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Monocarboxylate transporters in subsarcolemmal and intermyofibrillar mitochondria

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Abstract

Whether subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria contain monocarboxylate transporters (MCTs) is controversial. We have examined the presence of MCT1, 2, and 4 in highly purified SS and IMF mitochondria. These mitochondria were not contaminated with plasma membrane, sarcoplasmic reticulum or endosomal compartments, as the marker proteins for these sub-cellular compartments (Na^+–K^+-ATPase, Ca^{2+}-ATPase, and the transferrin receptor) were not present in SS or IMF mitochondria. MCT1, MCT2, and MCT4 were all present at the plasma membrane. However, MCT1 and MCT4 were associated with SS mitochondria. In contrast, the IMF mitochondria were completely devoid of MCT1 and MCT4. However, MCT2 was associated with both SS and IMF mitochondria. These observations suggest that SS and IMF mitochondria have different capacities for metabolizing monocarboxylates. Thus, the controversy as to whether mitochondria can take up and oxidize lactate will need to take account of the different distribution of MCTs between SS and IMF mitochondria.

Keywords: Lactate; Muscle; Pyruvate; Plasma membrane

The *trans*-membrane movement of lactate into and out of muscle involves a lactate-proton co-transport system that is stereo-specific for L-lactate [1,2]. Studies investigating the kinetics of lactate transport with small or giant vesicles have shown that the K_m values for lactate transport rates are within the physiological ranges of circulating and intramuscular lactate concentration (5–40 mM) [1]. Presently, 14 MCTs are known to exist (cf. [3]), but, the kinetics and/or tissue distribution of many remain unknown. MCT1 and 2 are ubiquitously expressed in many tissues, including skeletal muscle [4–11]. In contrast, MCT4 has a more limited tissue distribution, this transporter is expressed primarily in skeletal muscle [12–15]. The co-expression of MCT1, 2, and 4 in muscle suggests that they have different capacities for transporting monocarboxylates. MCT1 is a high-affinity lactate transporter ($K_m \sim 3.5$–8.3 mM) [6,16–19], MCT2 is a high-affinity pyruvate transporter (K_m 25–80 μM) [6,16,18] and MCT4 is a low-affinity lactate transporter (K_m 25–31 mM) [19,20].

MCT1 and 4 exhibit a somewhat different sub-cellular distribution. Both are located at the plasma membrane and t-tubules [12]. There is also an intracellular depot of MCT4, but not MCT1 [12]. This might indicate that the intracellular MCT4 depot can be induced to translocate to the plasma membrane with selected physiologic stimuli. However, muscle contraction, which can induce the

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translocation of the glucose transporter-4 (GLUT-4) [21,22] and the fatty acid transporter fatty acid translocase (FAT/CD36) [23], failed to induce the translocation of MCT4 to the plasma membrane [24]. It appears that MCT1 is also present in mitochondria [14,25], although some contamination of the mitochondria with the plasma membrane was evident in those studies. More recently, this group has confirmed their initial observations that MCT1 is present in mitochondria [26]. However, except for one study, in which MCT1 and MCT4 were both shown to be present in the mitochondria [14], the recent studies from the same group [25,26] make no mention as to whether MCT4 is also present in mitochondria, or whether detection of mitochondrial MCT4 in the prior study [14] was due to mitochondrial contamination with the plasma membrane.

It is important to note that there are two distinct populations of mitochondria in skeletal muscle, the subsarcolemmal (SS) and the intermyofibrillar (IMF) mitochondria. In IMF mitochondria, relative to SS mitochondria, state III respiration is elevated, and IMF mitochondria are more sensitive to inhibition with rotenone [27]. Additionally, IMF mitochondria appear to respond earlier to endurance training [28]. On the other hand, SS mitochondria appear to have a higher density of both UCP3 [29] and cardiolipin [27]. Thus, SS and IMF mitochondria may well have different substrate preferences.

Although, like Brooks et al. [25], we had also detected MCTs in mitochondria, the small contaminations in our work made us reluctant to publish these observations. However, recently, we have been able to obtain SS and IMF mitochondrial fractions from skeletal muscle that are free from contamination [30]. This prompted us to re-examine the presence of MCTs in SS and IMF mitochondria. Remarkably, our studies demonstrate that MCT1 and MCT4 are both present in SS mitochondria, while these proteins are completely absent from IMF mitochondria. In contrast, MCT2 is present in both SS and IMF mitochondria.

