

## THE pCa-TENSION CHARACTERISTICS OF SINGLE SKINNED FIBRES ISOLATED FROM THE ANTERIOR AND POSTERIOR LATISSIMUS DORSI MUSCLES OF THE CHICKEN

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The anterior latissimus dorsi (ALD) muscles of the chicken are mostly composed of multiply innervated slow fibres, and the posterior latissimus dorsi (PLD) muscles consist mostly of focally innervated fast fibres (Ginsborg, 1960). The rate of development of isometric tension is 8–10 times higher in the PLD than in the ALD, and the unloaded speed of shortening is 4–5 times higher (Canfield, 1971). On tetanic stimulation ALD fibres are able to maintain a steady isometric tension for more than 2 min, whereas the PLD begins to fatigue after only 1 s (Canfield, 1971). The fatigue resistance and economy (Goldspink, Larson & Davies, 1970) of the ALD when contracting isometrically are probably related to its postural role in holding the wings against the body. The rate of relaxation of PLD fibres following short tetani is eight times faster than for the ALD (Canfield, 1971). This difference is attributable in part to the less highly developed sarcoplasmic reticulum in the ALD (Page, 1969). The present study investigates the pCa-tension relationship of these fibres.

Male chickens (breed Thornber 404: Light Sussex cross Rhode Island Red), aged 90–100 days were used in all experiments. Single fibre segments (~3 mm length, 50–100  $\mu\text{m}$  diameter) were dissected from the anterior and posterior latissimus dorsi muscles. Fibres were isolated and chemically skinned for 30 min with 1% Brij (polyoxy-ethylene 20 cetyl ether) in relaxing solution as described previously (Altringham & Johnston, 1982). The basic relaxing solution contained 10 mM imidazole pH 6.95 (at 35 °C), 110 mM-KCl, 3 mM-MgCl<sub>2</sub>, 5 mM-EGTA, 10 mM phosphocreatine, 2.5 mM-ATP and 20  $\mu\text{g ml}^{-1}$  creatine phosphokinase (Sigma, Poole, England). Activating solutions were made by addition of 0–5 mM-CaCl<sub>2</sub> to the basic relaxing solution. The concentrations of the various ionic species were calculated using an iterative computer programme (Perrin & Sayce, 1967) as modified by White & Thorsen (1972). The sarcomere length of fibre segments was determined by laser diffraction and set to 2.3  $\mu\text{m}$ . Isometric tension was measured using an AE801 silicon beam strain gauge (A. M. E. Horton, Norway) (Fig. 1). Further details of the apparatus and method of fibre attachment are given by Altringham & Johnston (1982). All experiments were carried out at 35 °C.

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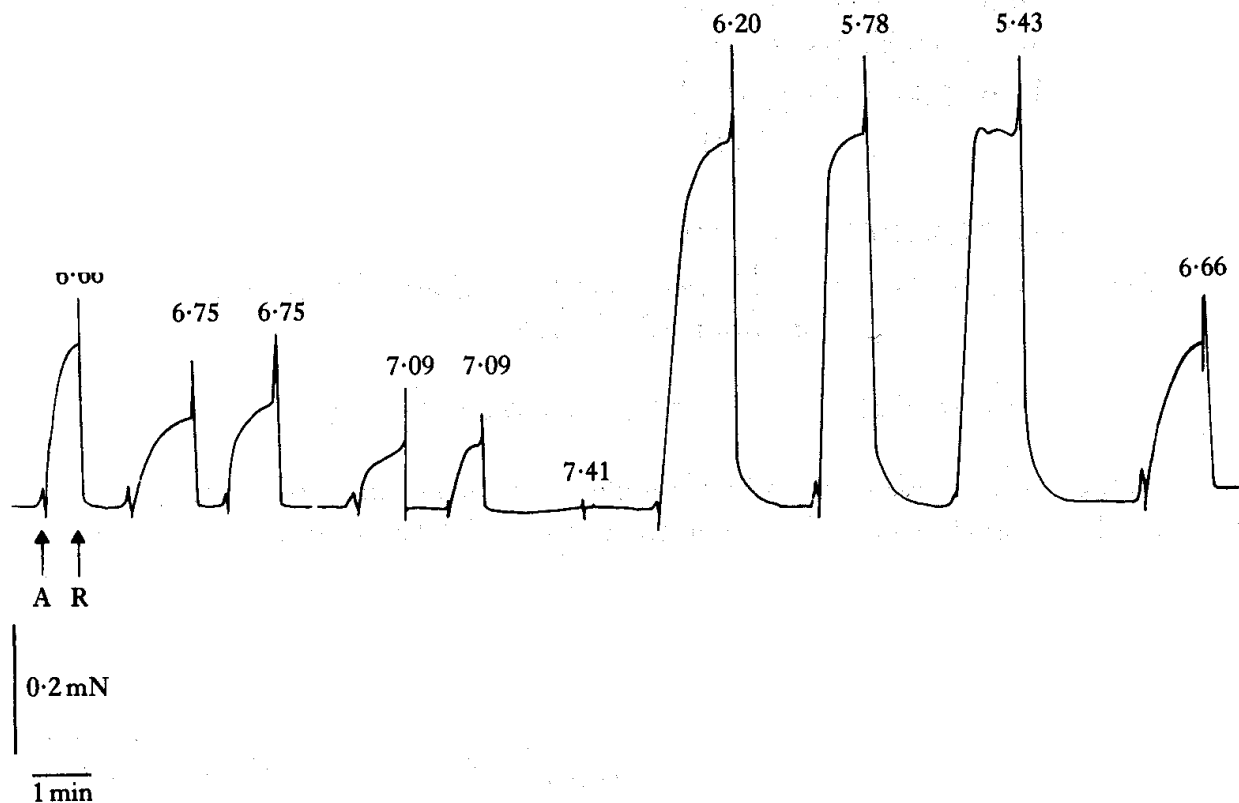


