Interaction between myoglobin and mitochondria in rat skeletal muscle

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Whether respiration increases immediately after the start of muscle contraction remains a highly debated topic (25, 31). Some investigators have posited a delay in pulmonary O₂ uptake and extraction and have interpreted the data to indicate an impedance to oxygen (8, 10, 16). Others disagree with the data interpretation. NMR studies show clearly that Mb releases immediately its O₂ at the start of muscle contraction, consistent with a rapid rise in O₂ consumption (VO₂) (4). Recent NIRS experiments on buffer perfused hindlimb also show Mb desaturating immediately (4, 26).

Why Mb desaturates so quickly, given the generally accepted inertia ascribed to oxidative phosphorylation, remains puzzling (9). However, the sudden increase in VO₂ at the onset of contraction does not appear to depend on muscle ADP concentration and implicates a direct role for Mb-mediated O₂ delivery (4, 26). Indeed, previous experiments have suggested that a direct Mb-mediated oxygen delivery might contribute to mitochondrial respiration. When the oxygen-binding function of Mb was blocked by CO in the presence of sufficient free O₂ to support oxidative phosphorylation, mitochondrial respiration was still suppressed by ~70% (32).

The present study shows that Mb can interact directly with mitochondria and can co-localize with complexes in mitochondria. This mitochondrial Mb interaction could have a potentially significant role in respiration at the start of muscle contraction.

MATERIAL AND METHODS

Animals. Male Wistar rats weighing 282–375 g (n = 12) were used for the experiments. Animals were housed in an air-conditioned room and exposed to a 12-h light-dark photoperiod. A standard diet (Oriental Yeast, Tokyo, Japan) and water were provided ad libitum. All procedures performed in the present study were approved by the Ethics Committee on Animal Experimentation of Kanazawa University (protocol AP-10187).

Immunohistochemistry. For immunohistochemistry, rats were anesthetized and perfused with Krebs-Henseleit buffer (118 mM NaCl, 5.9 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 1.8 mM CaCl₂, 20 mM NaHCO₃, and 15 mM glucose) into the abdominal aorta for 15 min at body temperature. The buffer was then switched to 4% paraformaldehyde (PFA) in phosphate buffer (PB; 0.1 M, pH 7.2). After 10 min of perfusion of 4% PFA, the fixed muscles were dissected. The muscles were then dipped in 4% PFA in PB overnight at 4°C. After that, the buffer was changed to 10, 20, and 30% sucrose in 0.1 M PBS one after another and incubated overnight at 4°C. Samples were stored at ~80°C until histochemical analysis. Serial cross and longitudinal sections (6 μm thick) of the muscles were cut at ~20°C in a cryomicrotome (CMR1510; Leica, Wetzlar, Germany), and the sections were adhered to glass slides. The glass slides were soaked in the antigen activator reagent at 92°C for 10 min and washed three times with PBS. Samples were soaked in PBS-T (PBS with 1% Tween 20) at room temperature for 30 min and then in methanol for 1 min and

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subsequently washed for 10 min in PBS. The samples were then incubated in blocking solution (1% BSA in PBS-T) for 30 min. After washing, samples were incubated overnight at 4°C with mouse anti-myoglobin and rabbit anti-voltage-dependent anion channel (VDAC)-I (Abcam, Cambridge, UK). The secondary antibodies for Mb and VDAC-I were anti-rabbit antibodies conjugated to Alexa Fluor 488 (Invitrogen, Carlsbad, CA) and anti-mouse antibodies conjugated to Alexa Fluor 568 (Invitrogen), respectively. The secondary antibodies were incubated with the slides for 1 h at room temperature. After being washed, a cover glass with the nuclear counterstain reagent Dapi-Fluoromount-G (Southern Biotech, Birmingham, AL) was applied to the samples.

The images of the sections were imported with a resolution of 1,360 × 1,024 pixels, and the exposure time was adjusted depending on the negative control. The colocalization areas were calculated with JACoP [plugin software of Image J; version 1.33, NIH, Bethesda, MD (2)]. Single fibers were distinguished along the lines of the plasma membrane from the cross-section image. Thresholds were set visually, according to the intensity of the yellow aspect of each fiber. Fifty fibers were identified from each muscle, and the regions of colocalization (yellow regions) and total Mb (yellow plus green parts) were calculated.