Materials and methods

Mature Sprague–Dawley rats 250–300 g were used in these studies. Muscles were removed while animals were anesthetized (Somnotol, i.p. 60 mg/kg). Thereafter the animals were immediately euthanized with an overdose of Somnotol. From mixed rat muscles we prepared giant sarcolemmal vesicle plasma membranes as well as highly purified SS and IMF mitochondria. The procedures for harvesting the muscle tissue were approved by the Animal Care Committee at the University of Guelph.

Giant sarcolemmal vesicles. Giant sarcolemmal vesicles were prepared to allow the detection of MCTs at the plasma membrane. The procedure for obtaining giant sarcolemmal vesicles from rat hindlimb muscles was performed as we have previously described [23,31,32]. Briefly, muscles were cut into thin layers (~1–3 mm thick) and incubated for 1 h at 34 °C in 140 mM KCl, 10 mM Mops (pH 7.4), and aprotinin (10 mg/ml), and collagenase type VII (150 U/ml) in a shaking water bath. The tissues were then washed with KCl/Mops and 10 mM EDTA and the supernatant was collected. Percoll (final concentration 16%) and aprotinin were added to the supernatant. This supernatant was placed at the bottom of a density gradient consisting of a 3 ml middle layer of 4% Nycodenz (wt/vol) and a 1 ml KCl/Mops upper layer. The samples were centrifuged (GS-15 centrifuge, Beckman, USA) at 60*g* for 45 min at room temperature. After centrifugation, the vesicles were harvested from the interface of the two upper solutions. The vesicles were diluted in KCl/Mops and recentrifuged (Sorvall MC 12V, DuPont, USA) at 12,000*g* for 5 min. Vesicles (~50 μg) were stored at −80 °C until analyzed by Western blotting.

SS and IMF mitochondria. Highly purified SS and IMF mitochondria were prepared as we [30] have recently described, using modifications of the procedures published by Cogswell et al. [27]. Briefly, minced muscles were diluted 10-fold in buffer 1 (100 mM KCl, 50 mM Tris HCl, 5 mM $MgSO_4$, and 5 mM EDTA, pH 7.4) and were homogenized at the setting of 3 (Kinematica, Switzerland) for 2×15 s. The SS mitochondria were isolated from the myofibrils by centrifugation at 800*g* for 10 min. The SS mitochondria were pelleted from the supernatant at 10,000*g* (10 min). The pellet was washed twice in buffer 2 (buffer 1 containing 1 mM ATP), spun at 10,000*g* (10 min), and resuspended in a final volume of 150 μL. The myofibrillar pellet containing the IMF mitochondria was rehomogenized using the polytron and spun again at 800*g* for 10 min. The supernatant was discarded and the pellet was diluted 10-fold in buffer 2 and treated with protease (Sigma P5380, 0.025 ml/g tissue) for exactly 5 min. Addition of 15 mL of ice cold buffer 2 arrested the protease, and the samples were centrifuged at 5000*g* for 5 min. The pellet was resuspended in a 10-fold dilution of buffer 2 and spun at 800*g* (10 min). The supernatant was spun at 10,000*g* for 10 min, and the pellet was washed twice in buffer 2, re-spun at 10,000*g*, and resuspended in a final volume of 150 μL. Samples were further purified using a Percoll gradient. For these purposes, samples were spun at 20,000*g* for 2 h and the mitochondrial layer was removed. The Percoll was removed from the sample by further spinning at 21,000*g* for 5 h. At this point highly purified mitochondria are obtained and they can be used for Western blotting.

Western blotting. MCTs on plasma membranes and mitochondria, as well as other proteins, were detected using Western blotting as we have previously described [23,33–35]. Antibodies against MCT2 were obtained from Chemicon International (Temecula, California, USA). Antibodies against MCT1 and MCT4 were obtained from Qiagen (Tokyo, Japan) and have been used in our previous work [34,35].

