The Inhibition of Skeletal-Muscle Fructose 1,6-Diphosphatase by Adenosine Monophosphate

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1. The present study extends the finding of Krebs & Woodford (1965) that muscle fructose diphosphatase is more sensitive to AMP inhibition than liver fructose diphosphatase. 2. Hen breast fructose diphosphatase has a K_i for AMP of $0.1 \mu\text{M}$; the plot of percentage inhibition is non-sigmoid and the reciprocal plot of activity against AMP concentration is sometimes linear. 3. Percentage inhibition plots for other muscle fructose diphosphatases are sigmoid curves which exhibit different threshold responses to the AMP concentration. 4. The intracellular content of AMP in all muscles tested exceeds the inhibition concentration range of AMP. 5. The sensitivity of muscle fructose diphosphatase to AMP inhibition is decreased by the presence of Mg^{2+} or Mn^{2+} ions; in the presence of Mn^{2+} the inhibition curve for hen breast fructose diphosphatase becomes sigmoid. 6. From the formation constants for the Mg^{2+} and Mn^{2+} chelates, the effect of these ions in chelation of AMP can be calculated. Although chelation of AMP can explain the Mg^{2+} effect, it cannot explain the marked relief of AMP inhibition by Mn^{2+} . 7. It is suggested that Mn^{2+} has a specific effect on this enzyme which reduces the sensitivity to AMP inhibition.

The presence of FDPase‡ (D-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) in skeletal muscle was reported and discussed by Krebs & Woodford (1965). Further investigation showed that the activity of FDPase is highest in white muscle and very low in red, and it was suggested that this distribution is related to the greater activity of the α -glycerophosphate-dihydroxyacetone phosphate cycle in white muscle (Opie & Newsholme, 1967). The activity of FDPase from rat liver and cat leg muscle may be controlled by inhibitory effects of fructose diphosphate and AMP (Taketa & Pogell, 1965; Krebs & Woodford, 1965; Underwood & Newsholme, 1965a).

Krebs & Woodford (1965) showed that cat leg FDPase is considerably more sensitive to inhibition by AMP than the liver enzyme. In the present investigation endogenous AMP was removed from muscle extracts of various species; FDPase of these muscles is exceptionally sensitive to AMP inhibition, and therefore the response of muscle FDPase from various species to AMP inhibition

was investigated in detail. The intracellular content of AMP in resting skeletal muscle *in vivo* far exceeded the concentration required to half-inhibit muscle FDPase activity. This suggests either that muscle FDPase is always strongly inhibited by the intracellular content of AMP even during rest, or that factors might be present in skeletal muscle that relieve the AMP inhibition. It was found that the sensitivity to AMP is modified by both Mg^{2+} and Mn^{2+} ions, but that Mn^{2+} is considerably more effective than Mg^{2+} .

METHODS

Materials. AMP (sodium salt, 98.3% pure), tris, NADP, fructose 1,6-diphosphate (tetracyclohexylammonium salt), glucose 6-phosphate dehydrogenase and phosphoglucose isomerase were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Other compounds listed in Table 1 came from Sigma Chemical Co., St Louis, Mo., U.S.A., except that malonyl-CoA was kindly provided by Dr N. Kuhn of this Department. 2-Mercaptoethanol was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks. EDTA (disodium salt), MgSO_4 and MnSO_4 were the AnalaR grade of British Drug Houses Ltd., Poole, Dorset. All chemicals were used without further purification.

Preparation of tissue extracts. In general, tissue extracts were prepared and enzyme assays carried out as described by Opie & Newsholme (1967). Supernatants (114000g) were

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‡ Abbreviation: FDPase, fructose 1,6-diphosphatase.

prepared from extracts of the leg muscles of pigeon, rabbit, cat, turtle, crab (*Maia*), Syrian hamster, frog (*Xenopus*) and from the leg and pectoral (breast) muscle of the hen (White Leghorn). Before measurement of the inhibition by AMP, the supernatant was passed through a Sephadex G-25 column (equilibrated with 50 mM-tris, 1 mM-EDTA, 5 mM-MgSO₄ and 20 mM-mercaptoethanol) to remove small molecules such as AMP and ATP.

