# EXCHANGE OF ACTIN-BOUND ADP IN FROG MUSCLE DURING ACTIVITY

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## 1. Introduction

Straub and Feuer [1] described the association of ATP with G-actin and the dephosphorylation of this prosthetic nucleotide to ADP on the cation-induced polymerisation of the protein to F-actin. Martonosi et al. [2] showed that the ATP of G-actin was freely exchangeable, whereas the ADP of F-actin was firmly bound. Szent-Györgyi and Prior [3] found an exchange of actin-bound ADP in actomyosin and glycerated myofibrils during superprecipitation and contraction respectively in the presence of ATP and $Mg^{2+}$. They thus provided support for the suggestion [1] that repetitive depolymerisation and polymerisation of actin, with concomitant splitting of ATP, was a feature of muscle contraction. We recently found [4] a change of labelling during potassium contracture of bound ADP in the rectus abdominis of frogs injected with [$^{14}$C]-glucose. This observation has now been confirmed and extended.

## 2. Methods

Frogs (*Rana temporaria*) were injected subcutaneously with 25 $\mu$Ci [U-$^{14}$C] glucose. After prescribed times, the animals were pithed and the sartorius, gastrocnemius or rectus abdominis muscles dissected out and transferred to Ringer's solution. Experiments were made with paired muscles, one of which was brought into unloaded contracture in isotonic (0.765%) KCl. Test and control muscles were minced rapidly with fine scissors in Ringer's solution. For isolation of ATP, about a quarter of each muscle was extracted for 10 min with 3 ml 10% (w/v) trichloroacetic acid (TCA). After centrifugation, TCA was removed from the supernatant by 10 extractions with ether and the solution adjusted to pH 8.2 with NaOH. 0.3 ml 10% (w/v) barium acetate was added. After 16 hr, the Ba salts, including that of ATP, were spun down and dissolved in 10 $\mu$l 2 N formic acid. This solution was used for chromatography. The remaining muscle was extracted with 0.05 M $Na_2CO_3$ – 0.05 M $NaHCO_3$ for 20 min at 0°. The residue was washed 10 times with water at 0° over about 2 hr with centrifugation after each washing. The residue was stirred for 10 min with 3 ml 10% TCA. The centrifuged extract was freed from TCA by shaking with ether and freeze-dried. The residue, containing the ADP, was dissolved for chromatography in 10 $\mu$l water. The nucleotides were isolated by the two-directional paper chromatography procedure of Krebs and Hems [5]. Control runs were made with possible contaminants such as NAD and glycolytic intermediates. The ATP spot from the direct tissue extract and the ADP spot from the washed muscle residue were eluted in 1 ml water. Aliquots were taken for assay in a Locarte fluorimeter by the method of Estabrook et al. [6] and for scintillation counting by the emulsion technique of Patterson and Greene [7].

In curarisation experiments, (+)-tubocurarine (20–25 $\mu$g per g body weight) was injected subcutaneously.

F-actin with bound [$^{14}$C]ADP was prepared from the pooled leg muscles of 8 labelled frogs by the method of Mommaerts [8]. The specific activity of ADP isolated by TCA-extraction of this protein was compared with that of bound ADP from the washed residue of the same muscle.

## 3. Results

The data from 16 experiments, all but two in a

Table 1
Effects of potassium contracture on specific activities of total ATP and bound ADP in muscles of frogs injected with [$^{14}$C]glucose.