Results

In both SS and IMF mitochondria cytochrome oxidase (COX) was present (Fig. 1A). In contrast, neither the plasma membrane marker Na^+–K^+-ATPase (Fig. 1B) nor the sarcoplasmic reticulum marker Ca^{2+}-ATPase (Fig. 1C) was present in these two mitochondrial fractions. Similarly, the transferrin receptor, an endosomal marker, was also not found in the SS and IMF mitochondria (Fig. 1D). On the other hand, the fatty acid transporter FAT/CD36 was present in both the SS and IMF mitochondria (Fig. 1E), as we [30] have recently reported.

Monocarboxylate transporter proteins

The monocarboxylate transporters MCT1, MCT2, and MCT4 were all present at the plasma membrane of the giant sarcolemmal vesicles (Fig. 2). MCT1

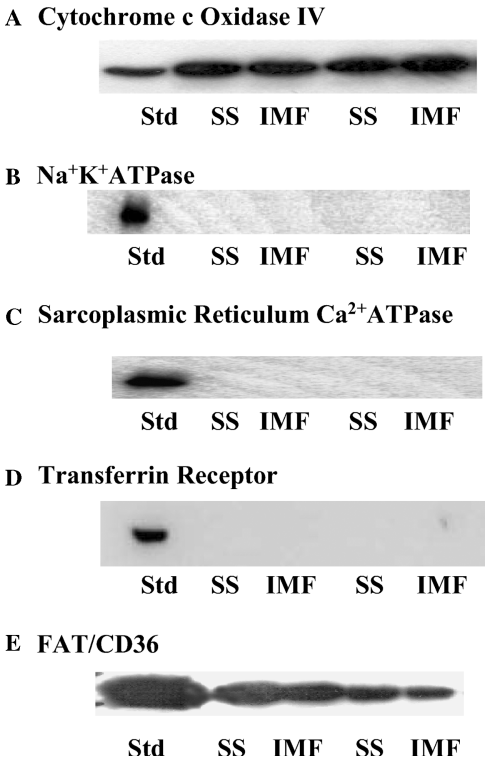

Fig. 1. Western blots of selected proteins in SS and IMF mitochodria. These blots are representative of 12 independent experiments (100 μg mitochondrial protein was loaded into each lane, and 30 μg muscle homogenate for each standard).

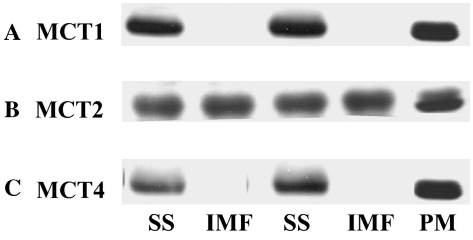

Fig. 2. Western blot showing MCT1, MCT2, and MCT4 in skeletal muscle plasma membranes and mitochondria. These blots are representative blots of 4–10 independent experiments (100 μg mitochondrial protein was loaded into each lane, and 20 μg protein for plasma membrane fractions).

(Fig. 2A) and MCT4 (Fig. 2C) were present in SS mitochondria. However, neither MCT1 (Fig. 2A) nor MCT4 (Fig. 2C) was detected in the IMF mitochondria. In contrast, MCT2 was present in both the SS and IMF mitochondria (Fig. 2B).

Discussion

The present studies confirm a recent report showing that MCT1 is present in skeletal muscle mitochondria [26]. However, the present study extends these recent observations considerably. Specifically, in mitochondria we have shown (a) that MCT1 is only present in SS, but not in IMF mitochondria. Similarly, (b) MCT4 is also exclusively present in SS mitochondria. In contrast, (c) MCT2 is present in both SS and IMF mitochondria.

Although we had already discovered some years ago that MCT1 and 4 were confined to SS mitochondria but not IMF mitochondria, we opted not to publish these data, as we were concerned that the results could have been attributed to some contamination of SS mitochondria with plasma membrane fragments. Initial studies [25] reported the presence of MCT1 at the mitochondria in heart and skeletal muscle that were contaminated with plasma membrane fractions, as shown by the appearance of GLUT1 on SS mitochondria [25]. To remedy the contamination of SS mitochondria with the plasma membrane we spent some time investigating the means to obtain highly purified SS and IMF mitochondria. It is clear that this objective was met with as we have shown that neither the SS nor the IMF mitochondria were contaminated with known plasma membrane, sarcoplasmic reticulum, and endosomal markers, namely Na^+–K^+-ATPase, Ca^{2+}-ATPase, and transferrin receptor, respectively.