Measurements of nucleotides in tissues. The rabbit and the frog were anaesthetized by ether inhalation, the hen and the cat by intraperitoneal Nembutal (1 ml./kg.). Care was taken to avoid too deep a degree of anaesthesia, with the risk of cardiovascular depression and tissue ischaemia. The skin was dissected off the thigh muscles, which were clamped in aluminium tongs chilled in liquid N₂. Crab pincers were also freeze-clamped and the exoskeleton was removed before further treatment. The thinnest parts of the frozen muscle, about 2–4 mm. thick, were pulverized at –70° in a percussion mortar. The frozen powder was extracted by grinding in a mortar with frozen actone-perchloric acid–5 mM-EDTA (50:12:38, by vol.) This extract thawed at a temperature of approx. –20° (see Lowry, Passonneau, Hasselberger & Schulz, 1964). After centrifuging at 1500g for 20 min. at 0°, the supernatant was removed by filtration through Whatman no. 1 paper in a cold room at 2°. No adenylate kinase activity could be detected in the filtrate, which was neutralized with 30% (w/v) KOH in saturated KCl, and the KClO₄ precipitated by freezing in solid CO₂. The mixture was thawed in melting ice, centrifuged as above and the supernatant stored at about –20°. Analyses for adenine nucleotides were performed within a few hours. ADP and AMP were assayed by the method of Adam (1963) and ATP by the method of Lamprecht & Trautschold (1963).

Assay of fructose 1,6-diphosphatase. FDPase was assayed as described by Opie & Newsholme (1967), by following the reduction of NADP at 340 mμ in a Gilford recording spectrophotometer at 25°.

RESULTS

Inhibition of muscle FDPase from various species by AMP. In Fig. 1 the percentage inhibition of muscle FDPase from various species is plotted against the AMP concentration. The curves are sigmoid for muscle FDPase from cat, turtle, crab, hamster and frog, and for liver FDPase from the rat (Fig. 1); the reciprocal plots of activity of muscle FDPase of these species (and of the rabbit and pigeon) against the AMP concentration are non-linear (Fig. 2). However, the percentage inhibition plot for hen breast FDPase is non-sigmoid (Fig. 1 and Table 4). Although different extracts of hen breast yielded apparently similar results when percentage inhibition against AMP concentration was plotted, the plots of reciprocal of activity against AMP concentration were sometimes linear, as is found in classical inhibition kinetics (see Dixon & Webb, 1964), and sometimes non-linear (Fig. 3). In extracts in which the reciprocal plot was linear, the modified Hill plot (see Monod, Changeux &

Fig. 1. Percentage inhibition of FDPase activity of various species by AMP: ○, hen breast and leg; ▲, cat thigh; □, turtle leg; ●, crab pincer; ■, hamster thigh; △, frog thigh; ▽, rat liver. Values in parentheses are the maximum FDPase activities, measured in the absence of AMP, expressed as μmoles of substrate transformed/g. wet wt./hr. at 25°. Conditions were as described by Opie & Newsholme (1967). The values for rat liver are taken from the data of Underwood & Newsholme (1965a).

Fig. 2. Reciprocal plots of activity of FDPase from various species against the AMP concentration: ■, white thigh muscle of rabbit; □, turtle leg muscle; ●, crab pincer; ○, pigeon leg; △, frog thigh. Conditions of assay were as described by Opie & Newsholme (1967).

Jacob, 1963) gave a slope of 1; with non-linear reciprocal plots, the slope of the Hill plot exceeded 1. Technical errors were not responsible for this

Fig. 3. Reciprocal plots of activity of hen breast FDPase against AMP concentration. Δ and $\circ$ represent different extracts of hen breast muscle. Each point is the mean of three or more enzymic analyses. Conditions of extraction are discussed in the Results section and conditions of assay were as given by Opie & Newsholme (1967).

change, because each extract was analysed repetitively (from 3 to 17 times). The age of the extract, omission of Sephadex treatment, and freezing and thawing the extracts, could not be correlated with the shape of the reciprocal plot of hen breast FDPase. In all other types of muscle FDPase tested, the reciprocal plots were non-linear (Fig. 2).