**Electron microscopic imaging.** Isolated gastrocnemius muscle was fixed with 4% paraformaldehyde (PFA), 0.05% glutaraldehyde (GA) (distilled EM grade; Electron Microscopy Sciences, Hatfield, PA) in 0.1 M PB, pH 7.4, at 4°C for 90 min, and then the sample was rinsed three times with 0.1 M PB for 15 min each. Then belly muscle was cut out into forms of some block. The muscles were dehydrated through a series of graded ethanol (50%, 70%) at 4°C for 20 min each. The samples were infiltrated with a 50:50 mixture of ethanol and resin (LR white; London Resin, Berkshire, UK) three times for 20 min each. After this infiltration, they require three changes of 100% LR white at 4°C for 1 h each. The samples were transferred to a fresh 100% resin and were polymerized at 50°C overnight. The blocks were ultra-thin sectioned at 80 nm with a diamond knife using an ultramicrotome (Ultracut UCT; Leica), and sections were placed on nickel grids. The grids were then placed into the rabbit anti-Mb antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1% BSA at 1:1,000 dilution, PBS for 90 min at room temperature followed by rinsing with 1% BSA, and PBS three times for 1 min. And then they were floated on drops of the secondary antibody attached with 10 nm-colloidal gold particles (Anti Rabbit IgG: British BioCell International) for 1 h at room temperature. After rinsing with PBS, the grids were placed in 2% GA in 0.1 M PB. Afterward, the grids were dried and stained with 2% uranylacetate for 15 min, and then rinsed with distilled water followed by being secondary-stained with Lead stain solution (Sigma-Aldrich) at room temperature for 3 min. The grids were observed by a transmission electron microscope (JEM-1200EX; JEOL) at an acceleration voltage of 80 kV. Digital images (2,048 × 2,048 pixels) were taken with a charge-coupled device (CCD) camera (VELETA, Olympus Soft Imaging Solutions).

**Western blotting.** Animals were anesthetized (50 mg pentobarbital sodium/100 g body wt). For biochemical studies, four calf muscles [the soleus (Sol), plantaris (Pla), and gastrocnemius surface (GasS) and deep portion (GasD)] were quickly isolated; the tissues were washed in ice-cold saline, removed from the connective tissues and nerves, and then frozen in liquid nitrogen. Tissues were stored at −80°C. Muscles (~300 mg) were homogenized in lysis buffer A (in mM: 250 sucrose, 5 NaCl, 2 EGTA, 100 PMSF, 1 pepstatin A, 10 leupeptin, 20 HEPEs-Na, pH 7.4) with the use of a loose-fitting Dunce (Teflon-glass) homogenizer (Asahi Techno Glass, Tokyo, Japan), and then centrifuged at 600 g for 10 min at 4°C to remove nuclei and debris. One fraction of the supernatant was used as the total tissue fraction in the immunoblots, whereas the other was centrifuged at 10,000 g for 30 min at 4°C to precipitate mitochondrial fragments. The result of the supernatant was diluted with 0.75 vol of buffer B (1.167 M KCl and 58.3 mM NaPO4·12H2O, pH 7.4), and centrifuged at 230,000 g for 120 min at 4°C. The supernatant from this high-speed spin was used as the cytosolic fraction in the immunoblots. The mitochondrial pellet was washed initially by resuspension in buffer A and repelleted at 10,000 g for 30 min at 4°C. This pellet was then washed twice in buffer C (1 mM EDTA and 10 mM Tris, pH 7.4) and resuspended in buffer C with 1% NP-40. Then, it was centrifuged at 1,100 g for 20 min at room temperature, and the supernatant containing this mitochondrial fraction was used for immunoblots and adjusted to contain equal protein amounts in all samples.

Western blot analysis was performed as previously described (7). In brief, equal protein amounts (4.5 μg/lane) of samples were loaded onto 15% SDS-PAGE gels, and proteins were transferred to a polyvinylidene fluoride (PVDF) membrane. The membrane was incubated in blocking buffer and then with an anti-Mb antibody, an anti-VDAC-I (Abcam, Cambridge, UK), and an anti-β-actin (Abcam) at 4°C overnight. It was then reacted with the secondary antibody, and the signals were visualized by enhanced chemiluminescence (ECL) (GE Healthcare, Piscataway, NJ). The signal intensity was quantified with ImageJ imaging software.