Fig. 1. A series of activations of a single ALD fibre,  $50\ \mu\text{m}$  diameter. The numbers above each isometric tension record refer to the pCa of the bathing solution. Arrows show transference of the fibre into activating (A) and relaxing (R) solutions.

Maximum isometric tensions (Mean  $\pm$  s.e.) (pCa 4.38) were  $10.0 \pm 1.5\ \text{Ncm}^{-2}$  (6 fibres) and  $11.3 \pm 1.7\ \text{Ncm}^{-2}$  (7 fibres) for ALD and PLD fibres, respectively. For both fibre types some decline in tension was observed with successive activations at high calcium concentrations (pCa 5.6) but not at low ones (Fig. 1). A similar deterioration of mechanical performance at saturating  $[\text{Ca}^{2+}]$  has been noted for various other skinned fibre preparations (Kushmerick & Krasner, 1982). To investigate the pCa-tension relationship, fibres were transferred in a random order to baths containing different free  $\text{Ca}^{2+}$  concentrations (Fig. 1). Sarcomere length was monitored throughout each experiment and reset to  $2.3\ \mu\text{m}$  if necessary. Any decay in tension with successive activations was monitored by maximal or near maximal activations (two or three per series) performed at intervals during the experiment. In this way the fall in  $P_0$  with each activation could be determined and used to obtain normalized values of  $P/P_0$  (Fig. 3). Fibres were discarded if  $P_0$  fell below 80% of the maximal isometric tension of the first activation.

A steep sigmoidal relationship between free  $[\text{Ca}^{2+}]$  and tension generation was

Fig. 2. The effect of successive activations on tension generation by an ALD fibre ( $72\ \mu\text{m}$  diameter) at pCa 5.31 and pCa 6.39. The inset shows records from activations 6 and 7 at pCa 6.39. A, activating solution; R, relaxing solution.

Fig. 3. pCa-tension relationship of PLD ( $\blacktriangle$ ) and ALD ( $\bullet$ ) fibres. (A) All the data (see text), and (B) paired data from five ALD and five PLD fibres. Values are mean  $\pm$  s.e. of mean.

