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# Tension Transients in Fibrillar Muscle Fibres as Affected by Stretch-Dependent Binding of AMP-PNP: A Teinochemical Effect?

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**Abstract.** The recovery in tension after release of a fibrillar muscle preparation as well as the fall in tension after restretch was found to be greater in presence of AMP-PNP than in its absence (rigor). The effect of AMP-PNP was concentration-dependent with an optimum at 0.1 mM corresponding to the dissociation constant of AMP-PNP from the myosin heads. This evidence supports the validity of the teinochemical principle which predicts a stretch-dependent AMP-PNP binding. The stiffness calculated per cross bridge was similar to that found by Huxley and Simmons (1971). It was further calculated that only 15% of the cross bridges are in a force-maintaining state in rigor.

**Key words:** ATP analogue – Insect fibrillar muscle – Stiffness per cross bridge – Quick release-recovery – Mechano-chemistry of muscle.

## Introduction

The relation between the tension and the stiffness of striated muscle fibres has been studied by a large number of investigators (Huxley and Simmons, 1971; Podolsky and Nolan, 1973; Julian et al., 1974; Rüegg et al., 1975; Ford et al., 1977; Yamamoto and Herzig, in press; Güth and Kuhn, 1978; cf. also Huxley, 1974). Huxley and Simmons (1971) showed that the immediate stiffness of a fibre is a measure of the number of attached cross bridges linking the myosin and actin filaments. The tension within the preparation reflects the mechanical state of the contractile elements within the cross bridges.

The ATP analogue AMP-PNP ($\beta$-$\gamma$-imido ATP) is not split by the myosin heads (Yount et al., 1971). Addition of this analogue to glycerinated fibrillar muscle fibres in the rigor state induces a decrease of isometric tension without a concomitant decrease of immediate elastic modulus (Barrington Leigh et al., 1973; Beinbrech et al., 1976). Washing out of AMP-PNP from these muscle preparations partially restores the inital rigor tension (Kuhn, 1973).

In a study demonstrating reversible transformation of chemical into mechanical energy by glycerol-extracted fibrillar insect flight muscle it was suggested that the

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affinity of cross bridges for AMP-PNP is stretch-dependent as predicted by the teinochemical principle (Kuhn, 1973). According to this principle (Kuhn et al., 1960), reagents which induce isometric relaxation are absorbed by the contractile system when it is stretched at constant thermodynamic activity of that reagent. Since tension will be reduced by this stretch-dependent binding (teinochemical effect), the teinochemical principle predicts that the elastic modulus decreases with time after the length change (Kuhn et al., 1963). After a release of the preparation AMP-PNP dissociates from some of the myosin heads. These cross bridges within the fibre should then return to the force-generating position, i.e. a state of rigor tension. Due to this effect, there should be a greater recovery of tension in presence of AMP-PNP after a release, than in its absence.

In an attempt to verify this hypothesis, quick releases of fibrillar muscle fibres in rigor solution and in the presence of AMP-PNP were compared as reported in the present paper. The study was designed to check the validity of the teinochemical principle. The effects of AMP-PNP were analysed at the level of the cross bridge in terms of the stiffness per cross bridge and the number of cross bridges attached to actin in rigor.

## Methods

Dorsal longitudinal muscle fibres from Lethocerus maximus were chemically "skinned" by extraction in a 50/50 v/v glycerol-water mixture pH 7 and then stored for up to 5 months in the extraction solution at −16° C.

Rigor tension was generated following the procedure of White (1970): the bundles (6–10 fibres) were first immersed in an ATP-saline at low $Ca^{2+}$ concentration (15 mM ATP, 15 mM $MgCl_2$, 1 mM $NaN_3$, 20 mM histidine, 10 mM KCl, 4 mM EGTA, pH 6.7, 18° C) in which they relaxed. After removal of the MgATP by incubation of the bundle in an ATP-free rigor solution (1 mM $NaN_3$, 20 mM histidine, 20 mM KCl, 4 mM EGTA, pH 6.7, 18° C, $I = 0.06$ M) rigor tension was isometrically generated. After addition of AMP-PNP (adenylyl-imidophosphate purchased from Boehringer, Mannheim) the ionic strength of the AMP-PNP salines was adjusted to $I = 0.06$ M with KCl. To prevent disproportionation of ADP (AMP-PNP was contaminated with up to 5% ADP) into ATP and AMP by the myokinase still active in glycerol-extracted fibres (Abbott and Leech, 1973) the myokinase inhibitor $P^1$, $P^5$-di-(adenosine-5′)-pentaphosphate (Feldhaus et al., 1975) purchased from Boehringer Mannheim was added to the AMP-PNP containing solution in a final concentration of 0.1 × [AMP-PNP].

