MUSCLE FRUCTOSE-1,6-DIPHOSPHATASE*

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Fructose-1,6-diphosphatase (FDPase), a key enzyme in neoglycogenesis, is generally considered as essentially restricted to liver and kidney although a small activity had been early detected in skeletal muscle (Gomori, 1943; Hers and Vanden Berghe, 1957). Interest in the regulation of the activity of FDPase has been recently aroused by studies with liver and kidney preparations (Taketa and Pogell, 1963; Mendicino and Vasarhely, 1963; Krebs, 1964 a; Newsholme, 1963).

This communication reports the occurrence of significant amounts of FDPase in skeletal muscle of frog and rabbit. The frog muscle enzyme has a very great affinity for fructose-1,6-P (FDP) as substrate and for AMP as allosteric inhibitor. The possible physiological significance of these kinetic properties and of the occurrence of this enzyme in muscle is discussed.

EXPERIMENTAL

Frogs kept for several weeks at 2-4º were killed by decapitation and spinal transection. The muscle of the legs was homogenized in a Kontes Duall grinder with 5 vols. of 50 mM imidazole - 5 mM ethanethiol, pH 7.5. Extracts were obtain-

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ed by centrifugation of the homogenate at 20,000 x g for 15 min. Rabbit muscle was homogenized in a Waring Blendor with 3 vols. of 50 mM Tris, pH 7.5, and centrifuged as above. In the conditions indicated in Fig. 2 the FDPase activities at about 20º were 0.7 to 1.0 and 0.5 to 0.7 μmole of fructose-6-P formed per minute per gram of frog and rabbit muscle, respectively. A preparation of FDPase which had on 1 mM glucose-6-P or α-glycero-P less than 3% of the activity on FDP, as measured by inorganic phosphate formation, was obtained by fractionation of frog muscle extract with ammonium sulfate between 60 and 80% saturation.

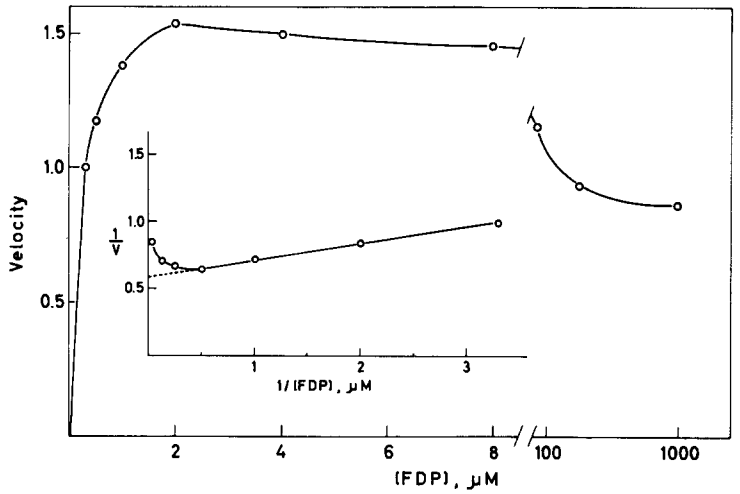

Figure 1.- Effect of FDP concentration on the activity of frog muscle FDPase. The reaction mixture contained, in a final volume of 1.5 ml, the following reagents: 50 mM histidine, pH 7; 10 mM $MgCl_2$; 1 mM ethanethiol; 0.16 mM NADP; 0.5 international unit of glucose phosphate isomerase and glucose-6-P dehydrogenase (Boehringer), respectively, and FDP as indicated; the reaction was started by the addition of 0.17 μg of a preparation of frog muscle FDPase (obtained by ethanol fractionation of the extract between 25 and 40% and elution from calcium phosphate gel with 40 mM phosphate, pH 7.5; this preparation of FDPase had only about 50% of aldolase contamination). NADPH formation was followed at about 20º with a Beckman Ratio Fluorometer. Results are not corrected for decrease in substrate concentration during the estimation of initial rates, which was about 50% in the case of the lowest initial concentration. Velocity is expressed in millimoles of NADPH formed per 20 minutes.

Frog muscle FDPase has an extremely great affinity for FDP, as shown in Fig. 1. A Km value of approximately 0.1 μM has been obtained; a precise evaluation would require a more sensitive method. There is a moderate inhibition by excess of FDP. The enzyme is very strongly inhibited by AMP, as shown in Fig. 2; at about 2.5 μM AMP there was a 50% inhibition. The inhibitory effect of AMP seems to be very specific: 0.1 mM cyclic-3′,5′-AMP, GMP, CMP, and UMP were not inhibitory. The activity in the presence of 5 μM AMP of a muscle FDPase preparation free of myokinase[*] was not affected by ATP (2 mM), citrate (3 mM), or lactate (6 mM).