Based on the highly purified mitochondrial preparation we can now report with confidence that MCT1 and MCT4 are both present at the mitochondria, but only at the SS mitochondria. Interestingly, neither of these MCT proteins is associated with IMF mitochondria. On the other hand, MCT2 is associated with both SS and IMF mitochondria. The presence of FAT/CD36 in SS and IMF mitochondria confirms previous observations [30]. These different associations of MCT1, 2, and 4 with SS and IMF mitochondria may be indicative of different metabolic capacities for oxidizing monocarboxylates by mitochondria.

In a recent study [26], it was argued that the presence of MCT1 at the mitochondrion validated the idea that lactate could be transported into mitochondria to be oxidized. While evidence has been presented in support of lactate oxidation within mitochondria [36], others have questioned this study on both theoretical and methodological bases [37,38]. Unfortunately, some of these criticisms were not addressed, when given the opportunity to do so [39]. A system for removing excess lactate from mitochondria via a lactate/pyruvate antiport mechanism with high efficiency has also been reported [40]. Thus, whether skeletal muscle mitochondria import lactate, as opposed to pyruvate, remains to be resolved.

It should be remembered that MCTs transport a variety of monocarboxylates. In skeletal muscle the most important are lactate and pyruvate. While MCT1 and MCT4 exhibit transport capacities for lactate that are within physiological limits of this substrate, a similar case can be made for the transport capacities of

Table 1
K_m values of different MCT isoforms for lactate and pyruvate

Investigator	MCT1 K_m		MCT2 K_m		MCT4 K_m	
	La (mM)	Pyr (mM)	La (mM)	Pyr (mM)	La (mM)	Pyr (mM)
Broer et al. [18]	3.5	0.74	0.74	0.080	—	—
Dimmer et al. [19]	6.4	—	—	—	33.7	18.9
	—	—	—	—	30.9	25.4
Garcia et al. [6]	8.3	3.1	8.1	0.080	—	—
Lin et al. [16]	6.0	2.5	6.5	0.025	—	—
Manning-Fox et al. [17]	4.4	—	—	—	28	153

pyruvate via MCT1 ($K_m \leqslant 3.1$ mM) (Table 1). Moreover, the presence of MCT1 and MCT4 at the mitochondria provides no definitive information as to whether lactate is preferentially taken up relative to pyruvate. However, the selective presence of MCT1 and 4 at the SS mitochondria might indicate that if indeed lactate is taken up directly into mitochondria, as has been proposed [36], it may only be into the SS mitochondria. The possible advantage is that lactate, when taken up from the circulation, is metabolized as soon as it enters the muscle cell, since SS mitochondria are in close proximity to the plasma membrane.

The absence of MCT1 and 4 from IMF mitochondria would appear to suggest that these mitochondria likely do not participate in the uptake of lactate. MCT2, a high-affinity pyruvate transporter (pyruvate K_m 25–80 μM) (Table 1), is associated with both IMF and SS mitochondria. The K_m for mitochondrial pyruvate oxidation in muscle ranges from 12 to 26 μM [41]. Thus, the MCT2 K_m for pyruvate is within the range of pyruvate concentrations observed in vivo. But, whether MCT2 functions as a pyruvate transporter in mammalian muscle tissue has not been established. There may well be a specific pyruvate transporter, as recently a mitochondrial pyruvate carrier was identified in *Saccharomyces cerevisiae* [42]. A BLAST search for mammalian homologues has identified two putative mitochondrial carriers which are ubiquitously expressed [42]. Thus, for this recently discovered putative transporter, as well as for MCT2, their physiological roles with respect to pyruvate transport need to be established in mammalian tissue.

In summary, we have shown that in highly purified preparations of SS and IMF mitochondria, the monocarboxylate transporters MCT1 and MCT4 are associated only with the SS mitochondria. In contrast, the monocarboxylate transporter MCT2 is associated with both SS and IMF mitochondria. The current controversy as to whether mitochondria take up lactate [36–38] will need to take account the very different distribution of MCTs between SS and IMF mitochondria. In view of the present finding, we are currently examining the possibility that SS and IMF mitochondria have different capacities with respect to lactate uptake and oxidation.

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