### *Mechanical Measurements*

For measurements of tension the bundles (6–10 fibres) were glued between two glass rods. One glass rod was connected to a RCA 5734 transducer (resonance frequency 1.5 kHz, compliance 0.75 μm/mN) or to a strain gauge (Aksjeselskapet, Type AE 802, resonance frequency 3.0 kHz, compliance 1.5 μm/mN). The force transducer was fixed on a micromanipulator the position of which could be moni-

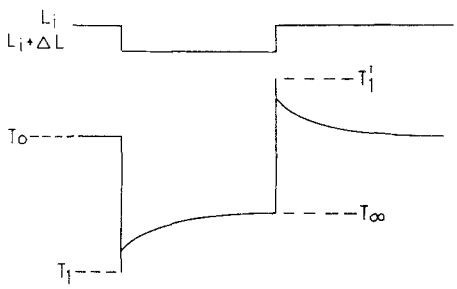


**Fig. 1.** Schematic representation of tension transients following a release and restretch to $L$; within 5 min; $T_0$ and $L_i$ are tension and length before release. $T_1$ the extreme tension reached during release or restretch of amplitude $\Delta L$. $T_\infty$ is the asymptotic tension value reached after equilibration in the released state. In the presence of AMP-PNP the amplitude of tension recovery following release ($T_\infty - T_1$) as well as the amplitude of tension fall following restretch ($T_1' - T_0$) are greater than in its absence, i.e. static stiffness, $(T_0 - T_\infty) \times L_i/\Delta L$, is greater in the absence of AMP-PNP (rigor) than in its presence

tored by the displacement of a Grass force transducer FTOC3. The second glass rod was connected to a Ling Dynamics 101 Vibrator which could perform length changes within 4 ms. The position of this second glass rod was measured by resistors sensitive to a magnetic field (Siemens, FP17L100).

Immediate elastic modulus ($E_i$) was measured when fast length steps (rise time 4 ms, duration less than 100 ms) of amplitudes $\pm$ 5 μm, $\pm$ 10 μm, $\pm$ 15 μm were applied to fibre bundles of initial length ($L_i$) varying between 4.8 and 5.2 mm. The extreme tensions reached during quick length changes were then plotted in lengthtension diagrams ($T_1$ curves, cf. Huxley and Simmons, 1971). The immediate elastic modulus was calculated from the product of the slope of the $T_1$ curve at $L_i \times$ initial length [$E_i = (T_1 - T_0) \times L_i/\Delta L$, cf. Fig. 1].

For estimations of static elastic moduli ($E_{\text{stat.}}$) of the fibres it is important that the bundle is *not* stretched beyond its elastic limit. Since the yield point of chemically skinned fibres from Lethocerus maximus is practically identical to the isometric rigor tension (or to the isometric tension maintained in fibres irrigated with AMP-PNP; Kuhn, 1978), only releases should be applied for static measurement of the elastic modulus.

Static elastic modulus was calculated by

$$E_{\text{stat.}} = (T_\infty - T_0) \times L_i/\Delta L\,,$$

where $T_0$ (cf. Fig. 1) is the tension (force per fibre) before the release, $T_\infty$ is the tension observed 5 min after the release was performed, $L_i$ is the initial length and $\Delta L$ is the amplitude of the length change.

## Results

A glycerol-extracted bundle containing eight fibres from Lethocerus maximus dorsal longitudinal muscle relaxed (*A*, Fig. 2) when it was immersed in a bath (2 ml, 18° C) containing a MgATP saline at low $Ca^{2+}$ concentration (4 mM EGTA). By increas-

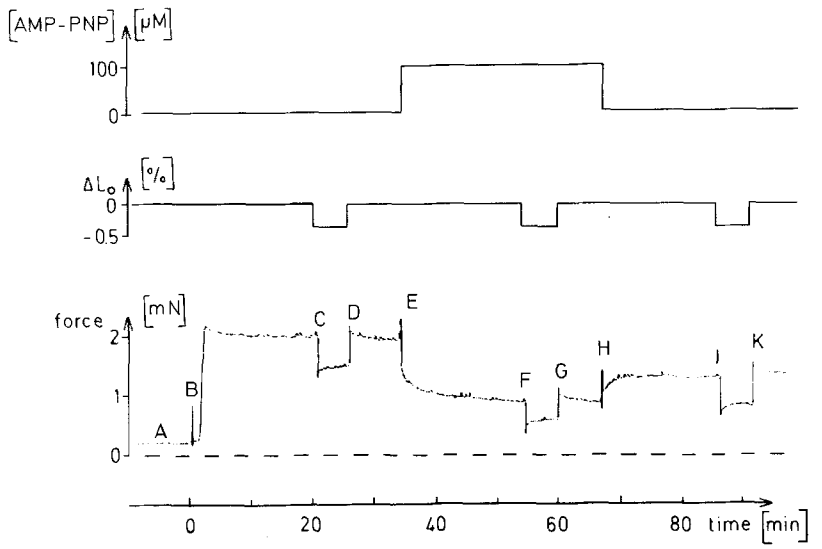


**Fig. 2.** Changes in the force exerted by a bundle of eight fibres when releases and restretches (0.3% $L_0$) were performed in absence and in presence of AMP-PNP. The sequence *A–K* is described in detail in text. Note the much more pronounced recovery in presence of AMP-PNP (trace *F–G–H*) than in its absence (*C–D–E*). Conditions: pH 6.7; $I = 0.06$ M; 18° C

ing the length of the bundle, tension was adjusted to 10 μN/fibre: in this particular preparation this value was reached at $L_i = 5.15$ mm.