Hen breast FDPase was the enzyme most sensitive to AMP inhibition and, assuming non-competitive inhibition, the K_i was $0.1 \mu\text{M}$. For the other muscles and rat liver 50% inhibition was produced by AMP concentrations which varied from $0.125 \mu\text{M}$ (pigeon leg) to approx. $30 \mu\text{M}$ for rat liver (Fig. 1). This difference in sensitivity to AMP was due to the increase in sigmoid character of the response to AMP, and cannot be correlated with the maximum activity of FDPase, or with the phylogenetic position of the species investigated (Fig. 1).

Effect of compounds structurally related to AMP on hen breast FDPase. As hen breast possessed the highest activity of FDPase of the muscles tested (see Fig. 1, or Opie & Newsholme, 1967), the specificity of AMP inhibition of this enzyme was investigated (Table 1). As some of the compounds which inhibited muscle FDPase might contain AMP as a contaminant, the AMP contents of CoA, acetyl-CoA and ADP-ribose were measured. The CoA contained 0.56% AMP, the acetyl-CoA 0.43% and the ADP-ribose 1.62%; this contamination could explain part of the inhibition of FDPase. No compound tested was able to inhibit hen breast FDPase

Table 1. *Effect of various compounds structurally related to AMP on hen breast fructose 1,6-diphosphatase activity*

Extracts were not passed through Sephadex columns. Compounds were added to the cuvette to the final concentrations shown, and the pH was readjusted to 7.5 if necessary. Otherwise, conditions of assay were as described by Opie & Newsholme (1967). Mg^{2+} concentration was 6 mM.

Compound	Concn. (μM) required to inhibit FDPase		
	25% inhibition	50% inhibition	75% inhibition
AMP	0.06	0.09	0.18
FAD	1.3	2.6	4.4
ADP-ribose	1.6	4.0	8.0
CoA	—	5.0	28
Acetyl-CoA	5.0	10	44
NAD	2.5	15	25
Malonyl-CoA	14	25	43
UMP	16	34	72
GMP	65	165	390
IMP	100	200	500
NADP	100	300	540
Adenosine	135	400	800
Cyclic 3,5-AMP	205	350	900
Palmitoyl-CoA	300	—	—
Ribose 5-phosphate	900	—	—

with a potency comparable with that of AMP; thus FDPase is about 25 times less sensitive to FAD than to AMP, 60 times less sensitive to CoA and 200 times less sensitive to acetyl-CoA (allowing for AMP contamination of CoA and acetyl-CoA).

Although FDPase is sensitive to inhibition by compounds such as NAD and NADP in concentrations which might well occur *in vivo*, it was not possible to exclude breakdown of these compounds to yield very small amounts of AMP during the assay of the enzyme. Such quantities of AMP could only be assayed with great difficulty. For these reasons, significance cannot be attached to inhibition of FDPase by compounds structurally resembling AMP.

Intracellular contents of adenine nucleotides in muscles *in situ*. The intracellular contents of adenine nucleotides were measured in thigh muscles of the cat, rabbit, hen and frog and in the pincer muscles of the crab, by freeze-clamping the tissue *in situ* (Table 2). The values of ATP and ADP are similar to those reported for abdominal muscle of the rat (Hohorst, Reim & Bartels, 1962). The values of AMP vary from 14 to $31 \mu\text{moles/g.}$ of frozen tissue, which, assuming intracellular water represents 70% of the frozen wt., gives a variation from 20 to $45 \mu\text{M}$ for the intracellular concentration. These AMP concentrations are similar to those

Table 2. *Contents of adenine nucleotides in muscle*

Conditions are as described in the Methods section. Muscles are thigh muscles except for crab pincer. Results are given as means $\pm$ S.E.M. and the numbers of tissue extractions are given in parentheses. Numbers in parentheses after animal species indicate numbers of animals tested. K'_{ad} , Apparent equilibrium constant of adenylate kinase reaction calculated from ATP, ADP and AMP concentrations.

