

# Properties of the Fragmented Sarcoplasmic Reticulum from Fast Twitch and Slow Twitch Muscles

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**ABSTRACT** Skeletal muscle of guinea pigs is composed of at least three types of fibers which are distinguishable by their kinetic and histochemical properties. Fast twitch muscles may be histochemically red or white; fragmented sarcoplasmic reticulum (FSR) prepared from both types of fast twitch muscles has identical calcium-accumulating capacity (maximal calcium uptake) and calcium-concentrating ability. FSR from slow twitch muscles accumulates less total calcium, but the calcium-concentrating ability is equal to that of fast twitch muscles. The slower rate of calcium accumulation by FSR of slow twitch muscles correlates with a prolonged relaxation time.

## INTRODUCTION

The role of the sarcoplasmic reticulum (SR) in the relaxation of white, fast twitch skeletal muscle is well accepted (1, 2), but there is no agreement (3-7) about the importance of the SR in slow twitch muscles, e.g., the soleus of the rabbit (8) or guinea pig (9). Studies from this group have recently established that fast twitch muscles may be red both by gross inspection and by histochemical techniques (9). The present study was designed to compare fragmented sarcoplasmic reticulum (FSR) from fast twitch muscles, which are grossly and histochemically red or white, with FSR from slow twitch muscles, which are grossly red and classified as intermediate by histochemical techniques.

Previous studies of FSR from slow twitch muscles have yielded contradictory conclusions. Takauji, Yamamoto, and Nagai (3) and Harigaya, Ogawa, and Sugita (4) suggested that FSR from slow twitch muscles is fundamentally similar to that of fast twitch muscles, whereas Sreter and Gergely (5) and again Sreter (6)

and others (7) doubted that FSR of slow twitch muscles plays the same dominant role in relaxation as it does in fast twitch muscle.

In the abovementioned studies the maximal calcium uptake (total amount of calcium accumulated at saturation) by FSR from fast twitch or slow twitch muscles was compared. This assay does not take into account possible contamination by inactive protein of FSR from different muscles and, more important, does not measure the most critical function of SR which is its ability to lower the ionized calcium concentration to levels at which muscle will relax, about  $10^{-7}$  moles/liter. The assay for calcium-concentrating ability of FSR, as described herein, avoids the problem of differential contamination and shows that FSR from slow twitch muscles has a calcium-concentrating ability similar to that from fast twitch muscles. The rate of calcium accumulation under these conditions, however, is slower and correlates with the slower relaxation time of slow twitch muscles.

## METHODS

FSR was prepared from guinea pig muscles of known fiber-type composition (Table I and reference 9) according to Peter, and Peter and Worsfold (10, 11). The fraction used was obtained between 18,000 and 50,000 *g*. It was electron microscopically free of mitochondria (12) and contained negligible cytochrome oxidase activity determined polarographically. The maximal calcium uptake was determined at 26°C as follows. Different amounts of FSR were added to the uptake assay medium containing 0.1 mM  $\text{CaCl}_2$  (plus  $^{45}\text{Ca}$ ), 5 mM ATP (adenosine triphosphate), 5 mM  $\text{MgCl}_2$ , 5 mM oxalate, 40 mM KCl, and 20 mM histidine, pH 7.0. The calcium uptake was determined after 15 min. The maximal calcium uptake was calculated from the calcium taken up per milligram of FSR protein at a protein concentration where only 50% of the calcium in the solution was taken up at equilibrium.

Kinetic measurements of calcium transport were performed according to Peter and coworkers (10, 11, 13, 14) in a medium containing 20  $\mu\text{M}$   $\text{CaCl}_2$  (labeled with  $^{45}\text{Ca}$ ), 5 mM ATP, 5 mM  $\text{MgCl}_2$ , 5 mM oxalate, 40 mM KCl, and

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20 mM histidine at pH 7.0. In the assay for calcium-concentrating ability of FSR the protein concentration chosen was such that the FSR was filled to only one-fourth capacity by the calcium present. After the calcium was taken up and an equilibrium established, 1 ml of the solution was diluted 1:5 with a solution containing EGTA- $\text{Ca}^{++}$  (ethylene glycol bis[ $\beta$ -aminoethyl ether]- $N,N'$ -tetraacetic acid) (15) with a free calcium concentration similar to that found outside the FSR at equilibrium, i.e., about 0.1 micromole/liter. From the rate of release of radioactivity from the FSR, the calcium leakage at equilibrium was calculated (13, 14). This can also be considered the steady-state rate of exchange of calcium by the different preparations of FSR at equilibrium. Measurements of the rate of calcium uptake and calcium leakage under these conditions gave straight lines with double reciprocal plots of  $1/[\text{Ca}]$  vs.  $1/(u + L)$ , where  $u$  equals the net rate of uptake and  $L$  equals the rate of leakage measured at equilibrium and assumed to be constant during the experiment (13, 14).