In the relaxed state, stiffness of this fibrillar muscle preparation was low ($E_i = 1.5$ mN/fibre, $E_{stat.} = 1.1$ mN/fibre). Washing out the MgATP under maintenance of a low $Ca^{2+}$ concentration (4 mM EGTA) by suspending the bundle in a MgATP-free rigor solution caused the fibre tension to increase under isometric conditions. Tension reached a maximal value 3 min after incubation in rigor solution and then fell to a plateau value (240 μN/fibre) which was reached within the following 5 min. If the bundle (still suspended in rigor solution) was quickly released by 0.3% $L_i$ (release completed within 4 ms, at *C*, Fig. 2), a sudden fall of tension in phase with the length change was induced. There was a subsequent rise in tension which reaches a plateau (205 μN/fibre) over a period of 5 min. The difference between the tension value before the release and the plateau tension value reached in the released state divided by the relative length change gives the value of the static elastic modulus (13 mN/fibre) of the initial rigor state.

A restretch of the preparation to $L_i$ (at *D*, Fig. 2) resulted in an increase in tension in phase with the length change. This increase in tension was equal to the decrease induced by the release at *C*, i.e. the peak tension at *D* was higher than that observed before the release at *C*. Tension subsequently fell within 5 min to the initial value before release.

After this return of tension, 0.1 mM AMP-PNP was added to the bath (*E*, Fig. 2). There was a gradual fall in tension over about 5 min, again reaching a plateau value (95 μN/fibre). The bundle was then again released by 0.3% $L_0$ (*F*, Fig. 2) resulting in further sudden decrease of tension. Subsequently there was a larger

recovery of tension in AMP-PNP solution than in rigor solution, but the plateau values were reached in a similar time in both cases (cf. also Kuhn, 1977b). In consequence, static stiffness (measured 5 min after release) has a lower value in AMP-PNP solution than in rigor solution. Subsequent restretch of the muscle fibre in AMP-PNP saline to $L_0$ (at *G*, Fig. 2) again results in an increase in tension. This increase in tension is equal to the decrease during release (at *F*). Subsequent to the restretch there is a gradual return of tension to the inital tension value in AMP-PNP saline.

If the AMP-PNP is subsequently washed out from the fibres by immersing them once more in rigor solution (at *H*, Fig. 2) tension increases under isometric conditions. Over a period of 15 min tension reaches a new plateau in rigor solution (140 μN/fibre) which is lower than the initial rigor tension. Thus, rigor tension is partially restored after washing out the AMP-PNP from the preparation. A 0.3% $L_0$ release (at *I*, Fig. 2) and restretch (at *K*) of the bundle result in tension changes which are quantitatively nearly the same as those observed for the initial rigor state (cf. *C*, *I* and *D*, *K* respectively). This indicates that the static elastic modulus increased to the initial rigor value when AMP-PNP is washed out from the fibres.

Figure 3 shows the tension responses to quick releases for the same experiment as in Figure 2 for a much faster sweep time of recording (the letters *C*, *F*, *I*, of Fig. 2 corresponds to those of Fig. 2). The elastic tension responses and the tension recovery within the first 100 ms after release did not change appreciably when AMP-PNP was added or removed from the fibres. Thus, in contrast to isometric tension, the immediate elastic modulus did not appreciably depend on the presence or absence of AMP-PNP (0.1 mM, 18° C). In rigor, immediate elastic modulus was 21.4, 20.3, 20.4 mN/fibre at *C*, *I*, *M*; in bundles irrigated with AMP-PNP it was 19.7; 20.2 mN/fibre at *F* and *N*. On the other hand, isometric tension levels were greatly affected by the presence or absence of AMP-PNP; isometric rigor tension was 240, 165, 170 μN/fibre at *C*, *I*, *M*. Isometric tension levels of fibres loaded with (0.1 mM) AMP-PNP were 115, 112 μN/fibre at *F*, *I*. When AMP-PNP was then repeatedly added and removed from the fibres up to ten times, the mean tension level at $L_i$ was $165 \pm 5$ and $112 \pm 3$ μN/fibre for fibres in rigor and for fibres irrigated with (0.1 mM) AMP-PNP respectively whereas immediate elastic modulus was $20.4 \pm 0.3$ and $19.3 \pm 0.5$ mN/fibre ($\pm$ SD, $n = 10$). This finding indicates that once it has been conditioned by irrigating it with AMP-PNP a fibre subsequently responds reversibly on the addition and removal of AMP-PNP.