**Immunoprecipitation.** The mitochondrial fraction was obtained by the same procedure as Western blotting. In this preparation, buffer D [20 mM Tris-Cl (pH 7.4), 50 mM NaCl, 250 mM sucrose, 50 mM NaF, 5 mM sodium pyrophosphate, 1 mM DTT, 4 mg/l leupeptin, 50 mg/l trypsin inhibitor, 0.1 mM benzamidine, and 0.5 mM PMSF] was used instead of buffers A and C.

The mitochondrial fraction of protein was divided into 1-ml PBS aliquots. Dynabeads (Invitrogen, Carlsbad, CA) were added to the samples, and then the samples were incubated at 4°C for 1 h. Dynabeads were collected with magnets, and normal mouse immunoglobulin G (nIgG; Santa Cruz Biotechnology) was added to the supernatants and incubated at 4°C overnight. Samples were centrifuged at 7,000 g at 4°C for 30 s, and aliquots of this supernatant were reacted with primary antibodies against Mb, cytochrome c oxidase-subunit IV (COX-IV), and mouse nIgG at 4°C overnight. Dynabeads were then added and incubated at 4°C for 2 h. The pellet was collected with magnets and washed with PBS. The final pellet from immunoprecipitation (IP) was analyzed by SDS-PAGE and immunoblotting. The antibody used for Mb immunoprecipitation and detection was the same as used for Western blotting. Mouse anti-COX-IV (Abcam) was used for immunoprecipitation and detection. Mouse anti-GAPDH (Abcam) and mouse anti-cytochrome c (Santa Cruz Biotechnology) were used for detection of each protein.

**Statistical analysis.** All data are expressed as means ± SD. Variables among groups were compared using one-way ANOVA, and a Tukey-Kramer post hoc test was conducted if the ANOVA indicated a significant difference. The level of significance was set at P < 0.05.

**RESULTS**

Mitochondrial myoglobin detected by immunohistochemistry and electron microscopic observations. Each muscle tissue was well stained with the Alexa fluor 488-conjugated anti-Mb (green) and Alexa fluor 568-conjugated anti-VDAC-I [red; Figs. 1 and 2 show the immunofluorescent images of Mb and VDAC-I in the cross sections of rat soleus, gastrocnemius deep, plantaris, and gastrocnemius surface (Sol, GasD, Pla, and GasS, respectively)]. Mb (green) was detected in almost all muscle cells (Fig. 1, A, D, G, and J). VDAC-I (red), a specific mitochondrial protein (Fig. 1, B, E, H, and K), was observed throughout the stained tissue in all muscle groups. Superposition of the Mb and for VDAC-I images clearly showed colocalization of Mb with mitochondria (Fig. 1, C, F, I, and L). Sol contains more Mb than GasD or Pla. In GasS, signals of Mb and VDAC-I (especially Mb) were quite weak (Fig. 1, J and D). VDAC-I (especially Mb) were quite weak (Fig. 1, J and D).
The colocalization of the Mb and VDAC-I signals were also quite weak in this muscle group (Fig. 1L).

The longitudinal-sections of GasD have both Mb-rich and Mb-poor fibers (Fig. 2A), and a colocalization of VDAC-I with Mb was observed in the Mb-rich fibers (Fig. 2C). However, GasS has few fibers that express large amounts of Mb (Fig. 2G), and therefore the merged VDAC-I and Mb immunofluorescent images show very few colocalized regions (Figs. 1L).

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**Fig. 1.** Histochemical images of myoglobin and mitochondria in muscle cross sections. Immunofluorescent imaging of myoglobin (Mb) and voltage-dependent anion channel (VDAC)-I in the cross sections from four rat calf muscles [soleus (Sol), gastrocnemius deep portion (GasD), plantaris (Pla), and gastrocnemius surface (GasS)]. Mb is green (A, D, G, J); VDAC-I (mitochondrial marker) is red (B, E, H, K); colocalization of Mb with mitochondria is yellow in the merged images (C, F, I, L). Scale bars are 50 μm. Signals of colocalization are widely observed in the oxidative muscles (Sol and GasD), whereas the glycolytic muscles show minimal colocalization (GasS).