The rate of passive loss of calcium from calcium oxalate-preloaded FSR in the absence of significant concentrations

TABLE I  
Characteristics of Fragmented Sarcoplasmic Reticulum Prepared from Slow Twitch and Fast Twitch Muscles of the Guinea Pig

Muscle	Vastus lateralis (red portion)	Vastus lateralis (white portion)	Soleus
Gross coloration	Red	White	Red
Physiological and histochemical type (% of fibers)	Fast twitch red, 75	Fast twitch white, 80	Slow twitch intermediate, 100
Time-to-peak tension (msec) (cf. reference 9)	19.0	21.2	82.3
Half-relaxation time (msec) (cf. reference 9)	18.5	19.9	113.8
Yield of FSR (mg protein/g wet weight muscle)	1.9	1.9	1.16
Maximal calcium uptake ( $\mu\text{moles calcium/mg protein}$ )	5.2 (7.8)	5.5 (7.5)	2.8 (3.8)
Maximal rate of uptake ( $\mu\text{moles calcium/mg} \cdot \text{min}$ )	1.2	1.3	0.3
Calcium concentration outside after calcium uptake (moles/liter)*	$0.96 \times 10^{-7}$	$1.02 \times 10^{-7}$	$1.01 \times 10^{-7}$
$V_{\text{max}}$ ( $\mu\text{moles Ca}^{++}/\text{mg} \cdot \text{min}$ )	1.3	1.35	0.35
$K_m$ ( $\mu\text{M Ca}^{++}$ )	4.0	4.0	4.2
Rate of calcium exchange at equilibrium ( $\mu\text{moles/mg} \cdot \text{min}$ )	0.09	0.08	0.04
Rate of passive loss of calcium ( $\text{nmoles/mg} \cdot \text{min}$ )			
36°C	14	17	16
26°C	8.0	10.0	7.0

Kinetic turnover data are averages of three preparations; the other data represent the average from six different preparations. The highest value for the calcium uptake at saturation is shown in parentheses.  $V_{\text{max}}$  and  $K_m$  were obtained by plotting  $1/[\text{Ca}]$  against  $1/(u + L)$ , where  $u$  equals rate of uptake and  $L$  equals leakage or turnover at equilibrium.

\* Calcium-concentrating ability of FSR.

of calcium and ATP in the medium was studied according to Hasselbach, Fiehn, Makinose, and Migala (16) as adapted for small protein quantities, (100  $\mu\text{M Ca}$ , 100  $\mu\text{M ATP}$ , 5 mM oxalate, and 0.1 mg of FSR protein per ml). The uptake was monitored, and then 1 ml of the solution was diluted with 49 ml of 0.05 M KCl in 20 mM histidine at pH 7.0. At different times 10 ml were filtered through Millipore filters (Millipore Filter Corp., Bedford, Mass.), which were then counted to determine the amount of calcium remaining in the FSR and thence the rate of passive loss of calcium.

## RESULTS

Though the SR content of different muscles cannot be accurately assessed from the yield of FSR, Table I shows that the yield, expressed as milligrams of FSR per gram muscle, from both types of fast twitch muscles is almost twice as high as from slow twitch muscles. Techniques previously described for electron microscopic examination of small samples (12) showed that no mitochondria were present. The morphology of the FSR from all three types of muscle is very similar (17).

Data summarizing the characteristics of FSR from fast twitch red, fast twitch white, and slow twitch intermediate muscles are presented in Table I. As expected, the maximal calcium uptake of FSR from slow twitch muscles was not as high as that from fast twitch muscles, but the values found were about 8 times higher than those which Sreter and Gergely (5, 6) obtained in their best preparation from rabbit soleus. The maximal calcium uptake per milligram FSR protein of slow twitch muscles was half that by FSR of fast twitch muscles composed predominately of either red or white fibers. Studies in this laboratory of FSR from rabbit soleus, a muscle composed of approximately 95% slow twitch intermediate fibers, yielded values for calcium-accumulating capacity similar to FSR of guinea pig soleus; this shows that the methods used by Sreter (5, 6) resulted in damaged FSR.

Fig. 1 shows the results of a typical experiment which tests the calcium-concentrating ability of FSR from the three muscles. In this experiment enough calcium was added to fill each preparation of FSR to one-fourth its maximal capacity as predetermined in the presence of excess calcium. The difference in the rate of calcium uptake between FSR of the two types of fast twitch muscles and the FSR of slow twitch muscle is apparent. The maximal rate of  $\text{Ca}^{++}$  uptake by FSR from slow twitch muscle is only one-fourth that of FSR from fast twitch muscles. Correspondingly the  $V_{\text{max}}$  of FSR from slow twitch muscle calculated from the plot  $1/(u + L)$  against  $1/[\text{Ca}]$  was much lower, whereas no difference in  $K_m$  was detected (Table I). In spite of the difference in the maximal rate of uptake, the FSR of the slow twitch muscles showed the same calcium-concentrating ability as that of fast twitch muscles. Under the condi-

