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EFFECTS ON TENSION AND STIFFNESS DUE TO REDUCED pH IN MAMMALIAN FAST- AND SLOW-TWITCH SKINNED SKELETAL MUSCLE FIBRES

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SUMMARY

1. The pH dependence of the Ca^{2+} sensitivities of isometric tension and stiffness was investigated at 10 and 15 °C in skinned single fibres from rat and rabbit fast- and slow-twitch skeletal muscles. Stiffness was determined by recording the tension responses to sinusoidal length changes (3·3 kHz); peak-to-peak amplitude of the length change was monitored by laser diffraction and averaged approximately 1·3 nm (half-sarcomere)$^{-1}$. We have assumed that stiffness provides a measure of the number of cross-bridge attachments to actin.

2. At maximal Ca^{2+} activation, stiffness was depressed by 22 ± 2% (mean ± S.E.M.) in fast-twitch fibres but was unchanged in slow-twitch fibres when pH was lowered from 7·00 to 6·20. As reported previously, maximum tension was depressed by 20–45% at low pH, with the effect being greater in fast-twitch compared to slow-twitch fibres.

3. In fast-twitch fibres at 10 and 15 °C the Ca^{2+} concentrations for half-maximal activation of tension and stiffness were increased at low pH. In slow-twitch fibres, similar effects were observed at 15 °C, but at 10 °C there were no changes in the Ca^{2+} sensitivities of tension and stiffness when pH was lowered.

4. Linear relationships between relative tension and relative stiffness were obtained at all temperatures, with slopes of $1·04 \pm 0·01$ at pH 7·00 and $0·76 \pm 0·01$ at pH 6·20 in fast- and slow-twitch fibres, indicating that force per cross-bridge attachment is similarly reduced at low pH in both types of fibres.

5. In both fast- and slow-twitch fibres, rigor tension (no added ATP or creatine phosphate; pCa 9·0) was depressed by 35 ± 7% and stiffness by 12 ± 4% when pH was reduced from 7·00 to 6·20. Since cross-bridge cycling is inhibited in rigor the effect of low pH to depress rigor tension suggests that pH directly modulates the strength of the bond formed between actin and myosin.

6. The effect of low pH to reduce the apparent number of cross-bridge attachments during maximal Ca^{2+} activation in fast- but not slow-twitch fibres could account for the greater H^+-induced depression of maximum force in fast-twitch fibres. In both fibre types, the decrease in the number of cross-bridge attachments at submaximal concentrations of Ca^{2+} may in part account for the loss in Ca^{2+}

sensitivity of tension at low pH, due perhaps to a reduction in co-operative activation of the thin filament by bound cross-bridges.

INTRODUCTION

The basis of muscle fatigue is not known for certain, but it appears that the depression of contraction in the fatigue of living muscles involves at least two components: (1) a change in the composition of the myoplasm which directly modulates cross-bridge function and (2) disruption of excitation–contraction coupling. The relative contributions of these two components in fatigue is currently debated, but it is likely that dominance of one component depends upon factors such as the intensity and duration of the contractile activity, as well as the fibre-type composition of the muscle under study (Fitts & Metzger, 1988). During conditions of intense contractile activity several metabolic byproducts accumulate in the myoplasm (e.g. H^+, P_i, creatine, lactate) and some of these have been shown to directly affect contractile function. Studies using skinned fibres, from which the sarcolemma is removed so that the composition of the solution bathing the contractile elements can be controlled, provide direct evidence that developed force is affected by changes in myoplasmic pH (Donaldson & Hermansen, 1978; Fabiato & Fabiato, 1978; Robertson & Kerrick, 1979; Metzger & Moss, 1987, 1988; Chase & Kushmerick, 1988; Cooke, Franks, Luciani & Pate, 1988; Godt & Nosek, 1989).

