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Is the Chemomechanical Energy Transformation Reversible?*

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Summary. In glycerol-extracted insect fibrillar muscle suspended in ATP salt solution the incorporation of $^{32}P_i$ into ATP was studied during the performance of positive or negative oscillatory work and under a variety of mechanical and ionic conditions. An increase in calcium ion concentration from 10^{-8}–10^{-5} M increased the incorporation rate in proportion to the increase in ATPase activity, mean tension and immediate stiffness, which is a measure of the extent of actin-myosin interaction. Sinusoidal stretches (at 1% L_o) performed at 5 Hz induced the fibres to perform optimal positive oscillatory work and it caused a doubling of the incorporation rate (and ATPase activity). A decrease or increase of the frequency below or above the optimum of 5 Hz always decreased the power output as well as the incorporation rate which, however, was still noticeable even under conditions where work was done *on* the fibres. A similar frequency dependence was found when square-wave rather than sinusoidal stretches were applied and this effect could be related to the finding that the rate of stretch-induced incorporation was highest shortly after stretching and then declined to low values (after about 100 ms). These results suggest the formation of an energy-rich intermediate (actomyosin-ADP?) during the contraction process induced by stretching and this intermediate must be assumed to accumulate transiently after stretching.

Key words: Insect flight muscle – ATP-Phosphate exchange – Contractile mechanism – Calcium; activation of – Chemo-mechanical energy transformation, reversibility of.

INTRODUCTION

On the basis of myothermal experiments Hill and Howarth (1959) suggested that, if a contracting muscle is stretched, the work done *on* the muscle might be partly transformed into chemical energy during a reversal of the elementary contractile process. Subsequent work showed an incorporation of ^{32}P-phosphate into ATP in stretched glycerol-extracted muscle fibres (Gillis and Maréchal, 1971; Mannherz, 1970) which could, however, be more readily interpreted as an ATP-P_i exchange reaction (Gillis and Maréchal, 1974; Ulbrich and Rüegg, 1971; Ulbrich, 1975) particularly since the incorporation reaction is also demonstrable under strictly isometric conditions (Ulbrich and Rüegg, 1971) and in purified actomyosin (Hotta and Fujita, 1971; Ulbrich and Paulsen, 1973; Wolcott and Boyer, 1974).

However, these investigations did not exclude the possibility of a reversal of mechanical into chemical energy and further studies seemed to be urgently needed.

In the past glycerol-extracted insect fibrillar muscle was extensively used to study the coupling of mechanical power and rate of ATP splitting during an oscillatory contraction (Steiger and Rüegg, 1969; Pybus and Tregear, 1975).

This preparation also proved ideal for examining the question of whether or not the "back reaction" leading to P_i incorporation into ATP depended on the extent of actin-myosin interaction after activation by Ca^{2+} or stretch and whether it was coupled either with positive or with negative oscillatory work.

METHODS

Glycerol-extracted muscle fibre bundles (6 fibres of length 0.5 cm) of the dorsolongitudinal muscle (DLM) of the waterbug *Lethocerus maximus* were glued at one end to the arm of a RCA 5734 transducer valve and at the other end to glass rod connected with a Ling

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101 vibrator driven by a servo-controlled wave generator (Jewell and Rüegg, 1966).

Length changes (0.25–3% of the fibre length) were monitored by field plates and work and power output were determined as described by Steiger and Rüegg (1969).

The fibres were incubated in an ATP-salt solution (the composition of which is given in legend of Fig. 1) for periods up to 1 h; the P_i incorporated into ATP was estimated as described by Mannherz (1970) by measuring the amount of glucose-6-phosphate-^{32}P which was formed (quantitatively from ATP and glucose) after addition of hexokinase and glucose. Each fibre bundle was used for a series of up to 10 incubation periods in different baths and under different mechanical or ionic conditions. Calcium ion concentration was controlled by means of the calcium buffer system as described by Jewell and Rüegg (1966).

RESULTS AND DISCUSSION

As shown in Figure 1 the rate of incorporation of $^{32}P_i$ into ATP may be increased several fold by increasing the Ca^{2+} ion concentration from 10^{-8}–10^{-5} M (Fig. 1). A similar dependence on Ca^{2+} ion concentration is exhibited by the actomyosin ATPase activity and the immediate stiffness (Fig. 1) which may be taken as a measure of the number of cross-bridges attached at any one moment (Huxley and Simmons, 1971).