Animal	Content (μ moles/g. of frozen powder)			K'_{ad}
	ATP	ADP	AMP	
Cat (2)	2.36 ± 0.20 (4)	0.60 ± 0.24 (4)	0.031 ± 0.008 (4)	0.20
Rabbit (2)	4.24 ± 0.31 (3)	0.67 ± 0.03 (3)	0.024 ± 0.019 (3)	0.22
Hen (1)	4.46 ± 0.48 (4)	0.76 ± 0.06 (4)	0.021 ± 0.004 (4)	0.16
Frog (5) (<i>Xenopus</i>)	5.68 ± 0.83 (5)	1.13 ± 0.17 (5)	0.014 ± 0.005 (5)	0.05
Crab (5) (<i>Maia</i>)	5.64 ± 0.86 (3)	1.33 ± 0.13 (3)	0.027 ± 0.055 (7)	0.09

found in rat liver *in situ* (approx. $100 \mu\text{M}$; A. H. Underwood & E. A. Newsholme, unpublished work); however, they are many times greater than the concentrations required to inhibit muscle FDPase of all species that have been investigated.

Effect of metabolic intermediates on AMP inhibition of hen breast FDPase. Because hen breast FDPase is so very sensitive to AMP, it is possible that some intermediate of metabolism might relieve the AMP inhibition. The following compounds did not relieve the AMP inhibition of hen breast FDPase (in these studies the extract was not passed through a Sephadex column): citrate, 0.01–1 mM; succinate, 2.5 mM; α -oxoglutarate, 2.5 mM; glutamate, 2.5 mM; pyruvate, 1–2.5 mM; L-lactate, 2.5 mM; DL-alanine, 2.5 mM; phosphoenolpyruvate, 2.5 mM; 3-phosphoglycerate, 2.5 mM; fructose 1-phosphate, 2.5 mM; oleate, 0.005–0.05 mM; inorganic phosphate 1 and 10 mM; uridine triphosphate, 0.5 mM; cytidylic acid, 0.4 mM. Concentrations of ATP below 1 mM had no effect, but concentrations above this inhibited the enzyme. Such inhibition could have been caused by contamination with trace amounts of AMP. Malate (2.5 mM) and isocitrate (0.25 mM) relieved AMP inhibition by 25% and 20% respectively; these values were not increased by raising the concentrations to 25 mM.

Effects of Mg^{2+} and Mn^{2+} on AMP inhibition of FDPase. The effects of Mg^{2+} and Mn^{2+} on AMP inhibition of muscle FDPase were investigated because an increase in the Mg^{2+} concentration can relieve AMP inhibition of liver FDPase (Underwood & Newsholme, 1965a). Mg^{2+} decreased the sensitivity of both frog leg and hen breast FDPase to AMP (Fig. 4 and Table 4). Mn^{2+} , both in the presence and absence of Mg^{2+} , was much more effective in relieving AMP inhibition than was Mg^{2+} (Figs. 5 and 6). Also, increasing the Mn^{2+} concentration from zero to 0.1 mM produced relief of AMP inhibition comparable with that produced by an increase of 7.5 mM in the Mg^{2+} concentration (Tables 3 and 4). With hen breast FDPase the percentage inhibition plot

Fig. 4. Effect of various Mg^{2+} concentrations on percentage inhibition of frog leg FDPase activity by AMP: Δ , 3.5 mM- Mg^{2+} ; $\circ$, 6.0 mM- Mg^{2+} ; $\blacktriangle$, 11.0 mM- Mg^{2+} . $\bullet$ — $\bullet$, Response to AMP at 11 mM- Mg^{2+} after the chelation effect of 7.5 mM- Mg^{2+} has been subtracted from the AMP concentration; this plot can be compared with 3.5 mM- Mg^{2+} . This effect of Mg^{2+} was calculated as described in the Results section. Conditions of assay were as described by Opie & Newsholme (1967); 1 mM-EDTA was present in all assays.

became sigmoid in the presence of Mn^{2+} . The Mn^{2+} effect was dependent on the EDTA concentration (Table 3). With 0.025 mM-EDTA, low Mn^{2+} concentrations (0.1–0.2 mM) relieved the AMP inhibition, whereas in the presence of 1 mM- and 2 mM-EDTA the inhibition was completely relieved only by Mn^{2+} concentrations of 1.25 and 2.5 mM respectively (because of the high affinity constant of EDTA for Mn^{2+} it can be assumed that the free ion concentration is the total ion concentration less the EDTA concentration).