The molecular basis of the effect of H^+ to reduce force in muscle is unknown. In the present study we have tested the hypothesis that altered pH directly modulates force by affecting the number of cross-bridge attachments to binding sites on actin. Measurements of stiffness were obtained at pH 7·00 and pH 6·20 over a wide range of Ca^{2+} concentrations in skinned single fibres from rat slow-twitch soleus and fast-twitch superficial vastus lateralis (SVL) and rabbit fast-twitch psoas muscles. These muscles were studied since it has been shown that pH differentially affects force production in slow- and fast-twitch fibres (Metzger & Moss, 1987, 1988). We show here that at maximal levels of activation by Ca^{2+}, stiffness is markedly reduced at low pH in fast-twitch but not in slow-twitch fibres. The effect of low pH to reduce the apparent number of cross-bridge attachments in fast-twitch fibres could account for the greater effect of pH to modulate maximum force in fast- as compared to slow-twitch fibres (Metzger & Moss, 1987, 1988).

METHODS

Skinned fibre preparations and experimental apparatus

Animals were anaesthetized with Nembutal (50 mg kg^{-1} body weight, I.P.) prior to the isolation of muscles. Fast-twitch skeletal muscle fibres were obtained from the superficial portion of the vastus lateralis (SVL) muscles of adult female Sprague–Dawley rats and from psoas muscles of adult male New Zealand rabbits. Slow-twitch fibres were obtained from soleus muscles of adult female Sprague–Dawley rats. Bundles of approximately fifty fibres were dissected from each muscle while in relaxing solution (described below) and were then tied with surgical silk to glass capillary tubes. Bundles were stored for up to three weeks at −23 °C in relaxing solution containing 50% (v/v) glycerol. Before each experiment, bundles were placed in relaxing solution containing 0·5% (w/v) Brij-58 for 30 min to disrupt the sarcoplasmic reticulum (Moss, 1979). Individual fibres were carefully pulled free from one end of the fibre bundle and were mounted (Moss, 1979; Metzger, Greaser & Moss, 1989) between a force transducer (model 407; Cambridge

Technology, Cambridge, MA, USA; sensitivity, 0·2 mV μN^{-1}; 1–99% response time, 100 μs; resonant frequency, approximately 5 kHz; noise level at the output equivalent to 1 mg wt peak-to-peak) and a DC torque motor (model 300s; Cambridge Technology, Inc.). The fibre was viewed through a model IM inverted microscope (Carl Zeiss, Inc., Thornwood, NY, USA) and the overall length was adjusted to set resting sarcomere length by using a three-way positioner on which the motor was mounted. Complete details of the mounting procedure and experimental set-up have been reported elsewhere (Moss, 1979; Metzger *et al.* 1989). Control values of maximum isometric tension (P_0), stiffness, and elastic modulus obtained at pCa 4·5 and pH 7·00 are listed in Table 1.

Solutions

Relaxing and activating solutions contained (in mmol l^{-1}): EGTA, 7; free Mg^{2+}, 1; total ATP, 4·4; creatine phosphate (CP), 14·5; imidazole, 20; and sufficient KCl (total Cl^- 68 to 83) to yield a total ionic strength of 180 mmol l^{-1}. Solution pH was adjusted to 7·00 or to 6·20 with KOH, except for solutions of pH 6·20 and pCa 9·0 in which pH was adjusted with HCl. Relaxing solution had a pCa (i.e. $-\log [Ca^{2+}]$) of 9·0 while the pCa of the solution for maximal activation was 4·5. Rigor solutions made at pH 7·00 and pH 6·20 were similar to relaxing solution except that ATP and CP were not added; sufficient KCl was added to these solutions to yield a total ionic strength of 180 mmol l^{-1}. The calculator program of Fabiato & Fabiato (1979) was used to calculate the final concentrations of each metal, ligand, and metal–ligand complex, using the stability constants listed by Godt & Lindley (1982). The apparent stability constant for Ca^{2+}-EGTA was corrected for ionic strength, pH, and experimental temperature, i.e. 10 and 15 °C (Fabiato & Fabiato, 1979).

