Oxidation of DL-α-Glycerophosphate and β-Hydroxybutyrate in Red and White Skeletal Muscle

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It is now accepted that the enzymes and cofactors involved in glycolysis are primarily located in the soluble extramitochondrial portion of the cytoplasm in the cell. In the glycolytic chain a single oxidative reaction occurs, the oxidation of glyceraldehyde phosphate to 1,3-diphosphoglyceric acid. Nicotinamide-adenine-dinucleotide (NAD) is the obligatory acceptor of electrons in this reaction. Since NAD is present in only catalytic amounts in mammalian cells, the presence of an efficient mechanism for the continuous oxidation of the reduced nicotinamide-adenine-dinucleotide ($NADH_2$) generated is essential for the maintenance of glycolysis. The reduction of pyruvate to lactic acid is one such mechanism, but the normal cell apparently has other systems available which effectively compete with the lactic dehydrogenase reaction.

Three main possibilities have been proposed to explain how the cell oxidizes the $NADH_2$ which is formed in the cytoplasm.

(1) Direct oxidation of $NADH_2$ by the mitochondria.
(2) Through the operation of the α-glycerophosphate cycle.
(3) Through the operation of the β-hydroxybutyrate cycle.

As regards the first alternative, it has, as a result of Lehninger's studies on liver mitochondria,[4,5] become generally accepted that morphologically and metabolically intact mitochondria are not permeable to externally added $NADH_2$. However, Blanchaer *et al.*[1] have put forward evidence that in pigeon-heart mitochondria the oxidation of cytoplasmic $NADH_2$ is a normal property of these mitochondria and that the control of this process by changes in the intracellular levels of ATP and P_i may influence myocardial carbohydrate utilization.

In the case of skeletal muscle the question about direct oxidation of $NADH_2$ is still more intriguing, since skeletal muscle is not an homogeneous tissue but contains three distinct types of fibers; red, white, and intermediate types can be distinguished and differ both morphologically, enzymatically, and metabolically from each other.[6-9] Blanchaer [10] has determined the oxygen consumption of mitochondria isolated from red and white muscle of guinea pigs with pyruvate-malate, DL-α-glycerophosphate, succinate, DL-lactate, and $NADH_2$ as substrates. Although they showed similar rates of oxygen uptake with pyruvate-malate, the mitochondria from the two muscle types demonstrated a different pattern of response to the other substrates. White-muscle mitochondria had a higher rate of oxygen consumption with α-glycerophosphate than with lactate, succinate, and $NADH_2$. In contrast, the mitochondria from red muscle were less active with α-glycerophosphate than with the other substrates. The author suggests that in the white muscle the α-glycerophosphate cycle is operative, while the properties of red-muscle mitochondria suggest that the direct oxidation of $NADH_2$ may be more important in this tissue than the α-glycerophosphate cycle.

We prepared red- and white-muscle mitochondria in the following way. As a source of red muscle in guinea pigs the two masseters were taken and as a source of white muscle we selected the superficial layers of the triceps quadriceps, adductor femoris iliacus, and psoas. The muscles were cut into small pieces and homogenized for 30 sec in 100 ml of a medium containing 0.21 M mannitol, 0.07 M sucrose, 0.1 mM EDTA and 0.01 M tris-phosphate buffer [11] by using a blender. This muscle extract was rehomogenized for 30 sec in a Potter-Elvehjem homogenizer with a teflon pestle and filtered through a nylon cloth. The mitochondria were obtained by differential centrifugation, as described before.[1] Red- and white-muscle mitochondria were incubated in Warburg flasks with pyruvate + malate (17 mM + 1 mM), DL-α-glycerophosphate (17 mM) and β-hydroxybutyrate (17 mM) as substrates. Mitochondria from each type of muscle were run in duplicate, one flask containing ADP and hexokinase and the other not. The results are given in Table 1. With pyruvate + malate as substrate, the white-muscle mitochondria show a 24 % lower oxygen uptake than the red-muscle mitochondria. With α-glycerophosphate as a substrate the white-muscle mitochondria show an almost double oxygen consumption (42 % higher), as compared with the red-muscle mito-

Table 1. Oxygen uptake by mitochondria of red and white skeletal muscle given as μmoles per 10 mg of mitochondrial protein per hour. Rm = red muscle mitochondria. Wm = white muscle mitochondria. The results are given as mean values ± standard errors and have been compared by means of "students" t-test. Number of experiments within brackets.

Substrate	Rm	Wm	P
17 mM Pyruvate + 1 mM Malate	29.2±3.2 (13)	22.2±3.1 (13)	0.2−0.1
17 mM DL-α-Glycero-phosphate	12.4±1.5 (12)	21.4±3.2 (12)	0.02
17 mM β-Hydroxy-butyrate	14.1±2.5 (5)	0.0 (5)	<0.001

chondria. For the red-muscle mitochondria the oxygen consumption with β-hydroxybutyrate as a substrate is about half of that with pyruvate + malate, while in the white-muscle mitochondria β-hydroxybutyrate is not oxidized at all.

These findings may indicate that, while the α-glycerophosphate cycle is mainly operative in white skeletal muscle the β-hydroxybutyrate cycle is operative in rat skeletal muscle. Wirsen's finding,[12] that lipid deposition in muscle cells is correlated to the myoglobin content, is in favour of this hypothesis; red fibers store significantly more lipid than do intermediate and white fibers and this difference reflects a selective uptake of fatty acids from plasma. However, Lehninger *et al.*[13] have shown that the β-hydroxybutyrate dehydrogenase of the intact liver mitochondria does not react with acetoacetate and $NADH_2$, and no evidence for a soluble dehydrogenase was obtained. Devlin agrees that there is no soluble β-hydroxybutyrate dehydrogenase which functions in this cycle, but his recent work [14] suggests the presence of a β-hydroxybutyrate dehydrogenase on the surface of the mitochondria, which appears to have properties different from the well-known β-hydroxybutyrate dehydrogenase. It is quite apparent that the β-hydroxybutyrate-acetoacetate cycle requires further study before it can be established that it is at work in red skeletal muscle.

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Influence of *cis-trans* Isomerism on the Kinetics of Vinyl Ether Hydrolysis

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The kinetics and mechanisms of protolytic cleavage of vinyl ethers were discussed in a recent paper.[1] The open-chain vinyl ethers studied were compounds which have no isomeric *cis* and *trans* forms, and therefore no conclusions could be drawn about the possible structural effect of this isomerism on the kinetics of the reactions. The present paper describes the results of experiments which were conducted in