ON THE ACTIVITIES OF SOME ENZYMES CONCERNED WITH GLYCOLYSIS AND GLYCOGENOLYSIS IN EXTRACTS OF RABBIT SKELETAL MUSCLES*

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We find that red muscles of the rabbit contain up to 6 times as much extractable hexokinase activity as do white muscles. Hexokinase activity is inversely related to those of phosphohexoseisomerase, aldolase and total glycogen phosphorylase, which vary over a 10-15 fold range. The results are consistent with known physiological properties of the different muscle types.

MATERIALS AND METHODS

Male white rabbits were killed while anesthetized with pentobarbital. Muscles were excised, chilled, freed of extraneous tissue and blood and minced finely. Using a Servall Omnimixer at setting #7, they were homogenized for 30 sec. in 20 vols. of ice-cold phosphate-glucose buffer, pH 7.1 (Grossbard and Schimke, 1966). Except for hexokinase, all enzyme assays were performed on appropriate dilutions of whole homogenates. Between 40-60% of the hexokinase activity in homogenates, but not that of the other enzymes studied, sediments after centrifugation at 30,000g for 20 min. Bound activity was released by treating homogenates with 1% (v/v) Triton X-100 in ice for 60 min. After centrifugation at 40,000g for 20 min., total hexokinase activity was measured in the supernatants. In some experiments the detergent treatment was omitted and hexokinase was assayed in supernatants after centrifuging at 30,000g for 20 min. This was a measure of soluble hexokinase.

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Hexokinase, myoglobin, aldolase and phosphohexoseisomerase were assayed respectively by the methods of Grossbard and Schimke (1966), Reynarfarje (1963), Rajkumar et al. (1966) and Slein (1955). Total phosphorylase was measured by adapting the procedure of Bueding et al. (1962) to continuous recording of the glucose 1-phosphate formed from glycogen. Reactions were conducted in 1 ml of medium containing potassium phosphate buffer (50 mM, pH 7.5), EDTA (1 mM), NaF (10 mM), bovine serum albumin (0.3%), AMP (1 mM), $MgCl_2$ (2.25 mM), NADP (0.4 mM), rabbit liver glycogen (0.5%) and fructose 1,6-diphosphate (0.2 mg/ml) as a source of glucose 1,6-diphosphate. Phosphoglucomutase (1.0 I.U.) and glucose 6-phosphate dehydrogenase (0.6 I.U.) were added to each cuvette.

Maximal linear rates were always measured. All enzyme activities were stable over the 12 hr period required to complete the determinations and required the presence of muscle extract, specific substrates and ancillary enzymes. Rates were proportional to added extract and were additive when extracts showing extremes of activity were mixed in equal amounts. Reactions were conducted at optimal concentrations of the appropriate ancillary enzymes and substrates.

RESULTS

Table 1 presents estimates of total hexokinase, phosphorylase, aldolase and phosphohexoseisomerase activities in selected rabbit muscles. The corresponding myoglobin contents are also given. Semitendinosus, soleus and diaphragm can be classed visually as red muscles, while gastrocnemius, semimembranosus and adductor magnus range from pink to white, in that order. Hexokinase activity varies markedly among extracts of the muscles studied and correlates well with myoglobin content. Semitendinosus (red) and adductor magnus (white) invariably display the extremes of hexokinase activity which on average differ by 6-fold. Phosphorylase, aldolase and phosphohexoseisomerase activities show the reverse trend. Lowest

MUSCLE	MYOGLOBIN	HEXOKINASE	PHOSPHORYLASE	ALDOLASE	PHI	PHOSPHORYLASE/ HEXOKINASE
	(mg/gm muscle)	(units)	(units x 10^{-3})	(units x 10^{-3})	(units x 10^{-3})	
ST	2.9 ± 0.4	158 ± 33	0.4 ± 0.2	0.9 ± 0.1	5.6 ± 1.2	2.7 ± 1.6
S	2.5 ± 0.6	129 ± 22	0.8 ± 0.4	1.5 ± 0.3	7.2 ± 3.3	5.9 ± 2.6
D	1.6 ± 0.4	122 ± 22	3.1 ± 0.5	4.6 ± 1.3	22.8 ± 4.3	25.6 ± 7.1
MG	0.9 ± 0.2	58 ± 13	5.3 ± 0.7	7.8 ± 1.6	39.1 ± 6.2	90.5 ± 26.5
SM	0.7 ± 0.2	47 ± 15	5.6 ± 0.5	8.7 ± 1.9	39.5 ± 4.2	120.0 ± 10.5
AM	< 0.5	27 ± 4	6.6 ± 0.9	10.7 ± 2.3	49.8 ± 7.1	246.0 ± 18.8

Table 1. Extractable enzyme activities and myoglobin content of rabbit muscles. Figures are the means and standard errors of determinations on 5-8 animals. Enzyme units are μmoles substrate utilized at 37°/hr/gm muscle wet weight and myoglobin content is also related to muscle wet weight. Abbreviations: Semitendinosus, ST; Soleus, S; Diaphragm, D; Medial gastrocnemius, MG; Semimembranosus, SM; Adductor magnus, AM; Phosphohexoseisomerase, PHI. The rabbits ranged in weight from 2.5-4.3 kg.

activities are found invariably in extracts of semitendinosus and are progressively greater in muscles arranged by decreasing myoglobin content. Maximum activities are observed in adductor magnus and are 10-15 times greater than in semitendinosus. The results show similar trends when calculated as activity/gm wet weight tissue or activity/mg extractable protein.