Tension–pCa relationship

To obtain a tension–pCa relationship each fibre was transferred to a particular Ca^{2+}-activating solution and steady isometric tension was allowed to develop, after which the fibre was rapidly (< 1 ms) slackened so that tension fell to zero (Fig. 1). The fibre was then relaxed by transferring the fibre to pCa 9·0 solution. The difference between steady tension and the tension baseline following the slack step was measured as total tension. To obtain active tension, the resting tension measured at pCa 9·0 (about 1% of total tension) was subtracted from total tension. Tension–pCa relations were determined for each fibre by expressing tensions (P) at various submaximal Ca^{2+} concentrations as fractions of the maximum value, P_0, obtained in the same fibre at pCa 4·5 and pH 7·00. Every fourth contraction was done at pCa 4·5 to check for any deterioration in fibre performance (Moss, 1979). Typically, tension–pCa data were obtained from a single fibre at pH 7·00, then at pH 6·20, and again at pH 7·00. In this study, there was no fall in maximum tension during the collection of data, as shown previously (Metzger *et al.* 1989).

Fibre cross-sectional area was calculated assuming an elliptical fibre cross-section.

Stiffness–pCa relationship

Stiffness, defined as the instantaneous dependence of tension on length, was determined by applying small-amplitude (approximately 1·3 nm (half-sarcomere)$^{-1}$) sinusoidal changes in sarcomere length at a frequency of 3·3 kHz and measuring the magnitude of the resultant change in tension. Peak-to-peak changes in sarcomere length (ΔL) were determined by monitoring the first-order laser diffraction pattern obtained from the fibres as described previously (Metzger *et al.* 1989; Hofmann, Metzger, Greaser & Moss, 1990). Sarcomere length resolution was about 0·5 nm sarcomere^{-1} (Metzger *et al.* 1989). The resultant tension response (ΔP) was measured and the average of ten consecutive readings of ΔL and ΔP were used to determine stiffness (i.e. $\Delta P/\Delta L$). At each pCa, stiffness was determined and then scaled to the value obtained in the same fibre at pCa 4·5 and pH 7·00. There was a phase shift of approximately 60 deg between the length and force signals with force lagging length, which was due entirely to the use of a 4-pole filter in the force-detection circuit (to eliminate 2 MHz noise from capacitor signal; personal communication, Bruce Rohr, Cambridge Technology), as described previously (Allen & Moss, 1987). The phase shift was similar at all pCa values.

Statistics

A two-way analysis of variance (ANOVA) was used to test whether pH significantly affected the tension–pCa and/or the stiffness–pCa relationships. When a significant interaction between pH and

Ca^{2+} was indicated by ANOVA, Student's two-tailed t test was used to determine significant differences between two mean values. A probability level of $P < 0.05$ was selected as indicating significance. Values are listed as means ± standard error of the mean.

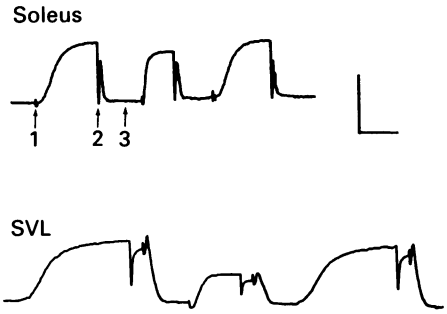

Fig. 1. Slow time base recordings of isometric tension during maximal activation by Ca^{2+} (pCa 4·5) at pH 7·00, at pH 6·20 and upon the return to pH 7·00 in a skinned soleus fibre (upper record) and an SVL fibre (lower record) at 10 °C. Soleus: isometric tensions were (in kN m^{-2}): 93·4 at pH 7·00 (left trace), 74·2 at pH 6·20 (middle trace) and 90·0 when the fibre was returned to pH 7·00 (right trace). Values for the calibration bar for the soleus data are: 88 kN m^{-2} for the y-axis and 34 s for the x-axis. End-to-end fibre length was 3·25 mm; sarcomere length was 2·52 μm at both pCa 9·0 and pCa 4·5. SVL: isometric tensions were (in kN m^{-2}): 70·9 at pH 7·00 (left trace), 33·2 at pH 6·20 (middle trace), and 70·9 at pH 7·00 (right trace). Values for the calibration bar for the SVL data are: 66·7 kN m^{-2} for the y-axis and 9·5 s for the x-axis. End-to-end fibre length was 2·35 mm; sarcomere length was 2·49 μm at pCa 9·0 and 2·37 at pCa 4·5. At each pH, at the arrow marked '1' the fibre was transferred from relaxing solution to maximum Ca^{2+}-activating solution. At point '2', overall muscle length was rapidly (< 1 ms) reduced by 400 μm and tension fell to zero so that total tension could be measured. The fibre was then returned to relaxing solution and muscle length was re-extended to the pre-release value at point '3'.

