Effect of 2:4-dinitrophenol and phenylmercuric acetate on enzymic activity of myosin

The mechanism whereby DPN* uncouples phosphorylation from oxidation and accelerates ATPase activity in mitochondria is unknown. After WEBSTER[1] had noticed that DNP, in rather high concentrations, increased the ATPase activity of myosin, we began to investigate this effect in the hope that ultimately light might be thrown on the interaction of DNP with an enzyme system. Some preliminary observations are given here.

Thrice precipitated L-myosin and four times precipitated actomyosin were prepared[2,3] from rabbit skeletal muscle. Myosin ATPase activity was usually measured by incubating the enzyme (0.08–0.15 mg protein/ml) for 5 min at 25°, with 0.065 M glyoxaline pH 7.0, 0.1 M KCl, 0.01 M $CaCl_2$ and $1-2\cdot10^{-3}$ M ATP. The reaction was started by addition of the myosin. The acceleration by DNP was 101% ($5\cdot10^{-3}$ M), 93% ($3\cdot10^{-3}$ M), 76% ($2\cdot10^{-3}$ M), 59% ($1.5\cdot10^{-3}$ M), 32% ($7.5\cdot10^{-4}$ M), 15% ($3\cdot10^{-4}$ M) and 1% ($3\cdot10^{-5}$ M).

Experiments with heavy metals and heavy metal combining agents gave no indication that DNP acts by removing inhibitory heavy metal ions. Measurements of the absorption between 230 and 280 mμ of DNP and ATP separately and together in presence of Ca^{++} gave no evidence of combination between DNP and the substrate.

As two SH-combining reagents, *p*-chloromercuribenzoate (LARDY AND WELLMAN[4]) and PMA[5] (CHAPPELL[6]), prevent the stimulation of mitochondrial ATPase by DNP, we have, at Dr. CHAPPELL's suggestion, studied the effect of PMA on myosin ATPase (Fig. 1). In absence of DNP, PMA in low concentrations ($5\cdot10^{-7}$ to $4\cdot10^{-6}$ M) was acceleratory, in higher concentrations inhibitory. The acceleration by PMA became progressively greater with increasing KCl concentration. LARDY AND WELLMAN[4] state that *o*-iodosobenzoate accelerated the ATPase of aged rat liver mitochondria, but CHAPPELL[6] found no acceleration by PMA with pigeon breast muscle mitochondria. Fig. 1 shows that PMA counteracts the acceleration by DNP. Indeed, whereas DNP ($2\cdot10^{-3}$ M) and PMA ($4\cdot10^{-6}$ M) were each acceleratory, when added together they caused a marked inhibition. With PMA in slightly inhibitory concentration, no concentration of DNP was found at which acceleration occurred.

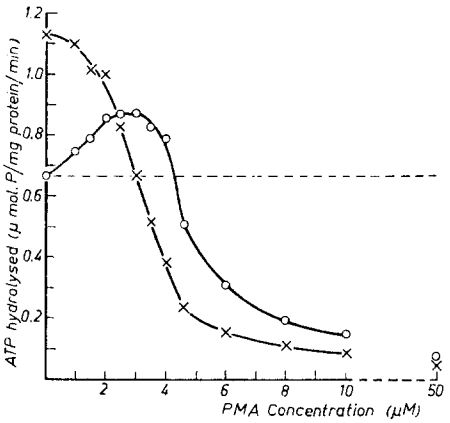

Fig. 1. Effect of DNP and PMA on ATPase activity of L-myosin. Protein, 0.1 mg/ml; ATP, $2\cdot10^{-3}$ M. For other conditions see text. —○— Without DNP; —×— $2\cdot10^{-3}$ M DNP.

CHAPPELL[6] found that ITPase activity of muscle mitochondria was unaffected by DNP in low concentrations (*cf.* ref.[4]); higher concentrations inhibited. The breakdown by myosin of ITP, more rapid than that of ATP under our conditions (*cf.* KLEINZELLER[7]) was unaffected by DNP up to $2\cdot10^{-5}$ M; above this there was inhibition, rising to 70% at $2\cdot10^{-3}$ M. No acceleration by PMA was observed, inhibition becoming progressively greater with increasing concentrations above 10^{-7} M.