Figure 4 shows the effect of varying the amplitude of the length change (given as length change per half sarcomere; initial sarcomere length = 2.4 μm; Reedy, 1968) on the extreme tensions reached during the length change (▲, △, ▽) and the tension plateau reached 5 min after a release (●, ○, □). The bundle (6 fibres, 18° C) was first conditioned by twice adding and removing AMP-PNP at a concentration of 0.5 mM. Subsequently the length tension diagrams for fibres in rigor (●, ▲), fibres irrigated with 0.4 mM AMP-PNP (□, ▽) and 0.1 mM AMP-PNP (○, △) were recorded. In each case, a straight line can be drawn through the points plotted.

Plots of values for extreme tension ($T_1$ curves) reached during the length changes (▲, △, ▽) enable immediate stiffness to be calculated. Since the small deviations of the $T_1$ values from a straight line seem to be negligible, Figure 4 indicates that Hooke's law is fulfilled. Extrapolation of the $T_1$ curves to the abscissa gives a value

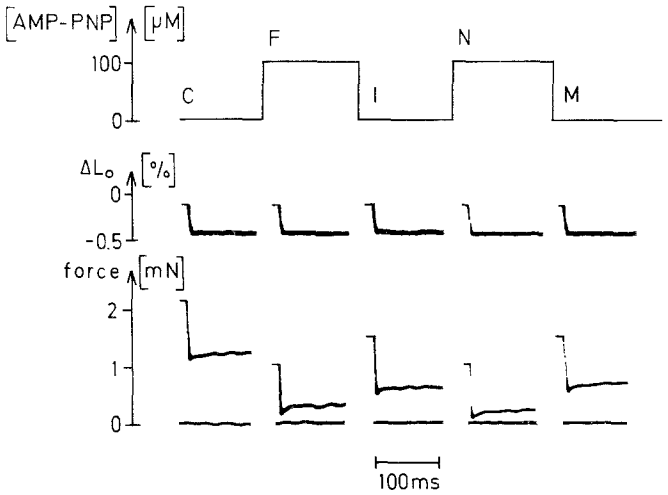


**Fig. 3.** Force values reached during releases performed in absence (*C*, *I*, *M*) and in presence (*F*, *N*) of AMP-PNP (cf. also Fig. 2). Note that once conditioned by AMP-PNP (*F*) the fibre subsequently responds reversibly on removal and readdition of AMP-PNP. Releases (followed by restretches, not shown) repeated alternatively in absence (*I*, *M*) and presence (*N*) of AMP-PNP do not give rise to appreciable changes in forces induced by the release. Same fibre bundle and conditions as in Figure 2

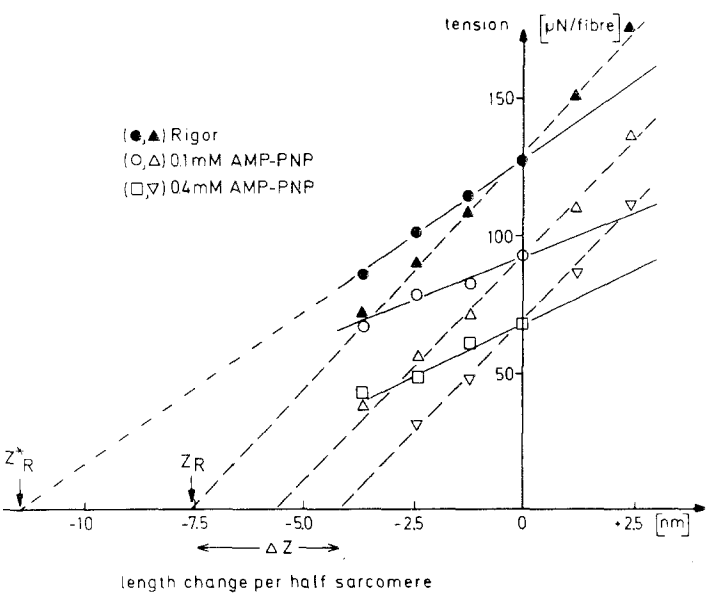


**Fig. 4.** Tension-length diagram in presence and absence of AMP-PNP. $T_1$ curves are plotted with triangles, while $T_\infty$ curves are plotted with circles and squares (see key). The points of intersection of the $T_1$ with the abscissa give the respective lengths at zero tension. Explanation see text. Conditions: Bundle of 6 glycerol-extracted dorsal longitudinal fibres from Lethocerus maximus; pH 6.7; $I = 0.06$ M; 18° C

