Preliminary Notes

PN 10063

The sarcotubular system and its relation to the control of Glycolysis

The stimulation of lactate formation which follows the addition of particulate fractions to soluble glycolytic systems has been hitherto referred to the supply of phosphate acceptor through mitochondrial and microsomal ATPases[1], or to the association of aldolase (EC 4.1.2.7) and or hexokinase (EC 2.7.1.1) with the mitochondria[2-4].

The present communication deals with the rate-limiting steps in the conversion of glucose 6-phosphate to lactate in frog-muscle extracts, and their relation to the fraction which derives from fragmentation of the sarcotubular system[5]. The sarcotubular fraction was isolated from 10% homogenates of frog *(Rana temporaria)* thigh muscles in 0.88 M sucrose, by centrifuging with forces between 40 000 × *g* for 30 min and 105 000 × *g* for 60 min. Lactate was determined by the BARKER AND SUMMERSON procedure[6]. Phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) was assayed spectrophotometrically[7].

Whole frog-muscle homogenates formed lactate at essentially the same rate, when either glucose 6-phosphate or fructose 1,6-diphosphate was the substrate used. However, the 105 000 × *g* supernatant fraction glycolyzed glucose 6-phosphate or fructose 6-phosphate at a markedly lower rate than it did the hexose diphosphate.

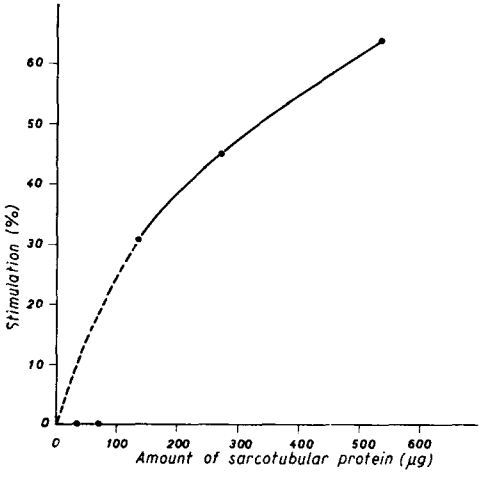

Fig. 1. Stimulation of glycolysis by sarcotubules. The reaction medium contained in a final volume of 3 ml: 50 mM Tris buffer (pH 7.4); 10 mM KCl; 6 mM $MgCl_2$; 10 mM nicotinamide; 0.53 mM NAD^+; 10 mM disodium arsenate; 1 mM ATP; 5 mM glucose 6-phosphate; 3.7 mg of high-speed (105 000 × *g* for 60 min) supernatant fraction, and variable amounts of sarcotubular protein, as indicated. The incubation was carried out aerobically at 30° for 30 min. The reaction was stopped by addition of 10% (final concentration) trichloroacetic acid and lactic acid was determined on the deproteinized trichloroacetic acid extracts by the BARKER AND SUMMERSON procedure[6]. Protein was determined by the biuret reaction.

In a system where the availability of phosphate acceptor or inorganic phosphate was made non-limiting by uncoupling the phosphorylation linked to the oxidation of 3-D-phosphoglyceraldehyde with arsenate, the addition of sarcotubules to the soluble fraction stimulated considerably the formation of lactate from the hexose monophosphates, but not from fructose 1,6-diphosphate. Fig. 1 shows the effect of the addition of sarcotubules to the soluble fraction of frog-muscle homogenate. The sarcotubules, which had very low glycolytic activity alone, stimulated the formation of lactate from glucose 6-phosphate at concentrations as low as 150 μg protein. The stimulation, here defined as the excess of activity of the mixture over the summed activities of the separate components, increased progressively with the concentration of sarcotubular protein, under conditions where the rate of glycolysis was linear with time.

These findings suggested that phosphofructokinase was the limiting step in the conversion of glucose 6-phosphate to lactate, and that the stimulating effect of the sarcotubules should be related to the association of phosphofructokinase with this fraction. As shown in Table I, the sarcotubular fraction was associated

TABLE I

DISTRIBUTION OF PHOSPHOFRUCTOKINASE ACTIVITY IN FRACTIONS OF FROG-MUSCLE HOMOGENATE

Experimental conditions: phosphofructokinase activity of fractions (250 μg of sarcotubular protein or 600 μg of supernatant fractions) was assayed spectrophotometrically[7] in a system which contained in a final volume of 3 ml: 33 mM Tris buffer (pH 8.0); 0.1 mM NADH; $MgCl_2$ as indicated; 2 mM ATP; 1 mM Fructose 6-phosphate; 5 μg of mixed crystals of α-glycerophosphate dehydrogenase (EC 1.1.1.8) and triosephosphate isomerase E.C. 5.3.1.1.); 0.45 unit of aldolase. Absorbancy values were measured at 340 mμ every minute for 5 min against a water blank. The average decrease in absorbancy per min occurring in control cuvettes containing all components except fructose 6-phosphate was substracted from that occurring in the experimental cuvettes.

Fraction	*μmoles fructose diphosphate formed per min*					
	per mg protein			*per g muscle*		
$MgCl_2$ concn. (mM)	*1*	*3*	*6*	*1*	*3*	*6*
Mitochondrial supernatant (40 000 × *g* for 30 min)	0.0141	0.0168	0.0168	0.91	1.08	1.08
Final supernatant (105 000 × *g* for 60 min)	0.0108	0.0132	0.0124	0.67	0.82	0.77
Sarcotubules	0.0672	0.0824	0.0824	0.17	0.21	0.21

with about 20% of the activity of the unfractionated mitochondrial supernatant, and the specific activity of sarcotubular phosphofructokinase was about six times as high as that of the soluble enzyme. The optimal conditions for phosphofructokinase activity in all three fractions corresponded to a ratio Mg^{2+}: ATP higher than 1.

It would then appear that the above reported stimulating effect of glycolysis by the sarcotubular fraction is accounted for, largely, by the association of phosphofructokinase to the sarcotubules. The phosphofructokinase activity associated with the sarcotubular fraction is only partially solubilized by physical means. Sucrose-

washed sarcotubules retained 60–80% of the original activity; treatment with freezing and thawing yielded comparable results.

The association of phosphofructokinase with the sarcotubules appears of interest because relaxation-factor preparations, which other studies have shown to derive from fragmentation of the sarcotubular system[5], are able to form adenosine 3′,5′-phosphate[8], a strong activator of phosphofructokinase[9]. Since phosphofructokinase is the rate-limiting enzyme of glycolysis[10], and phosphofructokinase activity is increased during muscular activity[11], it is conceivable that the sarcotubular system may be the basis for the integration of metabolic and physiological control mechanisms.

A detailed account of this work will be presented in a forthcoming paper[12].

This investigation has been supported by a grant from the Muscular Dystrophy Associations of America, Inc.

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Received June 10th, 1963

Biochim. Biophys. Acta, 77 (1963) 337–339

PN 10060

The flavin components of the NADH dehydrogenase of the respiratory chain

The relationship between the flavin prosthetic group of the NADH dehydrogenase of the respiratory chain[1–3] and that of the mitochondrial NADH:cytochrome *c* oxidoreductase (EC 1.6.2.1)[4–8] is obscure. First, the properties of the FAD in lipoflavoprotein[1] and in NADH dehydrogenase[2,3] are different. The FAD of lipoflavoprotein[1] is stable under the usual conditions of deproteinization preparatory to the flavin assay, while acid or thermal denaturation of NADH dehydrogenase yields FMN, AMP and riboflavin as well as FAD. SINGER *et al.*[2,3] suggest that the prosthetic group is FAD attached by multiple bonds to the protein and that unfolding of the