The small residual activity of myosin ATPase in absence of added Ca^{++} was slightly inhibited by $2\cdot10^{-3}$ M DNP. As the Ca^{++} concentration was lowered from the optimal, the percentage acceleration by $2\cdot10^{-3}$ M DNP progressively fell to zero. On the other hand, as the activity in presence of Ca^{++} was decreased by addition of increasing amounts of Mg^{++} (which competes with the Ca^{++}[8]) the percentage acceleration by $2\cdot10^{-3}$ M DNP progressively increased.

With actomyosin at low ionic strength (I) no activation by DNP was observed; but at high I (when the behaviour of actomyosin ATPase shows resemblances to that of myosin ATPase[9]) activation was obtained (Table I). With actomyosin (0.045 mg/ml) at low ionic strength (below 0.05), activated by Mg^{++} ($3\cdot10^{-4}$ M) or Ca^{++} ($5\cdot10^{-3}$ M), PMA was inhibitory above $6\cdot10^{-7}$ M, and lower concentrations failed to accelerate. Addition of DNP ($2\cdot10^{-3}$ M) somewhat increased this inhibition. At $I = 0.3$, with Ca^{++} activation as above, PMA accelerated between $2\cdot10^{-7}$ M and $2\cdot10^{-6}$ M; in higher concentrations it was inhibitory. When DNP ($4\cdot10^{-3}$ M) was added, results similar to those with myosin (Fig. 1) were obtained.

Similar effects of DNP on myosin and actomyosin ATPase are reported by Drs. J. B. CHAPPELL AND S. V. PERRY in an accompanying communication[10].

* Abbreviations: DNP, 2:4-dinitrophenol; ATP, adenosinetriphosphate; PMA, phenylmercuric acetate; ITP, inosinetriphosphate.

TABLE I

EFFECT OF DNP ON ACTOMYOSIN ATPASE AT VARIOUS IONIC STRENGTHS

No.	*I*	*Activity without DNP µmol P/mg protein/min*	*Acceleration by DNP*
1	0.050	0.88	−9
2	0.068	0.98	−2
3	0.088	1.00	+9
4	0.150	1.03	+23
5	0.300	0.60	+112

In No. 1: Actomyosin, 0.045 mg protein/ml; KCl, 0.011 M; $CaCl_2$, $5 \cdot 10^{-3}$ M; K-ATP, 10^{-3} M; glyoxaline-Cl pH 7.0, 0.0163 M; K-DNP, $4 \cdot 10^{-3}$ M (or equivalent KCl). In Nos. 2–5, I was raised by further addition of KCl. 5 min, 25°. Activity in absence of added Ca^{++}: 0.05 (I = 0.05), 0.22 (I = 0.30).

The deaminase activity of one of our L-myosin preparations was tested under the following conditions (*cf.* ref.[11]): 0.08 mg protein/ml; 0.04 M succinate buffer, pH 5.5; 0.02 M $CaCl_2$; $3 \cdot 10^{-3}$ M adenosine-5′-phosphate; 15 min at 25°. NH_3 was determined by microdiffusion (CONWAY[12]) followed by Nesslerisation. Q_{NH_3} (ref.[11]) was 1260, and unchanged by DNP ($2.5 \cdot 10^{-3}$ to $1.25 \cdot 10^{-4}$ M).

To summarise, with both mitochondria[6] and myosin DNP accelerates the ATPase activity and this effect is abolished by PMA; in neither system does DNP accelerate the ITPase activity. On the other hand, Ag^{++} in low concentrations greatly accelerates mitochondrial ATPase but only inhibits myosin ATPase[13]. Actomyosin at high ionic strengths behaves towards DNP and PMA similarly to myosin.

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The stimulation of the adenosinetriphosphatase activities of myofibrils and L-myosin by 2:4-dinitrophenol

In the course of studies on the metabolism of adenosinetriphosphate (ATP) by intracellular components of skeletal muscle[1,2,3] it was noted that whereas the adenosinetriphosphatase (ATPase) activity of freshly prepared pigeon breast muscle mitochondria was markedly stimulated by 10^{-4} M 2:4-dinitrophenol (DNP), this substance in concentrations ranging from 10^{-4} to $5 \cdot 10^{-3}$ M failed to increase the ATPase activity of rabbit myofibrils. As this latter observation did not appear to be consistent with WEBSTER'S[4] finding that myosin ATPase was stimulated by DNP, further investigations were carried out to discover why this difference in behaviour should exist between the purified enzyme and myosin occurring naturally as actomyosin in the myofibril.