The formation constant K for the AMP- Mg^{2+} complex is approximately 89 (Walaas, 1958), and from the formula

$$K = [\text{AMP-Mg}^{2+}]/[\text{AMP}][\text{Mg}^{2+}]$$

the concentration of free AMP can be calculated

Fig. 5. Percentage inhibition of frog leg FDPase activity by AMP: ●, 6mM-Mg²⁺; ○, 1.2mM-Mn²⁺; △, 6mM-Mg²⁺ combined with 1.2mM-Mn²⁺. □---□ and ▼---▼. Response to AMP after the chelation effect of 1.2mM-Mn²⁺ has been subtracted from the AMP concentrations. This effect of Mn²⁺ was calculated as described in the Results section. Other conditions of assay were as described by Opie & Newsholme (1967); 1 mM-EDTA was present in all assays.

Fig. 6. Percentage inhibition of hen breast FDPase activity by AMP: ●, 6mM-Mg²⁺; ○, 1.2mM-Mn²⁺; △, 6mM-Mg²⁺ combined with 1.2mM-Mn²⁺. □---□, Response to AMP at 6mM-Mg²⁺ plus 1.2mM-Mn²⁺ after the chelation effect of 1.2mM-Mn²⁺ has been subtracted from the AMP concentrations. This effect of Mn²⁺ was calculated as described in the Results section. Other conditions of assay were as described by Opie & Newsholme (1967); 1 mM-EDTA was present in all assays.

Table 3. Effect of Mn²⁺ on inhibition of hen breast fructose 1,6-diphosphatase activity by AMP

Conditions for assay of FDPase are as described by Opie & Newsholme (1967), except that EDTA concentration was varied. Mn²⁺ was added as MnSO₄. Mg²⁺ (6mM) was used throughout. The extract was passed through a Sephadex column before assay.

Concn. of AMP (mμM)	Concn. of Mn ²⁺ (mM)	FDPase activity (μmoles/g. wet wt./hr.)		
		EDTA (0.025 mM)	EDTA (1 mM)	EDTA (2 mM)
0	0	128	134	132
500	0	8	8	10
500	0.025	17	8	—
500	0.05	32	8	—
500	0.10	82	8	—
500	0.20	126	8	10
500	0.40	107	8	—
500	1.0	—	20	14
500	1.25	—	120	14
500	2.5	—	78	110
500	5.0	—	48	86

from a knowledge of the initial AMP and Mg²⁺ concentrations. Thus the effect of raising the Mg²⁺ concentration from 3.5 to 11.0mM on the free AMP concentration can be calculated. As shown in Fig. 4 (and from similar calculations for the data in Table 4) a large part of the Mg²⁺ effect can be explained by chelation of AMP. However, the formation constant for the AMP-Mn²⁺ complex

is only 204 (Walaas, 1958), and calculations show that the chelation complex-formation cannot explain the very marked effects of Mn²⁺ in relieving AMP inhibition (see Figs. 5 and 6).
Effect of substrate on muscle FDPase. Krebs & Woodford (1965) showed that the K_m of cat leg muscle FDPase for fructose diphosphate was approximately 2.0 μM. In the present investigation the K_m values for different types of muscle FDPase have been calculated from double reciprocal plots

Table 4. *Effect of Mg^{2+} on percentage inhibition of hen breast fructose 1,6-diphosphatase activity by AMP*

Results are expressed as percentage inhibition of FDPase activity in the absence of AMP at the indicated Mg^{2+} concn., with means $\pm$ s.e.m. and numbers of experiments given in parentheses. Conditions of assay were as described by Opie & Newsholme (1967). Extracts were not passed through a Sephadex column. 1 mM-EDTA was used throughout. Activities measured in absence of AMP were (μ moles/g. wet wt./hr.): 199, 211 and 208 at Mg^{2+} 3.5, 6.0 and 11 mM respectively.