Even at optimal Ca^{2+} concentration quick stretches further increase the immediate stiffness (Schädler et al., 1971) as a measure of the extent of actin-myosin interaction and they also increase the P_i incorporation into ATP. The rate of extra incorporation induced by stretching (1–3% L_0) may exceed 3–4 times (in general: about 2 times) the basal rate in unstretched preparations under the same ionic conditions. The extra exchange rate in a fibre bundle is barely influenced by azide and oligomycin (which are known to inhibit the mitochondrial exchange); it amounts to up to 0.2% P_i per h. This corresponds to an incorporation rate of 10–12 pmoles/min in 1 cm length of fibre or 1% of the ATP splitting rate, assuming that the P_i concentration within the fibre bundle was 3–4 mM. The stretch-induced incorporation increases with the amplitude of stretch (Table 1), but it also depended markedly on the frequency.

When fibres were sinusoidally stretched and released, they performed positive work in the frequency range of 1–20 Hz (with an optimal frequency around 5 Hz). At frequencies higher than 20 Hz or lower than 1 Hz work was done on the fibres during the application of sinusoidal forcings of the fibre bundle; in other words, the fibre bundle performed negative work. A frequency dependence similar to that obtained for the power output and the oscillatory work (Fig. 2) has now been found for the rate of $^{32}P_i$-incorporation during sinusoidal stretch-release cycles, as well as during rectangular stretches. The latter induced a delayed development of tension (as shown in Fig. 3, inset) but they produced no substantial negative or positive work.

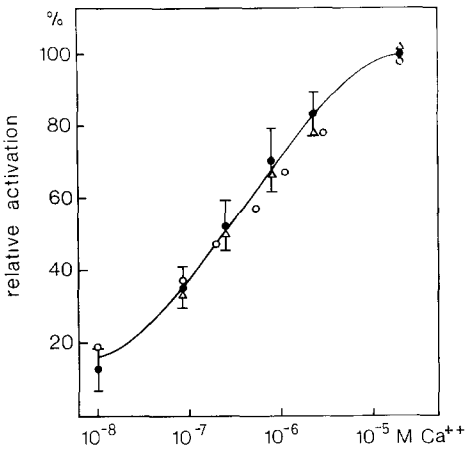

Fig. 1. Dependence of the rate of P_i incorporation into ATP (●) on calcium ion concentration (mean ± S.D., in % of maximal effect, $n = 8$). Also shown: calcium ion dependence of immediate stiffness (△) and of ATPase activity (○). Conditions: Glycerinated fibre bundles (6 fibres) incubated in 15 mM Mg-ATP, 10 mM Na azide, 20 mM imidazole/HCl buffer (pH 6.7), 4 mM Ca-EGTA buffer, 1 mM ^{32}P-P_i. 20° C

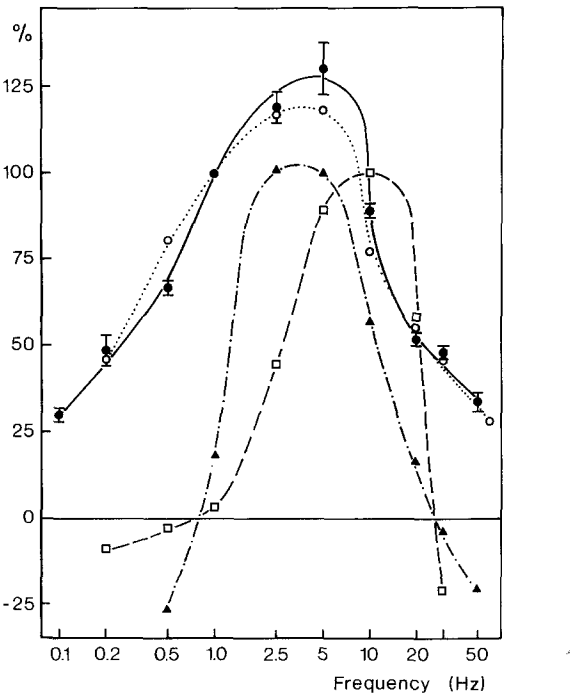

Fig. 2. Incorporation of ^{32}P-P_i into ATP in dependence on the frequency of sinusoidal (●——●) or square wave (○·····○) stretch-release cycles (mean ± S.D. in % of extra incorporation induced by 1 Hz stretches). Also shown: Power output (▲-·-·-▲) and work/cycle (□----□) during sinusoidal stretch-release cycles (in % of maximal values at optimum frequency). Conditions: fibre bundles (6 fibres) in ATP-salt solution (cf. legend Fig. 1)

