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Palmitate interaction with physiological states of myoglobin

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ABSTRACT

Background: Previous studies have shown that palmitate (PA) can bind specifically and non-specifically to Fe(III) MbCN. The present study has observed PA interaction with physiological states of Fe(II) Mb, and the observations support the hypothesis that Mb may have a potential role in facilitating intracellular fatty acid transport.
Methods: ^{1}H NMR spectra measurements of the Mb signal during PA titration show signal changes consistent with specific and non-specific binding.
Results: Palmitate (PA) interacts differently with physiological states of Mb. Deoxy Mb does not interact specifically or non-specifically with PA, while the carbonmonoxy myoglobin (MbCO) interaction with PA decreases the intensity of selective signals and produces a 0.15 ppm upfield shift of the PA methylene peak. The selective signal change upon PA titration provides a basis to determine an apparent PA binding constant, which serves to create a model comparing the competitive PA binding and facilitated fatty acid transport of Mb and fatty acid binding protein (FABP).
Conclusions: Given contrasting PA interaction of ligated vs. unligated Mb, the cellular fatty acid binding protein (FABP) and Mb concentration in the cell, the reported cellular diffusion coefficients, the PA dissociation constants from ligated Mb and FABP, a fatty acid flux model suggests that Mb can compete with FABP transporting cellular fatty acid.
General significance: Under oxygenated conditions and continuous energy demand, Mb dependent fatty acid transport could influence the cell's preference for carbohydrate or fatty acid as a fuel source and regulate fatty acid metabolism.

1. Introduction

Biochemistry textbooks have codified the function of Mb as an O_2 store or O_2 facilitated transporter. Yet, after half a century of research, questions still remain about Mb structure and function [29,72,73]. Certainly, experiments have demonstrated the importance of Mb in supplying O_2 in plants and in mammalian tissue. Indeed, in vivo NMR experiments have observed Mb releasing its O_2 store to sustain oxidative metabolism during apnea in seals and at the initiation of skeletal muscle contraction [11,52]. Yet the O_2 store of Mb can prolong respiration in a rat heart for only a few seconds during anoxia [10]. Upon CO inactivation of Mb function, the myocardium shows no compensating alteration in bioenergetics or contractile function response [9,20]. A mouse without Mb exhibits no striking impairments in its oxygen consumption rate, contractile function, bioenergetics, and metabolism [19,27]. Some researchers have now imputed a controversial NO bioscavenging and reductase function to Mb [18,35,37,57].

In the mouse model without Mb, myocardial metabolism switches its substrate preference from fatty acid to glucose. Fatty acid to glucose utilization ratio drops from 3/1 to 0.7/1 [17]. Given the conventional line of reasoning, the decline in oxidative fatty acid metabolism arises from a deficiency in Mb facilitated O_2 transport [17]. However, Mb appears to diffuse too slowly to compete effectively with free O_2 in normoxic heart [42,43,50,51]. Alternatively, the absence of Mb might indicate a diminished capacity to facilitate fatty acid transport. Indeed, early studies have suggested that Mb can bind fatty acid [25,26,28].

^{1}H NMR studies have recently interrogated the interaction of palmitate (PA) with Fe(III) MbCN and have found evidence for specific and non-specific binding [67]. Many studies use the paramagnetic Fe(III) MbCN as a structure–function model of the ligated physiological state of Mb, as represented by the diamagnetic Fe(II) MbO_2 or MbCO found in the cell, because the electron–nuclear interaction of the unpaired Fe(III) electron hyperfine shifts the heme and localized heme pocket amino acid residue signals into observable parts of the NMR spectral window [16]. The observation implies that PA also interacts with the physiological states of Mb.

Indeed, PA does interact specifically and non-specifically with MbCO, consistent with its interaction with MbCN. MbCO also increases PA solubility. However, PA does not appear to interact with deoxy Mb. The results suggest that ligated and unligated states of Mb exhibit distinct interactions with fatty acid and give rise to a modified view of intracellular

Abbreviations: PA, palmitate; FABP, fatty acid binding protein; Mb, myoglobin; MbCO, carbonmonoxy myoglobin

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fatty acid transport. Given the cellular Mb and fatty acid binding protein (FABP) diffusion coefficients, concentrations, and PA binding affinities, a fatty acid flux model indicates that ligated Mb can compete effectively with FABP to facilitate fatty acid transport [23,42,43]. Since deoxy Mb does not appear to interact with fatty acid, the differential interaction of ligated and unligated Mb suggests a convenient mechanism for fatty acid to load at the sarcolemma in the vicinity of a high PO_2 and unload the fatty acid and oxygen at the mitochondria in the environment of low PO_2. Mb can then follow the intracellular O_2 gradient from sarcolemma to the mitochondria to load and unload both fatty acid and oxygen without a need to invoke a complex explanation or mechanism as in the case with the high affinity FABP [71].

