscle. The major proteins present in slow-twitch muscle SR have the following molecular weights 57, 55, 47, 44,Kd, while the 100, 63 Kd appear as minors bands.This result is in accordance with those of zubrzycka-Gaarn et al. (1982).
The Ca^{2+}-dependent ATPase activity of fast-twitch white muscles TFL were higher than of fast-twitch red muscles PS (Table 2).
The present results agree with those of Cornforth et al.(1980) and Newbold and Tume (1981).
Table 3 represents Ca^{2+} accumulation and Ca^{2+}-uptake of TFL and PS. The initial rate of Ca^{2+}-uptake of SR from TFL(fast white muscle) measured in the presence or absence of oxalate was almost three times as hight as that found under similar conditions in PS (fast red muscle). The total Ca^{2+} accumulation capacity was also higher in the former muscles than in PS muscles and in accordance with those of kim et al. (1981) and Newbold and Tume (1981).
The PS represent an a ccumulation capacity equal to 65% of TFL capacity in 1mM Ca^{2+} concentration and 41% of TFL capacity in 0,4mM Ca^{2+} concentration.

CONCLUSION

The results of this experiment have shown that the yield of sarcoplasmic twitch muscles was almost three times as high as those from slow-twitch red muscles. Poly acrylamide-gel electrophoresis profiles of sarcoplasmic reticulum from fast and slow muscles showed a significant difference in the protein pattern. The Ca^{2+}-dependent ATPase activity and Ca^{2+} uptake and Ca^{2+} accumulation capacity of the fast-twitch white muscles (TFL) were higher than in the fast-twitch red muscles (PS).
Such kinetic measurments, could not be carried out in the slow-twitch red muscles (M) because of their relatively low content of sarcoplasmic reticulum.

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