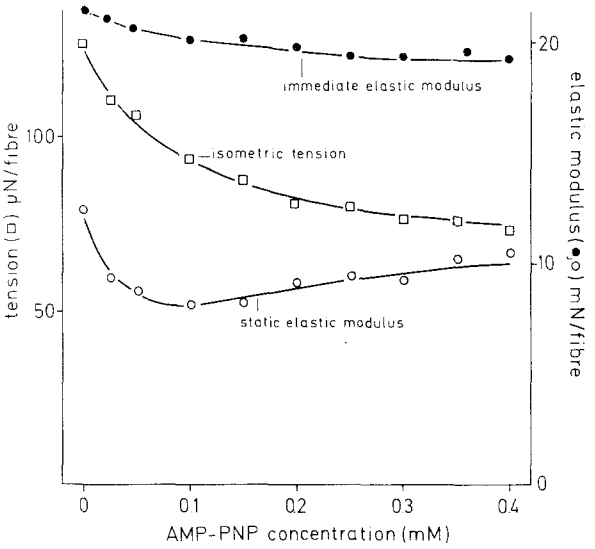


**Fig. 5.** Values of immediate elastic modulus, isometric tension and static elastic at different AMP-PNP concentrations (mean values from twelve experiments). Conditions: Bundles of 5–6 fibres; pH 6.7; $I = 0.06$ M; 18° C

of $Z_R = 7.5$ nm for the rigor state and 4.5 nm for the highest concentration of AMP-PNP (0.4 mM) used. Thus, AMP-PNP induces a right handed parallel shift of the $T_1$ curve of $\Delta Z = 3$ nm per half sarcomere.

The values of plateau tension ($T_\infty$, cf. Fig. 1) reached 5 min after releasing the fibre fit straight lines fairly precisely when plotted against length change. The slopes of the respective straight lines give the static stiffness for fibres in rigor (●), 0.4 mM AMP-PNP (□) and 0.1 mM AMP-PNP (○). Static stiffness is greatest in rigor (13 mN/fibre), although its value is lower than immediate stiffness. Static elastic modulus in 0.1 mM AMP-PNP is much lower (8.4 mN/fibre), but rises again in 0.4 mM AMP-PNP (10.7 mN/fibre). Extrapolation of the $T_\infty$ curve to the abscissa gives a value of $Z_R^* = +12$ nm for the rigor state. The experimental data are summarized in Figure 5. The mean values from a total of twelve experiments are plotted. The immediate elastic modulus remains high even when the AMP-PNP concentration is raised. In contrast the static elastic modulus falls to a minimum around 0.1 mM AMP-PNP and rises steadily up to 0.4 mM AMP-PNP. The isometric tension falls monotonically to reach approximately half its value in rigor at 0.4 mM AMP-PNP.

## Discussion

### *Effect of* AMP-PNP *on Isometric Tension*

Biochemical binding studies of AMP-PNP to the myosin and to the $S_1$ fraction of myosin (Schaub et al., 1975) have revealed that this ligand binds to the active centre of the myosin heads, but that AMP-PNP is not split by myosin (Yount et al., 1971). The reduction in tension induced by AMP-PNP under isometric conditions is further

evidence of an interaction between this ligand and the myosin heads. Figures 2 and 3 showed that this binding reaction is chemically reversible.

If it is assumed that *one* ligand binds to one myosin head (cf. Marston et al., 1976) and the decrease in tension is proportional to the number of bound ligands then the dissociation of AMP-PNP from the myosin heads can be described by the dissociation constant $K_d$ and the following relationship holds:

$$\Delta T = T_R - T = \Delta T_0 \times c/(c + K_d)\,. \tag{1}$$

According to Equation (1) the tension difference between the tension ($T$) at the analogue concentration ($c$) used and rigor tension ($T_R$) would be hyperbolically related to the AMP-PNP concentration ($c$). The solid line through the isometric tension values of Figure 5 is a Gaussian fit with respect to Equation (1), so that the conditions for the validity of Equation (1) are fulfilled. This fitting procedure gives a value for the dissociation constant $K_d = 85$ μM. Binding studies of AMP-PNP to the active centre of myosin in glycerol-extracted dorsal longitudinal muscle of Lethocerus cardofanus gave a value of $K_d = 115$ μM (Marston et al., 1976). From equatorial x-ray diffraction studies a value for $K_d$ of 95 μM was calculated (Goody et al., 1976). All three methods of investigations give rise to approximately the same value for $K_d$. It may therefore be concluded that the difference $\Delta T = (T_R - T)$ between rigor tension and tension in the analogue concentration used is proportional to the number of myosin heads loaded with AMP-PNP. As can be seen from Figure 5, the immediate elastic modulus is unaltered up to 0.4 mM AMP-PNP despite the concomitant decrease in isometric tension. In view of the relation mentioned in the Introduction between immediate elastic modulus and number of attached cross bridges, this confirms the suggestions made by Beinbrech et al., 1976, namely that AMP-PNP induces pseudorelaxation, i.e. a reduction in tension without change in the number of attached cross bridges. There are therefore at least two possible states of cross bridge attachment to actin filaments. It is probable that there is a transition from the angled to the perpendicular conformation in presence of AMP-PNP.