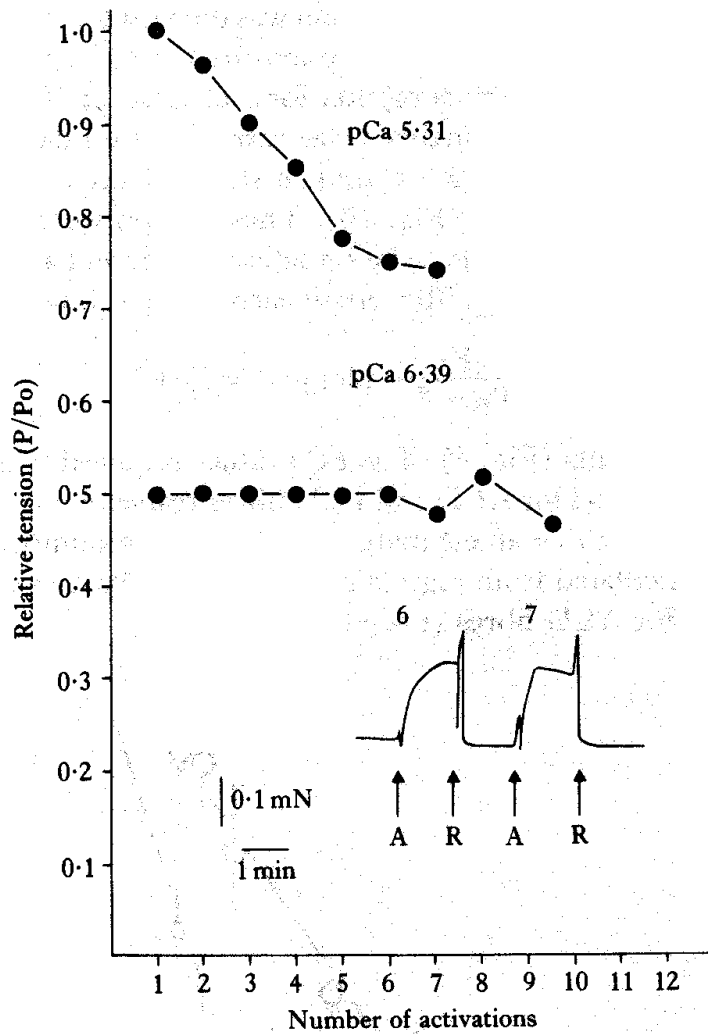


Fig. 2

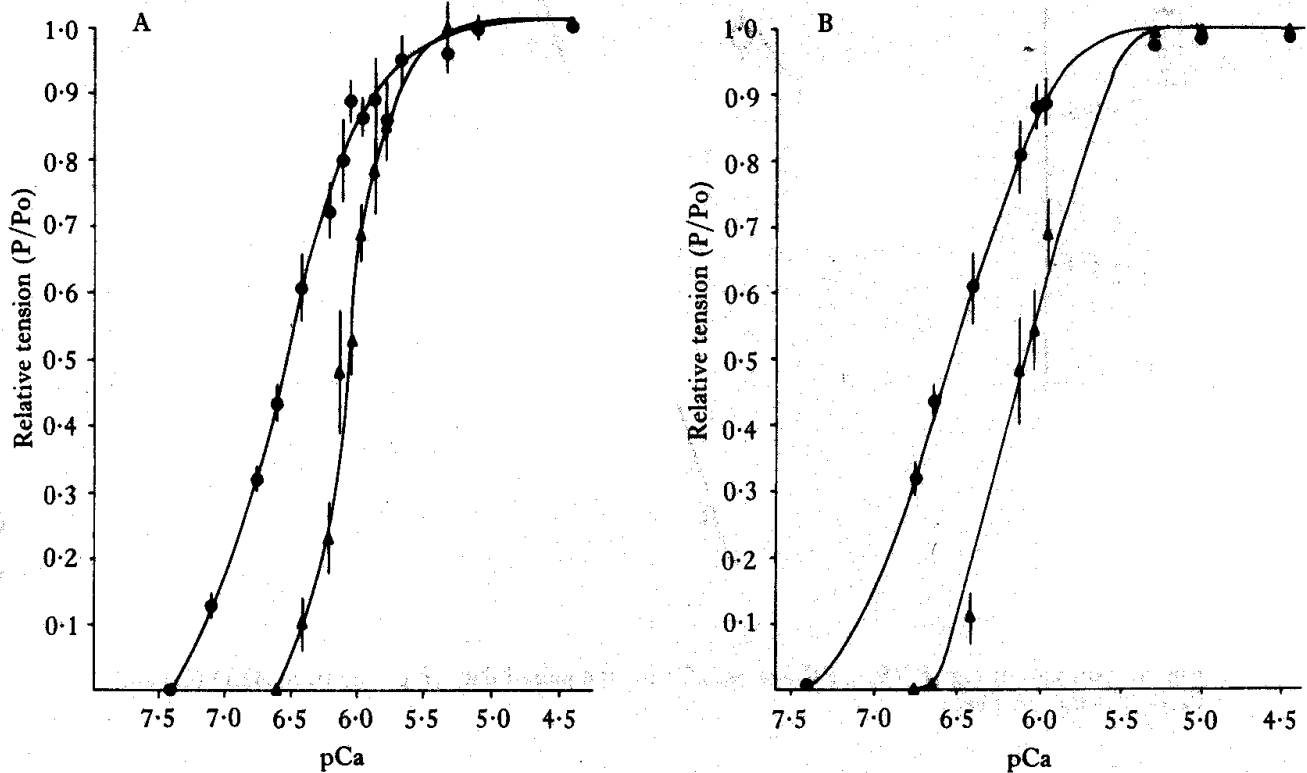


Fig. 3

obtained. The threshold  $[Ca^{2+}]$  for contraction was  $0.03 \mu M$  for ALD and  $0.23 \mu M$  for PLD fibres. Both fibre types were maximally activated at  $2-5 \mu M-Ca^{2+}$ . A 3rd degree polynomial equation was fitted by computer for each data set. The data for 16 fibres are shown in Fig. 3A. Statistical analyses was carried out on data from each of five ALD and five PLD fibres for which complete series of activations were obtained (eight different pCa's for each set) (Fig. 3B). These curves were subjected to a two-factor analysis of variance and found to be statistically different at the  $P < 0.001$  level ( $FC_{1,8} = 41.869$ ). The data in Fig. 3B were linearized according to the Hill equation

$$\log_{10} \frac{(P)}{P_0 - P} = n \log_{10} [Ca^{2+}] + h$$

where  $n$  and  $h$  are constants (Fig. 4). The pCa values required to give half maximal tensions were  $6.56$  and  $6.08$  for ALD and PLD fibres respectively. The constant  $n$  in the Hill equation may provide an estimate of the minimum number of  $Ca^{2+}$  binding sites. Values for  $n$  calculated from regressions of  $\log_{10} (P/P_0 - P)$  vs pCa were  $3.0$  for PLD fibres and  $1.5$  for ALD fibres ( $r = -0.99$ ).

