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SIGNIFICANCE OF METMYOGLOBIN REDUCING ENZYME SYSTEM IN MYOCYTES

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OBJECTIVES

Myoglobin is an oxygen-binding heme protein, found in muscle, that is responsible for intracellular oxygen storage and oxygen transport from the plasma membrane to the mitochondria. However, myoglobin is easily converted to an oxidized form (metmyoglobin). Importantly metmyoglobin is unable to bind oxygen and, thus, represents a defective state for physiological function. Also, in meat, undesirable discoloration of the meat surface during storage is due to the accumulation of brown metmyoglobin.

The significance of metmyoglobin reducing enzyme systems in preventing metmyoglobin accumulation has been demonstrated in muscle and meat. We have reported that the NADH-cytochrome b_5 reductase system is responsible for metmyoglobin reduction in muscle[1-5]. Purified NADH-cytochrome b_5 reductase reduces metmyoglobin rapidly using the electron transfer mediators OM cytochrome *b* or cytochrome b_5 *in vitro*[1,3]. Also, we demonstrated the localization of this enzyme system components in skeletal muscle by immunohistochemical techniques[5]. The data from our studies, along with previous work showing myoglobin localization in muscle, suggested that NADH-cytochrome b_5 reductase reduces metmyoglobin by using OM cytochrome *b* at the mitochondrial surface and, in part, by using cytochrome b_5 at the sarcoplasmic reticulum to prevent metmyoglobin accumulation in muscle and meat.

In this study, we have used isolated myocytes from rat hearts to elucidate the metmyoglobin reducing enzyme system in living muscle. The change of myoglobin derivatives in myocytes was measured to see the effect of inhibitors on the metmyoglobin reduction.

METHODS

Isolation of myocytes from rat hearts was performed essentially by the method of Farmer *et al.*[5]. Briefly, the hearts were taken from decapitated Wister rats weighing 200 to 250 g. They were attached to the apparatus for perfusion (Figure 1), and perfused with Krebs-Henseleit buffer containing glucose and bovine serum albumin to remove blood. Collagenase and hyaluronidase were added and the perfusion was further conducted. After perfusion, hearts were cut into slices and dispersed in the same solution containing two enzymes. Resulting cell suspensions were sieved through nylon mesh, centrifuged and washed. If necessary, to oxidize the intracellular myoglobin, nitrite ($NaNO_2$) was added to the cell suspension before the sieving procedure. Absorption spectra of the cell suspension were measured from 450 nm to 650 nm using spectrophotometer with opaline glass.

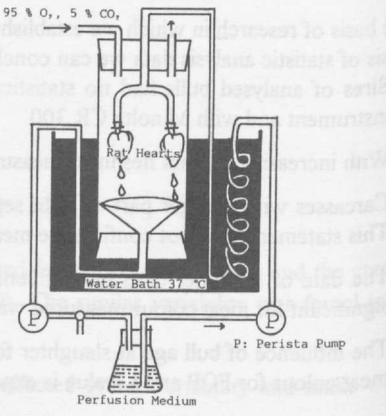

Figure 1. Apparatus for perfusion of rat hearts.

RESULTS

Although most of myoglobin derivative in living myocytes was oxymyoglobin, it was completely oxidized to metmyoglobin by the treatment with nitrite (Figure 2). Metmyoglobin in myocytes was reduced again by removal of the nitrite from cell suspensions (Figure 3). This reduction was thought to be an enzymatic reaction from the following experimental result.

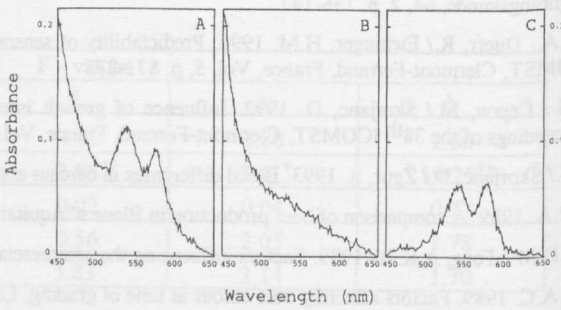

Figure 2. Spectra of rat heart myocytes.
A: control; B: treated with $NaNO_2$; C: difference spectrum (A-B).

The addition of 2-deoxy-D-glucose, the inhibitor of the glycolytic pathway, to the cell suspension inhibited the reduction of metmyoglobin in myocytes (Figure 4C, D). On the other hand, malonic acid, the inhibitor of the citric acid cycle, did not inhibit this reaction (Figure 4E).

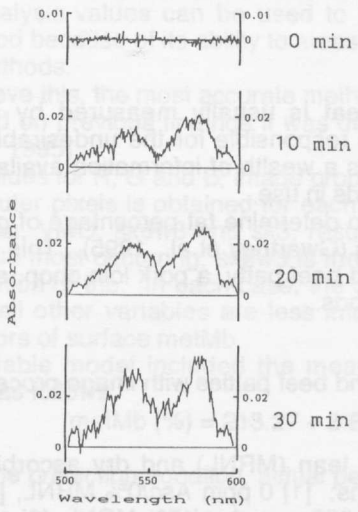

Figure 3. Reduction of metmyoglobin in rat heart myocytes during incubation.

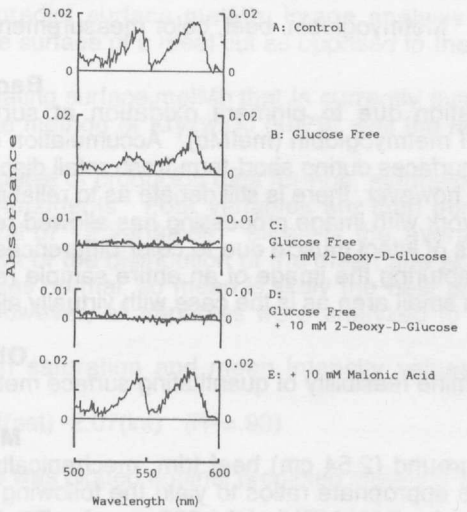

Figure 4. Effect of inhibitors on reduction of metmyoglobin in rat heart myocytes.

CONCLUSIONS

The glycolytic pathway is involved in the metmyoglobin reduction *in vivo*. Presumably, the glycolysis provides NADH for the enzymatic metmyoglobin reduction system. From the results of this study and our previous observations, we proposed the metmyoglobin reducing enzyme system at the mitochondrial surface shown in Figure 5.

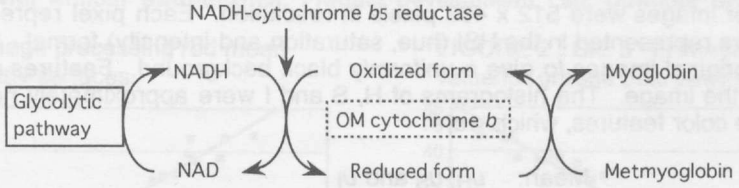

Figure 5. Proposed pathway of metmyoglobin reduction in myocytes.

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