| No. | Muscle | Period of labelling | Remarks | Specific activity (counts/μmole/min) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Total ATP | | Bound ADP | |
| | | | | Control | Contracted | Control | Contracted |
| 1 | Rectus abd. | 60 min | | 250 | 310 | 60 | 420 |
| 2 | Rectus abd. | 75 min | | 840 | 5800 | 1220 | 1840 |
| 3 | Rectus abd. | 75 min | | 2140 | 4800 | 70 | 340 |
| 4 | Rectus abd. | 3 hr | | 1480 | 1850 | 720 | 1390 |
| 5 | Sartorius | 60 min | | 370 | 620 | 1070 | 470 |
| 6 | Sartorius | 75 min | | 400 | 2130 | 2430 | 430 |
| 7 | Sartorius | 75 min | | 730 | 1200 | 1020 | 410 |
| 8 | Sartorius | 3 hr | | 1600 | 2150 | 900 | 780 |
| 9 | Sartorius | 18 hr | | 720 | 1300 | 1520 | 1100 |
| 10 | Gastrocnemius | 3 hr | | 300 | 3130 | 2500 | 1000 |
| 11 | Rectus abd. | 60 min | pre-curarised 1 hr before [$^{14}$C]glucose injection | 1140 | 1600 | 0 | 850 |
| 12 | Rectus abd. | 60 min | | 120 | 330 | 0 | 300 |
| 13 | Rectus abd. | 60 min | | 550 | 1550 | 0 | 770 |
| 14 | Rectus abd. | 60 min | | 8550 | 16300 | 0 | 4350 |
| 15 | Sartorius | 60 min | | 110 | 210 | 410 | 160 |
| 16 | Gastrocnemius | 6 hr | curarised 2 hr after [$^{14}$C]glucose injection | 350 | 270 | 7780 | 4250 |

Notes: Nos. 1–9 and 11–15 constitute a series of consecutive experiments. The rectus and sartorius muscles were taken from the same animal in nos. 1 and 5, 2 and 6, 3 and 7, 4 and 8, 12 and 15.

consecutive series, are shown in table 1. In short-term labelling experiments, the increased metabolic rate brought about by contracture gives an increased labelling of the ATP. The specific activity of bound ADP, which in the fast sartorius, but not in the slow rectus abdominis, usually exceeds that of total ATP, is altered in contracture. With the sartorius, this change tends to be a decrease, with the rectus abdominis an increase, but nearly always to a level closer to the mean of the values for ATP in control and contracted muscles.

Pre-curarisation of frogs before injection of labelled glucose inhibits subsequent incorporation of label into bound ADP in the rectus abdominis (table 1, nos. 11–14). This inhibition is relieved when the muscles are brought into potassium contracture. The effect is not evident with other muscles (cf. no. 15), but in experiments with the gastrocnemii of animals curarised 2 hr *after* injection of [$^{14}$C]glucose and kept for a further 4 hr before killing, we have found the activity of the bound ADP to remain high, while that of the total ATP has apparently fallen off (no. 16).

Fig. 1 shows the results of all experiments in potassium contracture performed up to the present, excluding a few, such as nos. 3 and 16 in table 1, where there was a difference of an order of magnitude between the specific activities of bound ADP and total ATP. The plot is designed to illustrate the finding that, in contracture, the bound ADP tends to equilibrate with a nucleotide pool, the mean specific activity of which is not very different from the mean of the specific activities of ATP before and after contracture. The regression line (slope −0.9), arbitrarily taken as rectilinear, should have corresponded with the abscissa axis if contracture had been without

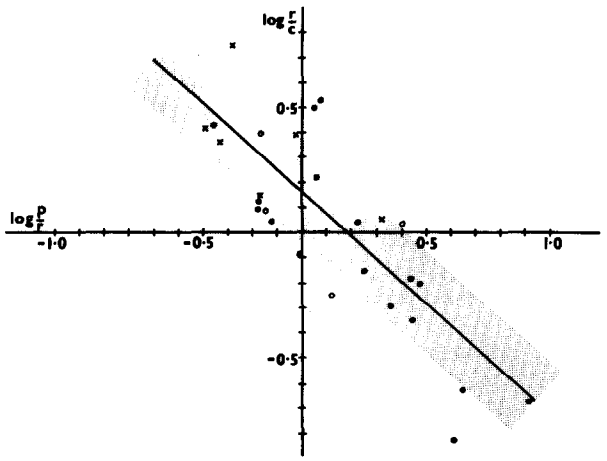


Fig. 1. Effect of potassium contracture on specific activity of actin-bound ADP in muscles of frogs injected with [$^{14}$C]glucose. Abscissae: $\log(p/r)$. Ordinates: $\log(r/c)$. $p$ = mean of specific activities of total ATP in control and contracted muscles, $r$ = specific activity of bound ADP in control muscle, $c$ = specific activity of bound ADP in contracted muscle. ● rectus abdominis, X sartorius, ○ gastrocnemius. Six results are taken from ref. [4]. The regression line is fitted by the method of least squares. The shaded area represents the standard deviation about the line. For $p = c$, the line should have a slope of $-1$ and pass through the origin.