**Fig. 2.** Histochemical images of Mb and mitochondria in muscle longitudinal sections. Immunofluorescent imaging of Mb and VDAC-I in the longitudinal sections of GasD and GasS muscles. Mb is green (A, D, G), VDAC-I (mitochondrial marker) is red (B, E, H), and colocalization of Mb with mitochondria is yellow in the merged images (C, F, I). Magnified images of GasD are the squared areas in A–C. Scale bars are 50 μm and 25 μm in the magnified images of GasD. Colocalization was widely observed in Mb-rich GasD fibers, whereas GasS fibers showed little colocalization.
and 2f). The magnified VDAC-I and Mb merged images of GasD show the stripe pattern indicative of colocalization of Mb with mitochondria (Fig. 2F). The colocalization area of Mb and VDAC-I in Sol, GasD, and Pla was significantly higher than in GasS. Furthermore, the colocalization area of Sol was higher than in Pla (Fig. 3).

Positive signals of Mb in electron microscopic imaging were detected in ultra-thin section of GasD tissue. Most of all Mb in images were present in cytosol, especially nearby I-bands (Fig. 4), which were in concordance with our immunofluorescent results (Fig. 2). Besides, it is noted that some Mb was close to or localized inside of mitochondria (Fig. 4). These results indicate that Mb was observed as points localized around or in the mitochondria.

Mitochondrial myoglobin detected by Western blotting. Western blot of β-actin assessed the cytosolic contamination in the mitochondrial fraction. The cytosolic protein marker β-actin was only found in the cytosolic fractions of Sol (Fig. 5A), GasD (Fig. 5B), Pla (Fig. 5C), and GasS (Fig. 5D). It showed no immunoreactivity to the corresponding mitochondrial fraction, which showed the mitochondrial fraction contained no significant cytosolic contamination (Fig. 5). Moreover, the mitochondrial outer membrane marker VDAC-I was detected only in the mitochondrial fraction. There was no immunoreactivity to VDAC-I in the cytosolic fractions of the muscles (Fig. 5).

Western blot revealed the presence of Mb in both the cytosolic and mitochondrial fractions. Comparison of the mitochondrial Mb levels of the four calf muscles as a fraction of VDAC-I revealed that slow-twitch oxidative muscles (Sol and GasD) have more mitochondrial Mb than fast-twitch glycolytic muscles (GasS; Fig. 6).

Interaction between Mb and COX-IV detected by immunoprecipitation. Immunoprecipitation experiments on the solubilized mitochondrial fractions determined the subcellular localization and the physical association between Mb and mitochondrial components. Protein complexes in the mitochondrial fractions were precipitated with antibodies against Mb and COX-IV, and then probed for COX-IV, Mb, or GAPDH [Sol (Fig. 7A), GasD (Fig. 7, B and E), Pla (Fig. 7C), and GasS (Fig. 7D)]. Mb co-precipitated with COX-IV from the mitochondrial fraction of each muscle tissue homogenate (Fig. 7, A–D). Also, COX-IV co-precipitated with Mb of GasD homogenate (Fig. 7E). The specificity of the complex composition was further demonstrated by the absence of COX-IV co-precipitation with IgG HC and IgG LC in Sol (Fig. 7A), GasD (Fig. 7B), Pla (Fig. 7C), and GasS (Fig. 7D), and the absence of GAPDH co-precipitations with Mb, nIgG, and COX-IV (Fig. 7F). GAPDH localizes mainly on cytosol, whereas a previous study shows it also localizes on mitochondria (27). We also detected GAPDH from mitochondrial fraction (Fig. 7F, lane of mito). The results of GAPDH detection can be used as a negative control of immunoprecipitation (cf. DISCUSSION). Cytochrome c was not detected in the immunoprecipitates of either Mb or COX-IV (Fig. 8), an indication that neither Mb nor COX-IV has interaction with cytochrome c.

**DISCUSSION**

Mb localization with mitochondria. In the present biochemistry view, Mb exists as a cytosolic protein. In contrast, the present study shows that Mb can localize in both the cytosol and the mitochondria. Detection of the colocalization of Mb with the mitochondrial marker protein VDAC-I (20) demonstrates the presence of Mb in the mitochondria. The mitochondrial fractions did not show any immunoreactivity to the cytosolic marker protein β-actin (5). These observations reveal that the mitochondrial fraction was well precipitated, and the cytosolic fraction, including cytosolic Mb, did not contaminate the mitochondrial fraction. Therefore, the Mb detected in the mitochondrial fraction by Western blotting did not include cytosolic Mb.