### *Teinochemical Effect as Reflected by Change in Static Stiffness*

The teinochemical principle (Kuhn et al., 1960) predicts that an additional amount of ligand will be bound to the fibre when it is stretched in a saline containing AMP-PNP. Bound AMP-PNP should be released into the saline when the fibre bundle is released. This prediction could recently be verified directly (Kuhn, 1977). When glycerol-extracted dorsal longitudinal fibres from Lethocerus maximus were stretched, the AMP-PNP concentration in the incubation bath decreased, whereas it increased when the preparation was released. From the concentration differences observed it was calculated that

$$\Delta N = 0.35 \text{ pmoles AMP-PNP} \tag{2}$$

are additionally bound to a fibre of 1 cm length immersed in a 0.1 mM AMP-PNP saline solution when it is stretched by 0.5% $L_0$. This teinochemical effect of the

AMP-PNP should influence the tension transient induced by a length change. When a fibre bundle is quickly released, part of the AMP-PNP is discharged from the myosin heads. Consequently tension recovery of fibres incubated in AMP-PNP solutions (Fig. 2, *F–G–H*) should be enhanced relative to fibres in rigor (Fig. 2, *C–D–E*), depending on the amount of AMP-PNP released from the myosin heads. Hence static stiffness should be lower for fibres charged with AMP-PNP than for fibres in rigor.

*Quantitative Treatment of the Teinochemical Effect*

From Equation (1), the effect of AMP-PNP on static stiffness may be described quantitatively as follows: at constant AMP-PNP concentrations ($c$), the amount of AMP-PNP bound to the myosin may be changed only when $\Delta T_0$ and/or the dissociation constant $K_d$ is altered by changing the fibre length. Changes of the axial strain component ($\varepsilon$), i.e. relative length, of the fibre would result through binding of AMP-PNP in a change in the difference ($\Delta T = T_R - T_0$) between rigor tension and the tension in AMP-PNP solution of a given concentration. Since immediate stiffness is practically unaffected by stretch (Fig. 4) or by adding AMP-PNP (Fig. 5) the difference ($\Delta E_{\text{stat.}}$) between static elastic modulus in rigor and presence of AMP-PNP becomes:

$$\Delta E_{\text{stat.}} = \frac{\partial \Delta T}{\partial \varepsilon} = \frac{c}{c + K_d}\left[\frac{\partial \Delta T_0}{\partial \varepsilon} - \frac{1}{c + K_d} \cdot \frac{\partial K_d}{\partial \varepsilon}\right]. \tag{3}$$

The last function of Equation (3) is obtained by differentiation of $\Delta T$ in Equation (1) with respect to the axial strain, $\varepsilon$. Substituting $\Delta T$ instead of the concentration $c$ then gives (cf. Eq. 1):

$$\frac{\Delta E_{\text{stat.}}}{\Delta T} = \left[\frac{\partial \ln \Delta T_0}{\partial \varepsilon} - \left(1 - \frac{\Delta T}{\Delta T_0}\right) \cdot \frac{\partial \ln K_d}{\partial \varepsilon}\right]. \tag{4}$$

Figure 6 demonstrates the validity of Equation (4). The values of the empirically determined ratio $\Delta E_{\text{stat.}}/\Delta T$ versus $\Delta T/\Delta T_0$ are consistent with a linear regression line. The regression line intersects the abscissa at $\Delta T/\Delta T_0 \sim 1$. This indicates that $\partial \ln \Delta T_0/\partial \varepsilon$ is nearly zero. The slope of the regression line gives

$$\partial \ln K_d/\partial \varepsilon = -140\,. \tag{5}$$

Releasing the fibre by 0.5% $L_0$ ($\varepsilon = -0.005$) thus increases the dissociation constant of AMP-PNP binding to the myosin heads by a factor of about 2, i.e. $K_d$ increases from 85 μM to about 170 μM. This is taken to mean that at [AMP-PNP] = 0.1 mM the fraction of myosin heads charged with AMP-PNP is decreased from 0.54 to 0.37 by this 0.5% $L_0$ release or 0.25 $N_s$ actomyosin sites are discharged from AMP-PNP when a total of $N_s$ actomyosin sites per cm fibre length are teinochemically active. Since the direct estimation of the stretch dependence of AMP-PNP binding gave a release of 0.35 pmoles AMP-PNP under comparable conditions (cf. Eq. 2), it seems that about $N_s = 1.4$ pmole (or about 15%, cf. Tregear and Squire, 1973) of the