Concn. of AMP (m μ M)	Concn. of Mg^{2+}	Percentage inhibition of FDPase		
		3.5 mM	6.0 mM	11.0 mM
12.5		12 $\pm$ 2.3 (3)	6 $\pm$ 2.9 (3)	—
15		18 $\pm$ 2.5 (3)	11 $\pm$ 3.5 (3)	—
25		22 $\pm$ 2.6 (3)	12 $\pm$ 2.3 (3)	9 $\pm$ 2.3 (5)
37.5		35 $\pm$ 1.1 (3)	22 $\pm$ 2.0 (6)	—
50		48 $\pm$ 3.5 (3)	26 $\pm$ 0.9 (4)	14 $\pm$ 2.8 (5)
62.5		54 $\pm$ 2.9 (3)	34 $\pm$ 3.8 (4)	—
75		63 $\pm$ 3.0 (3)	41 $\pm$ 4.5 (4)	16 $\pm$ 2.8 (5)
100		71 $\pm$ 5.5 (3)	54 $\pm$ 1.3 (4)	28 $\pm$ 3.3 (5)
125		80 $\pm$ 1.7 (3)	64 $\pm$ 1.5 (4)	30 $\pm$ 3.6 (5)
150		81 $\pm$ 1.0 (3)	69 (2)	35 $\pm$ 2.7 (5)
162.5		86 $\pm$ 5.7 (3)	73 (2)	—
175		—	—	43 $\pm$ 4.3 (5)
200		90 $\pm$ 1.5 (3)	79 $\pm$ 0.7 (3)	53 $\pm$ 3.9 (5)

Fig. 7. Effect of substrate concentration on activity of FDPase from hen breast (●) and turtle leg (○). Conditions of assay were as described by Opie & Newsholme (1967). Activities were measured on a recording fluorimeter.

Table 5. *Effect of substrate concentration on hen breast fructose 1,6-diphosphatase activity*

Conditions of assay were as described by Opie & Newsholme (1967).

Concn. of fructose diphosphate (μ M)	FDPase activity (μ moles/g. wet wt./hr.)
0.25	3
1.0	4.5
2.0	6.9
10	64
100	187
500	79
5000	60
10000	67

similar manner to the cat leg muscle enzyme (Krebs & Woodford, 1965).

DISCUSSION

from activities obtained at very low substrate concentrations by using a recording fluorimeter (Dalziel, 1962); the values are: rabbit and hamster leg, 0.4 μ M; turtle leg, 0.8 μ M; frog and cat leg, 0.9 μ M; pigeon leg, 1.2 μ M. A plot of FDPase activity against substrate concentration for hen breast FDPase is sigmoid (Fig. 7) and the double reciprocal plot is non-linear; 50% of maximum activity is reached at about 16 μ M (from (Fig. 7). Excess of substrate inhibits hen breast FDPase (Table 5) in a

FDPase may be important, especially in white muscle, for the removal of fructose diphosphate and triose phosphates during rest, particularly after the cessation of exercise (Krebs & Woodford, 1965; Opie & Newsholme, 1967). As muscle phosphofructokinase and FDPase have some similar properties to the enzymes from rat liver, there may be a mechanism in muscle for the control of both enzymes similar to that proposed for liver (Underwood & Newsholme, 1965b). The importance

of substrate inhibition of FDPase from liver or kidney cortex in the control of glucose formation is in doubt because the intracellular contents of fructose diphosphate are not in the inhibitory range (Exton & Park, 1965; Newsholme & Underwood, 1966; Gevers & Krebs, 1966). However, the content of fructose diphosphate in frog leg muscle increases from $20\mu\text{M}$ at rest to $1370\mu\text{M}$ during sustained contraction against a load (Özand & Narahara, 1964), which suggests that inhibition by fructose diphosphate may be a factor controlling the activity of muscle FDPase. This possibility has not been explored further, because the marked sensitivity of muscle FDPase to AMP inhibition seemed the major problem in understanding the control of muscle FDPase.