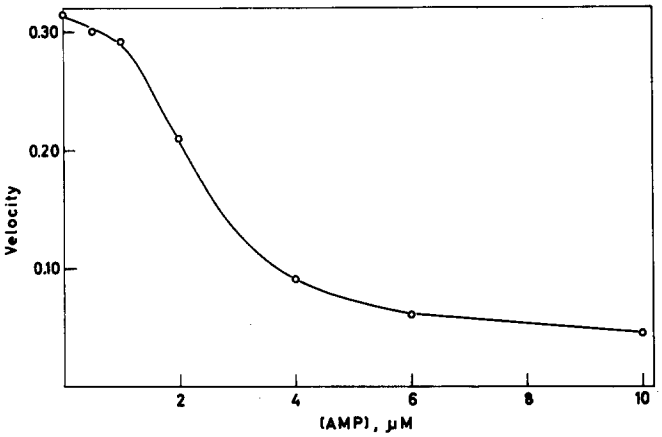

Figure 2.- Inhibition of frog muscle FDPase by AMP. The reaction mixture contained, in a final volume of 2 ml, the following reagents: 50 mM imidazole, pH 7; 10 mM $MgCl_2$; 1 mM ethanethiol; 0.25 mM NADP; 0.1 mM FDP; 0.2 international unit of glucose phosphate isomerase and glucose-6-P dehydrogenase, respectively, and AMP as indicated; the reaction was started by the addition of 22 μg of protein from the FDPase preparation described in Fig. 1 and the NADPH formation was followed spectrophotometrically at about 20º. Velocity is expressed as μmoles of NADPH formed per minute per mg of protein.

[*] This preparation was obtained by ethanol fractionation of the extract between 25 and 40% and elution from a CM-Sephadex column with 0.05 mM FDP.

Observations with a partially purified preparation of rabbit muscle FDPase indicate that this enzyme also has a great affinity for FDP and AMP.

DISCUSSION

The occurrence and properties of FDPase in frog and rabbit skeletal muscle raises the question of its physiological role in this tissue. Some years ago Hiatt et al. (1958) indicated that rat diaphragm can incorporate in vitro [2-^{14}C]-pyruvate in glycogen with little randomization. More recently Warnock et al. (1963) have shown that [2-^{14}C]-lactate can serve in vivo as a precursor of muscle glycogen, also observing very slight randomization.

The lack of a bypass for the pyruvate kinase reaction in muscle (Utter, 1959) has been a major difficulty for the neoglycogenesis from lactate in this tissue. In conditions that could have detected 0.2 unit per gram we have not found phosphoenolpyruvate carboxykinase in fresh extracts of muscle from either the rabbit or the frog*. Nevertheless, the reversibility of pyruvate kinase is well established in vitro (Krimsky, 1959) and the fact that there is nearly two orders of magnitude more pyruvate kinase in muscle than in liver in the rat (Utter,1959) and the rabbit* may be significant in relation to a possible reversion of pyruvate kinase in vivo. In optimal conditions the pyruvate kinase of rabbit muscle can work in reverse some three times faster than the FDPase. Whether conditions in vivo could eventually permit the operation of pyruvate kinase in reverse at a significant proportion of this rate remains to be ascertained.

* Unpublished work.

The very great affinity of FDPase for its substrate, found here with the muscle enzyme, may be of considerable importance for neoglycogenesis, because the glycolytic chain below FDP could hardly be efficient in reverse unless very low values of FDP could be maintained. This requirement can be fulfilled by the ability of FDPase to operate very efficiently at micromolar concentration of FDP.

Taketa and Pogell (1963, 1964) have emphasized the likely regulatory significance of the inhibition of liver FDPase by AMP. The muscle enzyme seems to be even more sensitive to the allosteric effect of AMP than the liver FDPase. The very low Ki value leads to the suggestion that there may be some way of counteracting AMP inhibition in certain physiological conditions, unless the actual AMP concentrations in rest muscle be lower than those reported up to now (Parmeggiani and Morgan, 1962).

Taking into account the relative masses of liver and muscle, a rabbit has at least as much total FDPase in muscle as in liver. Krebs (1964 b) has also found substantial amounts of FDPase in muscles of about half of several species of mammals examined. It seems possible that in some species a significant proportion of the lactate produced by muscular exercise could be converted into glycogen not only in liver (Cori and Cori, 1928) and in kidney (Krebs, 1964 a) but also in muscle (Meyerhof, 1930).

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