In rabbit muscle, but not in rat, up to 60% of the hexokinase activity in homogenates of skeletal muscle sediments at 30,000g. Rat muscle contains only type II isoenzyme, whereas rabbit muscle contains types I and II (Grossbard et al., 1966). We find that red and white muscles of the rabbit do not differ systematically in their proportions of soluble and particulate hexokinase, or in the proportions of types I and II isoenzymes in the soluble fractions as indicated by starch gel electrophoresis.

DISCUSSION

Muscles differ markedly in enzyme composition in a manner which can be related to their function, as illustrated by the differences between so-called red and white forms of skeletal muscle. Thus, of the rabbit muscles we have studied, red forms such as semitendinosus contract in a relatively slow, sustained manner, whereas white muscles contract more rapidly, but intermittently (see Needham, 1926). Red muscles are rich in cytochromes, myoglobin and Kreb's cycle enzymes (Lawrie, 1953), consistent with a more active aerobic metabolism. Compared with white muscles they also have a greater capacity to oxidize fatty acids and fat (George and Talesara, 1961; Dubowitz and Pearse, 1960). White muscles appear to maintain reserves of glycolytic and glycogenolytic activity against intermittent periods of rapid contraction. They contain more glycogen (see Domonkos, 1961; Ogata, 1960) and more extractable activity of enzymes in the pathway from hexose phosphate to lactate, as shown in the rabbit by Pette and Bücher (1963) and in rat by Dawson and Romanul (1964). The latter workers also found that extracts of white muscle contain more phosphorylase activity, a finding

we have confirmed in rabbit. None of these workers studied hexokinase, however. Under the assay conditions we have used, hexokinase is invariably the least active enzyme studied; its activity is greater in redder muscles while phosphorylase shows the opposite trend. Ratios of phosphorylase to hexokinase activity vary from 3 in semitendinosus (red) to 240 in adductor magnus (white). We have also found that activities of these enzymes show a similar inverse relationship where the proportion of morphologically distinguishable red and white fibers in a given muscle varies as a function of animal size and species.

If one assumes some direct relationship between enzyme activity in extracts and activity in vivo, then the rate of glucose uptake and phosphorylation should be greater in red muscles than in white, since the former contain more hexokinase. In support of this are the findings that blood flow is faster through red muscle in the resting cat (Reis et al., 1967), capillaries surrounding fibers of the red type in rat muscle are more abundant (Romanul, 1964) and up to 4 times more 3-O-methylglucose is taken up in vivo by predominantly red muscles of the rat than by white (Dr. A. Goldberg, personal communication). Furthermore, since phosphorylase activity is low in extracts of red muscle, we suggest that whereas glycogen breakdown via glycolysis is the major energy pathway during contraction of white muscles, red muscles expend energy at a rate which is met significantly by metabolism of blood glucose via glycolysis and the Kreb's cycle without prior conversion of glucose to glycogen. This hypothesis is not immediately reconcilable with findings that red muscle can incorporate more glucose into glycogen in vitro (Bar and Blanchaer, 1965; Bocek et al., 1966).

Clearly there is need to relate differences in extractable enzyme activity to metabolic events in red and white forms of skeletal muscle in vivo, both at rest and in contraction. The differences between red and white muscles would appear to be potentially useful for studying the interplay of total enzyme content and levels of metabolic intermediates in regulating energy metabolism.

REFERENCES

Bär, U. and Blanchaer, M. C. (1965). Am. J. Physiol. 209, 905.

Bocek, R. M., Basinger, G. M. and Beatty, C. M. (1966). Am. J. Physiol. 210, 1108.

Bueding, E., Bülbring, E., Kuriyama, H. and Gercken, H. (1962). Nature 196, 944.

Dawson, D. M. and Romanul, F. C. A. (1964). Arch. Neurol. 11, 369.

Domonkos, J. (1961). Arch. Biochem. Biophys. 95, 138.

Dubowitz, V. and Pearse, A. G. E. (1960). Histochemie 2, 105.

George, J. C. and Talesara, C. L. (1961). J. Cell. Comp. Physiol. 58, 253.

Grossbard, L. and Schimke, R. T. (1966). J. Biol. Chem. 241, 3546.

Grossbard, L., Weksler, M. and Schimke, R. T. (1966). Biochem. Biophys. Res. Commun. 24, 32.

Lawrie, R. A. (1953). Biochem. J. 55, 298.

Needham, D. M. (1926). Physiol. Rev. 6, 1.

Ogata, T. (1960). J. Biochem. (Tokyo) 47, 726.

Pette, D. and Bücher, T. (1963). Hoppe-S. Z. Physiol. Chem. 331, 180.

Rajkumar, T. V., Woodfin, B. M. and Rutter, W. J. (1966). In Methods of Enzymology, p. 491. Colowick, S. P. and Kaplan, N. O. (Eds). Vol. IX.

Reis, D. J., Wooten, G. F. and Hollenberg, M. (1967). Am. J. Physiol. 213, 592.

Reynarfarje, B. (1963). J. Lab. Clin. Med. 61, 138.

Romanul, F. C. A. (1964). Arch. Neurol. 12, 497.

Slein, M. W. (1955). In Methods in Enzymology, p. 299. Colowick, S. P. and Kaplan, N. O. (Eds). Vol. I.