A fraction of Mb in the myocytes was localized with the mitochondria, as evidenced by immunohistochemical and Western blotting analysis (Figs. 1, 2, 5). Electron microscopic images corroborated the colocalization. The immunohistochemical analysis of longitudinal sections showed striped pattern in the areas of colocalization (Fig. 2F). Previous work revealed that both Mb and mitochondria are abundant in the I-band of the cardiac cells (15). Thus the striped pattern noted in the longitudinal section would indicate that Mb and mitochondria interact on the I-bands in the skeletal muscles. The sarcoplasmic reticulum (SR) localizes around the I-band in the skeletal muscle cells, and, therefore, these data suggest that the mitochondria could potentially associate physically and functionally with the SR (6) on the I-band region. The electron microscopic images clearly show that Mb localize in both the cytosol and the mitochondria (Fig. 4).

The human heart mitochondrial proteome study has detected Mb in the mitochondria by osmotic shock methods (28). The results suggested that Mb is present in the mitochondrial outer membrane. However, the investigators could not confidently determine whether the detected Mb originated only from the mitochondria without any cytosolic contamination. Our study shows clearly that Mb can interact with the mitochondria.

**Mechanism of Mb colocalization.** How Mb localizes to the mitochondria remains moot. Most mitochondrial proteins are encoded by nuclear genes and are, therefore, imported from the cytosol. The protein import machineries of mitochondria are the translocase of the outer membrane (TOM) complex and the translocase of the inner membrane (TIM) complex. TOM

![Fig. 3. Comparison of the colocalization areas. The areas of colocalization of Mb and mitochondria are significantly higher in Sol and GasD than in GasS. Moreover, the colocalization area of Sol is higher than that of Pla. The data are presented as means ± SD of 4 rats and represent 50 muscle fibers. *Significant difference vs. GasS (P < 0.05). †Significant difference vs. Pla (P < 0.05).](http://jap.physiology.org/doi/10.1152/japplphysiol.00789.2012/fig3)
Fig. 4. Electron microscopic imaging of Mb in mitochondria. Mb immunogold labeling in GasD tissue shows mitochondrial localization of Mb. A: the wide view around sarcolemma (bar = 2 μm). B and C: magnified views of subsarcolemmal mitochondria (bar = 50 nm). D: the wide view of myofibril (bar = 2 μm) and its magnified view of intermyofibril mitochondria (bar = 500 nm). Sections are 80 nm thin.
recognizes and imports the signal sequences of precursor proteins (24); however, almost all studies on mitochondrial protein import systems have been conducted with yeast or bacterial cell models. In the mammalian cell, especially the skeletal muscle cell, the presence and function of any mitochondrial protein import system still require experimental verification. In addition, since mitochondria are dynamic organelles that constantly fuse and divide (30), Mb may be imported in the fusion process.

Previous studies (28, 29) have indicated that Mb might localize to the mitochondrial outer membrane via heme uptake by apomyoglobin, which occurs at the mitochondrial outer membrane (29). Other research has proposed that Mb binds to the mitochondrial outer membrane via an electrical charge effect (22). However, our data at present cannot determine the mechanisms by which the mitochondria interact with Mb. Nevertheless, the results show an interaction between the mitochondria and Mb, which may play an important role in influencing mitochondrial respiration and intracellular oxygen transport.

**Mb interaction with COX-IV.** The electron microscopic images do show some Mb located inside the mitochondria and suggest a potential Mb interaction with complex IV. The mitochondria locating under sarcolemma, fairly large mitochondria have more Mb than those locating interny whole. Indeed, COX-IV was detected in the immunoprecipitates of Mb and COX-IV, whereas it was not detected in the immunoprecipitates of nfgG (Fig. 7, A–D). Comparatively, GAPDH, which localizes mainly to the cytosol but also to the mitochondria (Fig. 7E, lane of mito (27)), was not detected in the immunoprecipitates of Mb (Fig. 7E). Since GAPDH localizes either the cytosol or the mitochondria, the results of GAPDH detection would be used as a negative control of immunoprecipitation. Undetected of GAPDH in the immunoprecipitates of Mb indicated that COX-IV detected from the same sample is not the mitochondrial contaminants. Additionally, undetected of GAPDH in the immunoprecipitates of COX-IV revealed that Mb detected from the same sample is not the cytosolic contaminants. Therefore, GAPDH can be considered as the negative control for the immunoprecipitation experiment. These results suggest that COX-IV specifically interacts with the Mb in the electron transfer system (ETS).