2. Materials and methods

2.1. Protein preparation

Myoglobin and albumin solutions were prepared from lyophilized horse heart protein and essentially fatty acid free bovine serum albumin (Sigma Chemical Inc., St. Louis, MO). Deoxy Mb was prepared from lyophilized metMb as described previously [36]. The preparation of MbCO solution followed a similar procedure. Dissolved oxygen from the metMb was removed and replaced with N_2. A 5 time excess of sodium dithionite was then injected to reduce the Fe(III) metMb to Fe(II) deoxy Mb and to remove any residual O_2. In the preparation of MbCO, the solution was equilibrated with CO. The resultant MbCO solution was loaded on a Sephadex G-25 column equilibrated with 30 mM Tris and 1 mM EDTA at pH 7.4. Elution with the same buffer removed the dithionite from the MbCO. Additional CO was then bubbled into the final MbCO solution. NMR tubes were sealed tightly with a rubber stopper.

2.2. Fatty acid-Mb preparation

Sodium palmitate (Sigma Chemical Inc., St. Louis, MO) was dissolved in 30 mM Tris buffer with 1 mM EDTA at pH 8.5 at 65 °C. Stock solutions of 10 mM and 100 mM were prepared and kept in a heating block (Thermolyne 17600 Dri-Bath) at 65 °C. An aliquot of 10 mM or 100 mM PA in Tris buffer at 65 °C was added to 600 μl of 0.2–0.8 mM myoglobin at 35 °C to yield a final solution with Mb:PA ratios from 1:0.1 to 1:4. All NMR experiments were then conducted at 35 °C. The time between PA addition and the start of the NMR measurement was approximately 5 min. The pH was measured at 35 °C using a calomel electrode (Orion 7110BN Micro Calomel pH, Thermo Electron Corporation).

2.3. NMR

Bruker Avance 500 and 600 MHz spectrometers measured the ^1H signals with a 5 mm probe. The ^1H 90° pulse, calibrated against the H_2O signal from a 0.15 M NaCl solution, was 9 μs. Watergate pulse sequence was used to obtain solvent suppression. Sodium-3-(trimethylsilyl) propionate 2,2,3,3 d4 (TSP) served as the internal chemical shift and concentration reference. All samples contained 5% D_2O to enable the deuterium lock during signal acquisition. All measurements were carried out at 35 °C. A typical spectrum required 1024 scans and used the following signal acquisition parameters: 12 kHz spectral width, 2560 data points, and 107 ms recycle time. Zero-filling the free induction decay (FID) and apodizing with an exponential window function improved the spectra. A spline fit then smoothed the baseline.

The ^{13}C signals collected at 151 MHz used the following acquisition parameters: 8.25 μs 90 pulse, a 33 kHz spectral window, and 16 K data point. A GARP pulse sequence decoupled the ^1H signals, and $^{13}C_2$ acetate provided an internal chemical shift reference at 24.2 ppm.

2.4. Intracellular fatty acid Transport

The intracellular fatty acid flux has contributions from free PA diffusion and protein mediated PA diffusion as expressed in the following equation, which approximates a zero free PA at the mitochondrial surface:

$$J = D_{PA}PA + D_X C_X \frac{PA}{K_D^X + PA} \quad (1)$$

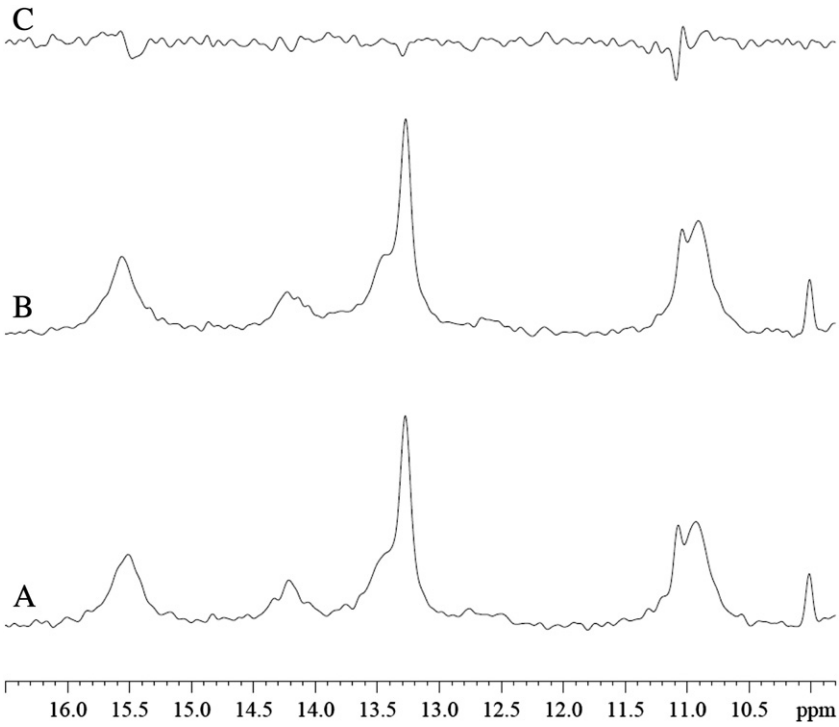

Fig. 1. ^1H NMR spectra of hyperfine shifted region of deoxy Mb Tris buffer at pH 7.4 at 35 °C: A. 0.8 mM deoxy Mb. B. 0.8 mM deoxy Mb and 3.2 mM PA. C. Difference of B − A.