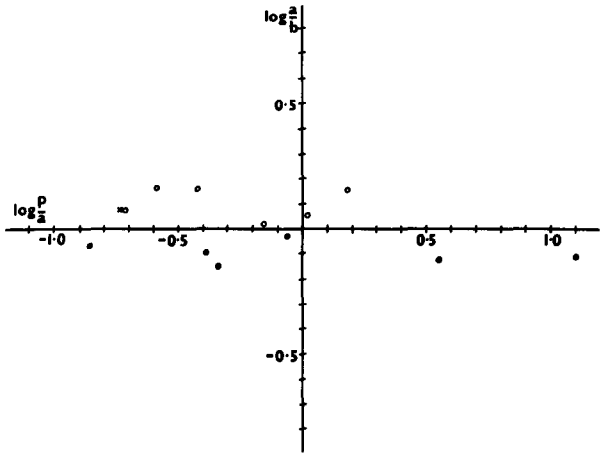


Fig. 2. Plot analogous to fig. 1 for blank experiments with paired, uncontracted muscles. Abscissae: $\log(p/a)$. Ordinates: $\log(a/b)$. $p$ = mean of specific activities of ATP in paired muscles, $a$ and $b$ = specific activities of bound ADP in the individual muscles. The role of control muscle is so allocated that points for recti appear below and those for other muscles above the abscissa axis.

effect on the activity of bound ADP.

An impression of the errors in the experiments is given by an analogous plot (fig. 2) for blank determinations on paired, uncontracted muscles. Each point in the figures incorporates the errors of 4 nucleotide assays and 4 activity determinations. An additional error lies in differences between paired muscles, which could be enhanced by mechanical stimulation.

The identity of the bound ADP with actin-bound ADP has been made probable by the procedure given in the "Methods" section. In two experiments, ADP isolated from washed muscle, and from actin prepared from the same muscle, showed the same specific activity to within 10%.

## 4. Discussion

In frogs injected with [$^{14}$C]glucose, the actin-bound ADP rapidly becomes labelled and, in fast muscles, may soon assume a specific activity higher than that of the total ATP of the tissue. It is clearly in exchange with a nucleotide pool undergoing rapid turnover through *de novo* synthesis. Exchange of actin-bound ADP *in vitro* occurs only by replacement of the nucleotide with ATP, which depolymerises the protein to G-actin. The ATP is then split, in the presence of cations, to ADP and orthophosphate, with reconversion of the protein to the polymerised F-form [1,2]. The ADP exchange is clearly energy-dependent. If the same conditions held in living muscle, it would be a wasteful process, unless the depolymerisation of actin were an essential feature of contraction.

In the rectus abdominis, where labelling of bound ADP is sluggish, curarisation completely inhibits exchange of this substance, which suggests that in this slow postural muscle the exchange is indeed dependent upon contractile activity. Such clear-cut inhibition has not been found with the sartorius and the gastrocnemius. Whether this is due to incomplete neuromuscular block in these muscles, or to an essential difference between the economies of phasic and tonic muscles, we cannoy firmly conclude. The retention of radioactivity by bound ADP in the gastrocnemii of frogs curarised after injection of [$^{14}$C]glucose and kept until the activity of ATP has declined, indicates that the former alternative is to be preferred.

While the demonstration of intensified exchange of

bound nucleotide during potassium contracture of the extirpated muscles is in itself inconclusive, the initiation of such exchange by the same process in the rectus abdominis of curarised animals may be taken as *prima facie* evidence that, during contraction, the actin enters into a repetitive depolymerisation–polymerisation cycle. This evidence is in direct conflict with that of Martonosi et al. [9].

One is tempted to enquire, in fact, whether the enzymic function of myosin in muscle may be not that of an ATPase, but rather that of an ADP-kinase with actin-bound ADP as substrate.

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