TABLE 1. Control tension and stiffness data

P_0 (kN m^{-2})	Stiffness (N m^{-1})	Elastic modulus (N m^{-2})
	Soleus	
86·9 ± 14·1 ($n = 5$)	32·0 ± 1·9 ($n = 5$)	$2.9 \times 10^7 \pm 0.7 \times 10^7$ ($n = 5$)
	SVL	
73·4 ± 15·1 ($n = 5$)	23·0 ± 5·8 ($n = 5$)	$1.4 \times 10^7 \pm 0.5 \times 10^7$ ($n = 5$)

Values were obtained at pCa 4·5 and pH 7·00, with temperature set at 10 °C. Values are means ± standard error of the mean, n is number of observations.

RESULTS

Our primary concern in collecting stiffness data was to minimize inhomogeneities in sarcomere length along the fibre during Ca^{2+} activation. Non-uniform sarcomere lengths during activation would yield a poor diffraction pattern and compromise our ability to determine changes in sarcomere length. To minimize this problem, the experiments were initially done at 10 °C, since non-uniformities are less likely to occur at lower temperatures (Hofmann *et al.* 1990), presumably due to the reduction

in force as temperature is lowered. However, in several fibres, data was collected at 15 °C for comparison with our earlier work (Metzger & Moss, 1987, 1988). The quality of the diffraction pattern was similar at these two temperatures (that is, in these experiments sarcomere homogeneity was similar); however, there were differences at