The FDPase from hen breast is extremely sensitive to AMP inhibition, and the plot of percentage inhibition against AMP concentration is not sigmoid (Fig. 1 and Table 4). The inhibition plots of the other types of muscle FDPase tested form a family of curves (Fig. 1) in which the sigmoid character increases as the sensitivity of the enzyme to AMP inhibition decreases. These curves indicate differing thresholds of various types of muscle FDPase to AMP inhibition. This is in contrast with the types of hepatic FDPase from various species, which have similar threshold responses to AMP (Wallace & Newsholme, 1967).

Two possible explanations are put forward to explain the disparity between the intracellular contents of AMP and the concentration of AMP required to inhibit FDPase *in vitro*, and the variation in threshold response. Firstly, if the AMP content of the cell were unevenly distributed owing to some form of 'compartmentation', the concentration of AMP in the immediate environment of FDPase might vary from muscle to muscle. With hen breast, an extremely low environmental concentration of AMP would permit a rapid response of FDPase to a change in the AMP concentration without the requirement of sigmoid response to AMP (as in Fig. 1). Other types of muscle FDPase may function at higher environmental concentrations of AMP so that a rapid response to a change in concentration would require a threshold effect of AMP, and therefore sigmoid inhibition kinetics would be expected. However, the present techniques of freeze-clamping and extracting frozen tissue preclude measurements of AMP in any of the known intracellular compartments.

The second possibility is that metal ions present in the cell can decrease the sensitivity of muscle FDPase to AMP inhibition. Underwood & Newsholme (1965a) showed that AMP inhibition of rat liver FDPase was relieved by raising the Mg^{2+} concentration, and this was assumed to be due to a decrease in the available AMP concentration,

because of complex-formation between AMP and Mg^{2+} . In this investigation, it has been shown that both Mg^{2+} and Mn^{2+} relieve the AMP inhibition of muscle. However, the effects of Mn^{2+} are unusual in at least three respects. Firstly, Mn^{2+} is approx. 100-fold more effective in relieving AMP inhibition than Mg^{2+} , although the formation constant for the Mn^{2+} -AMP complex is only 2.5 times that of the Mg^{2+} -AMP complex (see Walaas, 1958). Secondly, the relief of AMP inhibition by a combination of Mg^{2+} and Mn^{2+} is not additive and with frog leg FDPase the effect of Mn^{2+} was less in the presence of Mg^{2+} (Figs. 5 and 6). Thirdly, the non-sigmoid response of hen breast FDPase to AMP inhibition is changed to a sigmoid response in the presence of Mn^{2+} (Fig. 6).

Moreover, as shown in Fig. 4, the effect of Mg^{2+} in relieving AMP inhibition may be explained, to a large extent, by chelation of AMP, but, as shown in Figs. 5 and 6, the effect of Mn^{2+} cannot be explained by chelation complex-formation. This is supported by the results reported in Table 3; 0.2mm-Mn^{2+} completely relieved the inhibition produced by 0.5mm-AMP , although this concentration of Mn^{2+} could only chelate about 5% of the AMP, according to the formation constant of Walaas (1958).

The mechanism of action of Mn^{2+} in reducing the sensitivity of muscle FDPase to AMP is unknown, but it is possible that Mn^{2+} reduces the affinity of the enzyme for AMP; this might be analogous to the effect of inorganic phosphate on glucose 6-phosphate inhibition of erythrocyte hexokinase (Rose, Warms & O'Connell, 1964). That there is a specific effect of bivalent metal ions on FDPase is supported by the extremely strong Zn^{2+} inhibition of the cat muscle enzyme (Krebs & Woodford, 1965).

If Mn^{2+} is accessible to FDPase within the cell, the sensitivity of the enzyme to AMP inhibition may be reduced so that variations in the total intracellular concentration of AMP could modify the activity of FDPase. Moreover, variations in the intracellular concentration or 'compartmentation' of Mn^{2+} under different physiological conditions might be an important regulatory mechanism in the control of the activity of muscle FDPase.

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