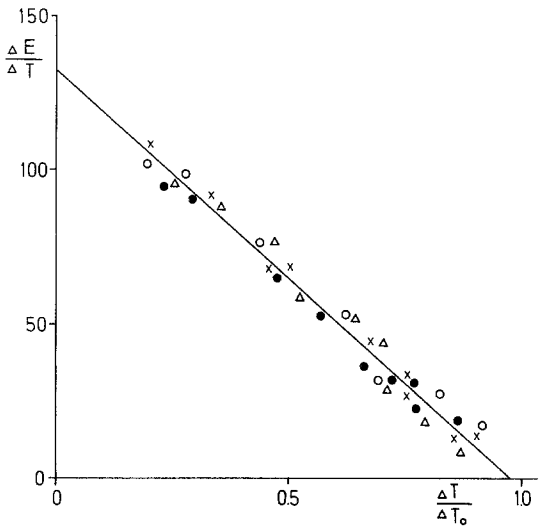


**Fig. 6.** Plot of the empirically determined ratio $\Delta E_{stat}/\Delta T$ against $\Delta T/\Delta T_0$. In connection with Equation 4 in the text, the point of intersection of the linear regression line with the ordinate (140) is a measure of the dependence of dissociation constant of AMP-PNP binding on fibre length. The symbols represent four different muscle preparations. The values of tension and static elastic modulus were obtained at concentrations from 0–0.4 mM AMP-PNP. Conditions: Bundles of 6–8 fibres; pH 6.7; $I = 0.06$ M; 18° C

myosin heads present in 1 cm fibre out of a total of 10 pmole sites are involved in the teinochemical reactions.

*Molecular Interpretation of Static Stiffness*

Since the dissociation constant $K_d$ decreases with increasing fibre length, the affinity of the AMP-PNP for the active centre of the myosin heads increases when the fibre is stretched. It is tempting to consider the described teinochemical effect in terms of the known structural effects of AMP-PNP (Marston et al., 1976; Beinbrech et al., 1976; Goody et al., 1975; Lymn and Huxley, 1973) as well as the theory of Huxley and Simmons (1971).

When a fibre is relaxed by AMP-PNP under isometric conditions, the cross bridges are probably rotated from an acute angled position (Reedy et al., 1965) to a perpendicular position (Huxley, 1969). It was suggested (Kuhn, 1973) that AMP-PNP stabilises the perpendicularly attached myosin head conformation, i.e. the standard free energy ($\Delta F^0$) of this conformation may be much less in the case of AMP-PNP charged heads than in the case of uncharged heads under rigor conditions. Removal of AMP-PNP from the fibre under isometric conditions induces the cross bridges to return to the angled position. Concomitantly the ligand dissociates from the heads while the cross bridges remain attached to the actin. Isometric tension is thereby generated. Conversely, when a fibre is stretched, the model of Huxley and Simmons (1971) predicts that the cross bridges acquire a tendency to rotate into the perpendicular position. In this position AMP-PNP binds to the myosin heads.

The standard free energy associated with this process is the sum of the free energy differences ($\Delta F^0$) of the conformational changes and of the elastic energy difference ($\Delta F_e$) stored when a cross bridge rotates on the actin filament. Under the conditions assumed in the model of Huxley and Simmons, this elastic energy was calculated by Huxley and Simmons as:

$$\Delta F_e = D \times \Delta Z \times \Delta y\,, \tag{6}$$

where $D$ is the stiffness of one attached cross bridge irrespective of its conformation, $\Delta Z$ is the increase in the length of the elastic elements in the cross bridges generated by the rotation from the perpendicular position to the acute angled position, and $\Delta y$ is the distance through which the actin filament is displaced relative to the myosin filament by a length change:

$$\Delta y = L_s \varepsilon\,, \quad L_s = 1.2\ \mu\text{m length of a half sarcomere} \tag{7}$$

(cf. Reedy, 1968; $\varepsilon$ = axial strain component).

According to van't Hoff's law the standard free energy ($\Delta F = \Delta F^0 + \Delta F_e$) divided by $kT (= 4.15 \cdot 10^{-21}$ Ws at 12° C) gives the natural logarithm of the dissociation constant for the conformational change of the cross bridges, i.e. for the transition from an uncharged myosin head in an acute angled position to an AMP-PNP charged myosin head in a perpendicular position. Hence, the slope, $\partial \ln K_d/\partial \varepsilon$, of the logarithm of the dissociation constant with respect to the fibre straining becomes:

$$\frac{\partial \ln K_d}{\partial \varepsilon} = - D \times \Delta Z \times L_s/kT\,. \tag{8}$$

Equation (8) is a means of describing the teinochemical effect of AMP-PNP without reference to the numbre of attached cross bridges.
According to Beinbrech et al. (1976), AMP-PNP induces a lengthening of the elastic elements of the cross bridges of $\Delta Z = 3$ nm (cf. also Marston et al., 1976). This value of $\Delta Z$ was confirmed by the present study (cf. $\Delta Z$, Fig. 4). From the value $\partial \ln K_d/\partial \varepsilon = -140$ estimated from static elastic modulus values (cf. Fig. 6) the stiffness of one cross bridge is then calculated [Eq. (7), (8)] as $D = 1.6 \cdot 10^{-4}$ N/m.