**Mb-mediated O2 delivery.** A previous study has hypothesized a direct Mb-mediated oxygen delivery to accompany mitochondrial electron flow (32). The observation of Mb bound to COX-IV appears consistent with a Mb direct role in affecting the ETS. Furthermore, the presence of mitochondrial Mb would enable the mitochondria to respire immediately and to stimulate ATP synthesis at the onset of contractions. Mb could then support mitochondrial electron transfer and facilitate O2 consumption. Thus the direct interaction of Mb with COX-IV may have a basic role in intracellular oxygen dynamics.

**Mb colocalization in muscle groups.** Immunohistochemical analysis revealed that the area of colocalization in the slow-twitch oxidative muscles was greater than that in the fast-twitch glycolytic muscles (Fig. 3), which was supported by the results of Western blotting. The Mb content in the mitochondrial fraction was significantly higher in slow-twitch oxidative muscles (Sol, GasD) than in the fast-twitch glycolytic muscles (GasS, Pla; Fig. 6). Previous work has demonstrated that the slow-twitch oxidative fibers possess large amounts of Mb and mitochondria, whereas fast-twitch glycolytic muscles possess significantly lesser amounts (23).

Gueguen et al. (12) reported a higher respiration rate in permeabilized fibers dissected from type I fiber-dominant muscle than those from type II-dominant muscle. Isolated mitochondria from type I muscle fibers also showed faster respiration than those from type II fibers (13). Type I fibers have more Mb than type II fibers. Our experiments show a larger Mb-mitochondria colocalization area in the oxidative muscles relative to the glycolytic muscles, which suggests that the interaction between the mitochondria and Mb may contribute to the enhanced oxidative capacity and respiration rate of type I slow-twitch oxidative fibers over type II glycolytic fibers.
Moreover, endurance training increases both CS activity and Mb concentration, implying that Mb may play a role in enhancing both O₂ storage and Mb-mediated O₂ delivery. The Mb concentration is also increased by endurance training in rat skeletal muscle (1, 19), implying an increase in O₂ storage and O₂ flux mediated by Mb.

Implication for respiratory control. The localization of Mb to the mitochondria and to COX-IV could potentially facilitate the rise in VO₂ at the start of muscle contraction. Much debate surrounds the VO₂ on-kinetics at the start of muscle contraction (25, 31). Proponents for a VO₂ or metabolic impedance point to delays observed in the pulmonary O₂ uptake and extraction data. Opponents point to an errant analysis in interpreting noisy breath-to-breath O₂ uptake data and in using changes in blood flow and oxygen extraction to correlate precisely to any metabolic surge, especially in respiration, without considering the role of Mb.

Indeed, the NMR and NIRS have observed the intracellular Mb deoxygenating immediately at the onset of muscle contraction (4, 26). The rapid release of O₂ from Mb implies that respiration has suddenly stepped up with no intervening delays. NIRS experiments on buffer perfused hindlimbs have corroborated the NMR findings and have also detected an immediate desaturation of Mb, consistent with a rapid surge in respiration.

However, recent research also shows that the diffusivity of Mb appears much slower than that previously expected (11, 14, 17, 18). These observations raise questions about how Mb can respond quickly to the increase in the mitochondrial oxygen demand. The presence of a myoglobin-mitochondria complex would provide an alternative route of Mb-mediated O₂ delivery to the mitochondria. Mb colocalization with the mitochondria, specifically with COX-IV, presents a new paradigm to understand the regulation of intracellular oxygen transport and mitochondrial respiration.
In conclusion, the present study presents experimental evidence that Mb localizes to the mitochondria and interacts with COX-IV. In addition, the mitochondrial Mb content and the area of colocalization are significantly higher in the slow-twitch oxidative muscles compared with the fast-twitch glycolytic muscles. Taken together, these findings suggest that Mb can directly interact with the mitochondria. The specific interaction of Mb and COX-IV (complex IV) remains an open question. Mb can bind to COX-IV from the matrix or intermembrane side and may be mediated by other proteins. Further studies must then clarify the Mb-mitochondria and Mb-COX-IV interaction, especially with respect to the impact on respiration.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.Y. and K.M. conception and design of research; T.Y. and H.T. analyzed data; T.Y., H.T., T.J., and K.M. interpreted results of experiments; T.Y. and K.M. prepared figures; T.Y. and K.M. drafted manuscript; T.Y., T.H., Y.H., T.J., and K.M. edited and revised manuscript; T.Y. and K.M. approved final version of manuscript.

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