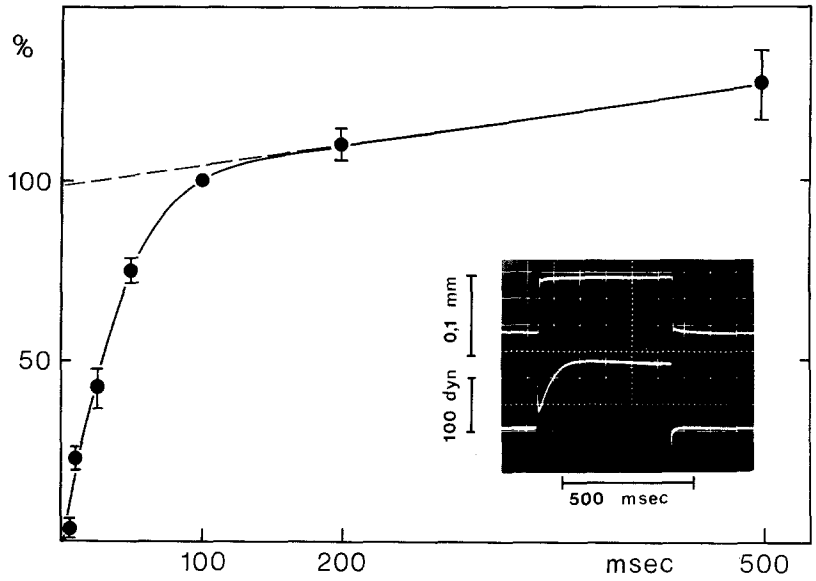

Fig. 3. Stretch-induced ^{32}P-incorporation into ATP as a function of stretch duration (time course of incorporation following quick stretch). In each experiment the amount of incorporation was measured after incubating the fibre bundles for 60 min during which it was subjected to repetitive rectangular stretch-release cycles (1 Hz, amplitude = 1% L_0) of the duration indicated on the abscissa. Results expressed in % (mean ± S.E., $n = 6$) of effect obtained with 100 ms stretches. Incubation conditions as in Figure 1. Inset: time course of tension following a quick stretch

Table 1. Dependence of stretch-induced incorporation rate in dependence on the amplitude (% L_0) of square wave stretches. Incorporation rate mean values ± S.E. in % of the reference value obtained at 1% L_0 (= 100%). Incubation conditions as in Figure 1

Amplitude (%L_0)	0.25	0.50	0.75	1.0	2.0	3.0
Rel. incorp.	13.3 ± 1.5	31.6 ± 7.8	70.0 ± 9.5	100	132.0 ± 6.3	150.5 ± 16.4

These results are not in agreement with the earlier hypothesis (Hill and Howarth, 1959; Mannherz, 1970) that the stretch caused a reversal of the chemomechanical energy transformation coupled with the resynthesis of ATP. Rather, the experimental results and in particular the parallelism of ATP splitting rate and formation of ATP-γ-^{32}P under a variety of conditions may be taken to indicate an ATP-P_i exchange reaction occurring on the level of a high-energy intermediate such as the enzyme-ADP complex. Such a complex has been described and analyzed by Lymn and Taylor (1971) as well as by Trentham and his colleagues (Trentham et al., 1972).

If the incorporation of $^{32}P_i$ predominantly occurred shortly after stretching, the observed frequency dependence of the exchange reaction, in particular the increase in the rate with increasing frequency in the range below the optimum, could be accounted for, many brief stretches per unit time would be far more effective in producing incorporation than a few prolonged stretches. This prediction could be verified by studying the time course of $^{32}P_i$ incorporation after stretch or the dependence of incorporation on the duration of stretch.

The experiment shown in Figure 3 illustrates the point that the amount of $^{32}P_i$ incorporated during one stretch becomes large as the stretch duration is prolonged from 5 – 100 ms, but any further prolongation barely increases the amount of incorporation, thus suggesting that most of the "back reaction" occurs soon after stretching. The rapid initial burst incorporation occurs with a time constant of 37 ms corresponding to an apparent first order rate constant of 27 s^{-1}. This rate constant is quite similar to the rate constant of the delayed tension generation (31 s^{-1}), suggesting that the speed of the force generation and the speed of exchange reaction are coupled. Perhaps both reactions are dependent on the concentration of a "high energy intermediate" of the actomyosin-ADP-P type, which must then be assumed to accumulate transiently shortly after stretch. A quick stretch also seems to increase transiently the vertically attached crossbridge configuration as indicated by stiffness and tension measurements (Herzig, 1975).

Therefore it is tempting to speculate that this cross-bridge species corresponds – on the biochemical level – to the actomyosin-ADP high-energy intermediate, thus giving rise to the ATP-P_i exchange reaction.

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