*Molecular Interpretation of Isometric Tension*

Isometric tension ($T_R$) may represent the sum of the tensions in $N$ force maintaining cross bridges per half sarcomere. If the elastic elements of each of these cross bridges are strained by isometric rigor contraction with displacement $Z_R^*$ (cf. Fig. 4), one force-maintaining cross bridge contributes the force $D \times Z_R^*$ to total rigor tension. Then:

$$T_R = D \times N \times Z_R^*\,. \tag{9}$$

The zero length $Z_R^*$ may be estimated if the following assumptions are made:

1) the immediate elastic modulus of a fibre in rigor is a measure of all cross bridges attached to the actin filament at the moment of release (cf. Huxley and Simmons, 1971);

2) part of these cross bridges (with compressed contractile elements) may change their locus of attachment on the actin filament, so that their elastic elements are

discharged and rigor tension recovers (therefore cross bridges exerting negative forces do not affect the estimation of $Z_R^*$);

3) other cross bridges – the force-maintaining cross bridges – remain fixed at the actin filament and contribute solely to static rigor tension and to static stiffness.

Under these assumptions the intersection of the extrapolated $T_\infty$ curve in the rigor state (Fig. 4) with the abscissa gives the zero length $Z_R^* = 12$ nm. From the empirical data ($T_R = 130$ μN/fibre, $D = 1.6 \cdot 10^{-4}\text{Nm}^{-1}$, $Z_R^* = 12$ nm) and Equation (9) the number $N$ of force-maintaining cross bridges per half sarcomere is calculated as $N = 7.4 \cdot 10^7$. This corresponds to $N_s = 1.1$ pmole force maintaining cross bridge sites in 1 cm fibre, i.e. practically the same small (in relation to 10 pmole/cm myosin heads, Tregear and Squire, 1973) density of force-maintaining cross bridges as revealed by direct binding studies (cf. Eq. 2).

The $T_2$ curves reported by Huxley and Simmons (1971) and by Ford et al. (1977) for tetanized frog semitendinosus fibres and tibialis anterior fibres respectively extrapolate to zero tension at releases near 12 nm per half sarcomere. According to the Huxley and Simmons interpretation (1971), the force-generating cross bridges in these living preparations were lengthened by about 12 nm under isometric conditions, i.e. the same value as found in this study for rigor bridges in glycerol-extracted fibrillar fibres. Furthermore, this value of the zero length (12 nm) and the zero slopes of the $T_2$ curves at zero release amplitudes reportes by Huxley and Simmons (1971) and Ford et al. (1977) suggest a value for the stiffness of one cross bridge $D = 1.2 \cdot 10^{-4}\text{Nm}^{-1}$ when interpreted by the Huxley and Simmons model (cf. also Julian et al., 1974; $D = 2.2 \cdot 10^{-4}\text{Nm}^{-1}$).

These stiffness values compare well with the value $D = 1.6 \cdot 10^{-4}\text{Nm}^{-1}$ found in this study by interpreting steady state tension changes induced by length changes and by adding (or by removing) the nucleotide AMP-PNP to chemically skinned fibrillar fibres. This quantitative equivalence of stiffness values and values of zero lengths in living fibres and skinned fibres gives evidence that tension generation after discharging the active centre of a myosin head from the analogue AMP-PNP (at $H$, Fig. 2) is – also on the molecular level – a complete mechano-chemical model for the force generating step in muscular contraction. It is then interesting to note that this nucleotide is stretch dependently bound to the myosin heads (Kuhn, 1977) thereby converting mechanical work into free chemical energy (Kuhn, 1973; cf. also Kuhn et al., 1960; Hill, 1974).

Recent interpretations (Goody et al., 1976; Holmes, 1977) of x-ray diffraction patterns recorded in the presence and absence of AMP-PNP (Goody et al., 1975; Marston et al., 1976) indicate that the structural changes induced by AMP-PNP in fibrillar fibres are certainly more complicated than merely a rotation of cross bridges between two conformations. It would be interesting to establish whether the mechano-chemical behaviour of the ternary actomyosin-AMP-PNP complex as revealed by this study corresponds to that of the actomyosin-product complex (Lymn and Taylor, 1971; White and Taylor, 1976) in muscular contraction.

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