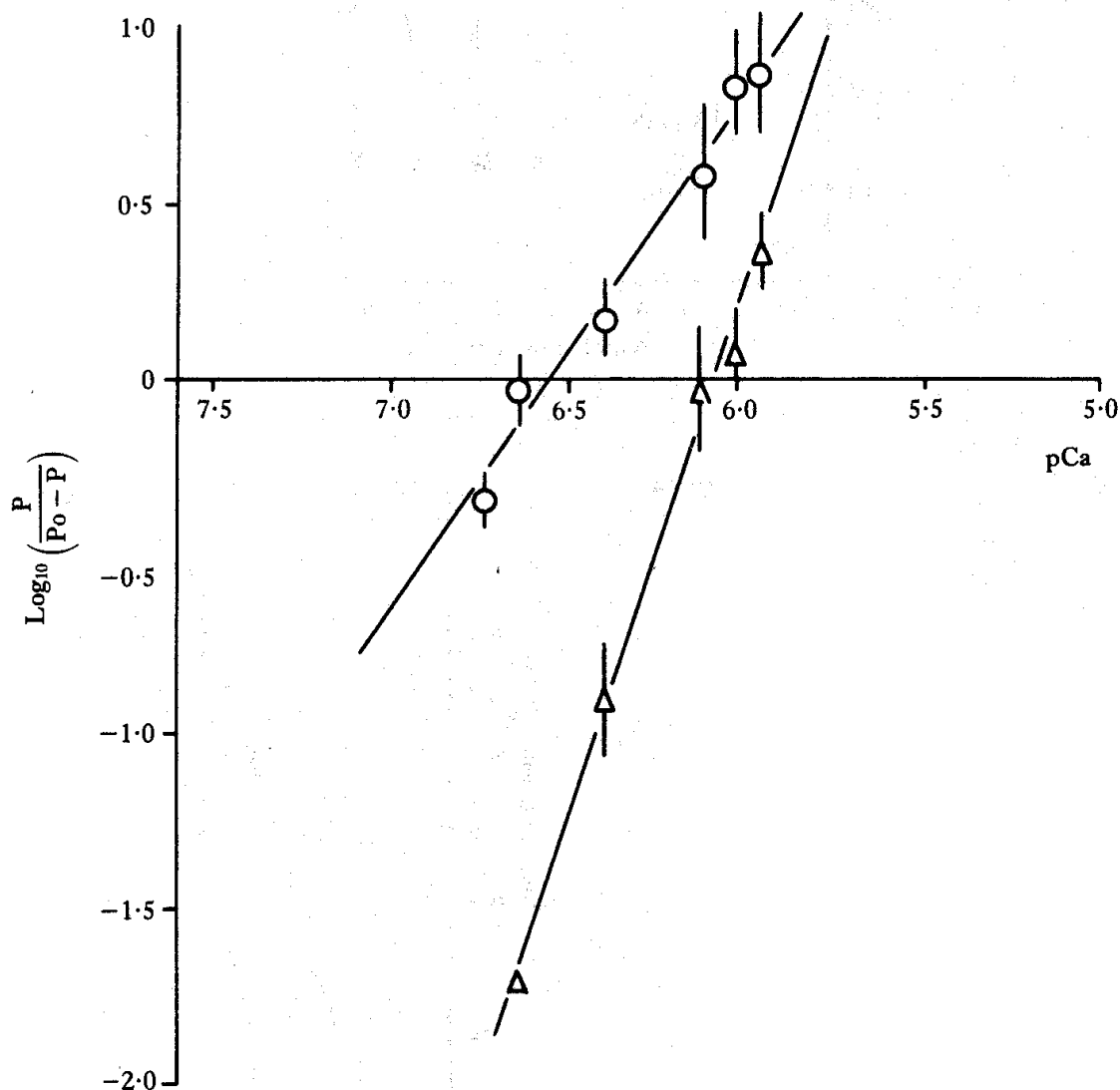


Fig. 4. Hill plot of  $\log [P/(P_0 - P)]$  against pCa for the paired data (Fig. 3B) from ALD (○) and PLD (△) muscle fibres.

*In vivo* pMgATP and pMg are thought to be in the millimolar range (Polimeni & Page, 1973; Cohen & Burt, 1977). The  $\text{Ca}^{2+}$ -sensitivity of skinned muscle fibres is rather insensitive to changes in pMgATP within this concentration range but is affected by relatively small changes in pMg (Donaldson & Kerrick, 1975; Ashley & Moiescu, 1977). For example, for skinned cardiac cells from rat ventricle, decreasing pMg from 4.5 to 2.5 (at constant pMgATP 2.5) increases the  $\text{Ca}^{2+}$  concentration required to produce half maximal tension by around 0.6 pCa units without significantly affecting the shape of the pCa-tension curve (Fabiato & Fabiato, 1975). In the present study, ALD and PLD fibres were compared under identical ionic conditions. The results indicate a minimum of three binding sites for  $\text{Ca}^{2+}$  for fast fibres and a minimum of two sites for slow fibres. The pCa-tension curves for fast fibres are shifted to higher free  $\text{Ca}^{2+}$  concentrations ( $\sim 0.48$  pCa units) than those for slow fibres and they also show a greater degree of co-operativity. These differences are similar to those reported for the fast and slow muscles of both rabbit (Kerrick, Secrist, Copy & Lucas, 1976) and cod (Altringham & Johnston, 1982). For example, Kerrick *et al.* (1976) found that the pCa-tension curve of adductor magnus (fast) fibres was shifted by around 0.3 pCa units to the right of that for soleus fibres (slow). These differences in  $\text{Ca}^{2+}$ -sensitivity probably reflect the presence of different isoforms of  $\text{Ca}^{2+}$  regulatory proteins in fast and slow fibres.

Each isoform of tropomyosin, troponin I, troponin C and troponin T, has a distinct chemical structure and is coded for by a separate gene (Perry, 1979). For example, two forms of troponin I have been identified in bovine fast fibres each with a different electrophoretic mobility from the characteristic forms found in slow fibres (Young & Davey, 1981). In addition to changes in the affinity of TNC for  $\text{Ca}^{2+}$ , Brandt, Cox, Kawai & Robinson (1982) have suggested that differences in the times cross bridges spend in the attached and refractory states could also contribute to shifts in the position of the pCa-tension curve. According to this hypothesis the  $[\text{Ca}^{2+}]$  required to give 0.5  $P_0$  would be affected by the ratio of the rate constant for the dissociation of the Ca-TNC complex to the rate constant for the movement of the cross bridge through the attached states (Brandt *et al.* 1982). Whatever the mechanism, these differences in pCa-tension characteristics will contribute (together with other factors such as differences in SR development, properties of the  $\text{Ca}^{2+}$ -pump, parvalbumin concentration and cross-bridge kinetics) to the more rapid rates of relaxation of chicken fast fibres than slow fibres (Page, 1969; Stephenson & Williams, 1981; Hager, Bárány, Bárány & Homa, 1982).

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