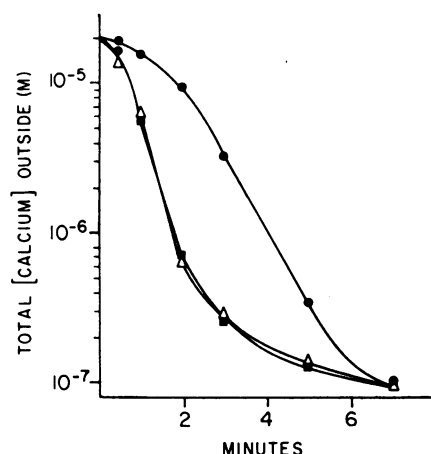


FIGURE 1 Calcium-concentrating ability of FSR from slow twitch and fast twitch muscles. The initial  $\text{Ca}^{++}$ :FSR protein ratio was chosen so that at equilibrium the FSR was filled to one-fourth capacity. Details of the assay are given under "Methods." FSR from the fast twitch white ( $\Delta$ — $\Delta$ ) and from fast twitch red ( $\blacksquare$ — $\blacksquare$ ) part of vastus lateralis. FSR from the soleus, a slow twitch intermediate muscle, ( $\bullet$ — $\bullet$ ).

tions chosen, FSR from all muscles studied were able to lower the total calcium concentration in the medium to at least  $10^{-7}$  moles/liter. The equilibrium rate of calcium exchange (leakage at equilibrium) was significantly lower in FSR of slow twitch muscles, whereas the rate of passive loss of calcium out of calcium oxalate-preloaded vesicles in the absence of ATP was almost the same for the FSR of different origin. For all preparations this rate of passive loss was temperature dependent as described recently by Hasselbach et al. (16).

## DISCUSSION

Much confusion exists in the biochemical literature because of the generalization that white muscles are fast contracting and red muscles are slow contracting. Previous studies from this group (9) show that this generalization is not valid; some red skeletal muscles composed predominantly of histochemically red fibers contract and relax as rapidly as muscles composed of white fibers. Other grossly red muscles which contract and relax slowly, e.g., the soleus of guinea pigs and rabbits, are classified histochemically as intermediate. The latter muscles have a low specific activity myosin ATPase in contrast with the high specific activity of myosin from fast twitch muscles, which may be red or white grossly and histochemically (9). It is thus imperative that the generalization relating color of muscle and speed of the contraction-relaxation cycle be dropped. This generalization has already caused some investigators (18) to infer that the type of myosin need not cor-

relate with contraction speed because myosin from some red muscles (assumed, but not shown to be slow twitch) of chickens is similar to that of white muscles (assumed to be fast twitch muscles). The danger of such a generalization is also emphasized by other studies from our group showing that chronic exercise can convert fast twitch white fibers to fast twitch red fibers which contain much more mitochondrial protein (19).

Previous investigators have not defined the histochemical characteristics or contraction kinetics of the muscles they employed in their studies which were designed to compare FSR of fast twitch and slow twitch muscles. Consideration of the muscles used by these investigators (3–6), however, suggest to us that misidentification of muscle speed does not account for the discordant results. Rather, the dispute about the role of the SR in the relaxation kinetics of slow twitch muscles apparently derives from the inadequate techniques for preparation of FSR used by some investigators. In addition too much emphasis has been given to maximal calcium uptake by FSR as a tool for comparing FSR from different muscles. The assay for calcium-concentrating ability of FSR avoids the vexing problem of differential contamination by inactive protein of FSR prepared from different muscles, either normal or diseased. In addition, the latter assay is physiologically more relevant to the role of the SR *in vivo* which is to lower the free  $\text{Ca}^{++}$  concentration to the very low levels required for relaxation of muscle.

The lower yield of FSR from slow twitch muscles and its slow rate of calcium uptake in the calcium-concentrating assay (Fig. 1) may well explain the slowed relaxation of this type of muscle. Our kinetic data show no fundamental difference in the calcium transport complex of FSR from fast or slow twitch muscles, at least as assessed by the similar Michaelis constants. Our suggestion that a decreased number of transport units per milligram of FSR protein might explain the slower calcium uptake is supported by the data of Sreter (6), who found less phosphoprotein per milligram of FSR protein of slow twitch muscles.

At equilibrium the rate of calcium uptake equals the rate of calcium leakage from the FSR. These rates, which measure the exchange or turnover of calcium at equilibrium, are higher in fast twitch muscles than in slow twitch muscles. On the other hand, the rate of passive loss of calcium from FSR in the absence of ATP at different temperatures is similar for FSR from fast or slow twitch muscle. This suggests that there are no gross differences in the mechanisms of passive efflux of calcium from different types of FSR. Thus the differences in calcium exchange at equilibrium are due to a higher steady-state rate of calcium uptake by FSR from fast twitch muscles rather than to a more rapid efflux of calcium from FSR of such muscles.

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