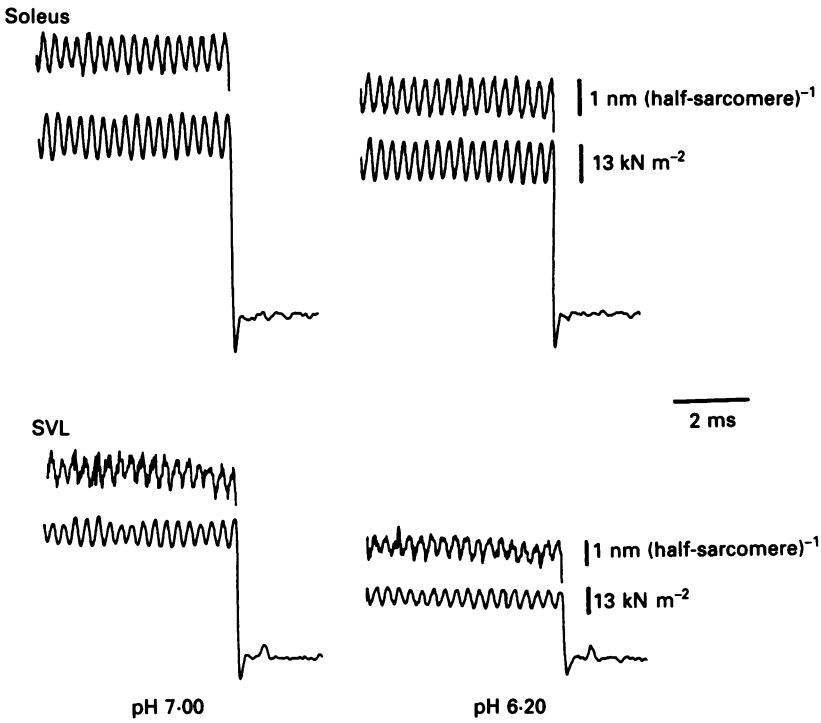

Fig. 2. Fast time base recordings of tension (lower traces) and sarcomere length (upper traces) during maximal activation with Ca^{2+} (pCa 4·5) at pH 7·00 (left traces) and pH 6·20 (right traces) in a soleus fibre and an SVL fibre at 10 °C. Soleus: the amplitude of the sarcomere length change averaged 1 nm (half-sarcomere)$^{-1}$. End-to-end fibre length was 3·80 mm; sarcomere length was 2·65 μm at pCa 9·0 and 2·60 μm at pCa 4·5. SVL: the amplitude of the sarcomere length change averaged 1·27 nm (half-sarcomere)$^{-1}$. End-to-end fibre length was 2·99 mm with sarcomere length set at 2·56 μm at pCa 9·0. The drop in tension during the later part of each tension trace resulted from a rapid release of muscle length as described in Fig. 1.

the two temperatures in the effect of pH upon some of the mechanical properties. These differences are reported below.

Results obtained at 10 °C

Slow time base records showing the effect of altered pH on maximum Ca^{2+}-activated isometric tension in rat soleus and SVL fibres are shown in Fig. 1. In agreement with our findings at 15 °C (Metzger & Moss, 1987, 1988; present study), isometric tension was markedly reduced when pH was lowered from 7·00 to 6·20, with the effect being significantly greater in the fast-twitch fibres. At pH 6·20, mean

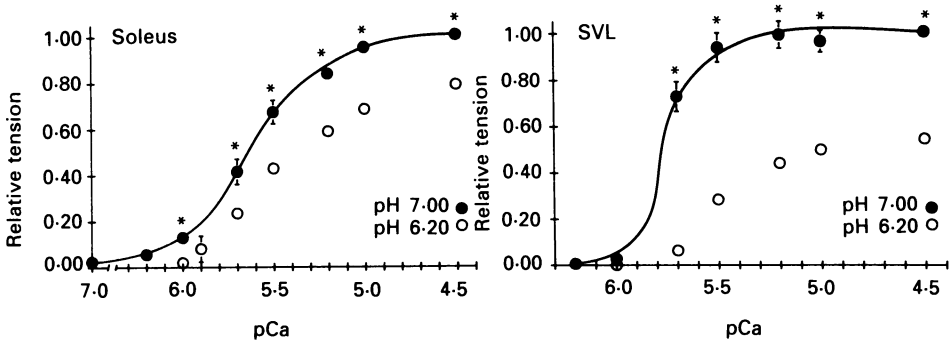

Fig. 3. Summary of the effect of altered pH upon the isometric tension–pCa relationships of soleus and SVL fibres at 10 °C. Values are scaled relative to the maximum tension (P_0) at pCa 4·5 and pH 7·00 in each fibre. Values are means ± standard error of the mean; n is 5 for soleus and 3 for SVL. In some instances the error bar was smaller than the symbol size. An asterisk at any pCa indicates that the mean value at pH 7·00 is significantly greater than the mean value at pH 6·20, $P < 0·05$.

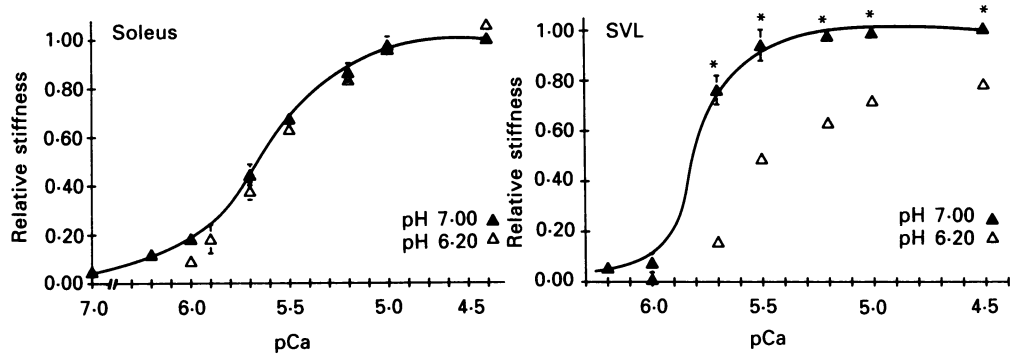

Fig. 4. Summary of the effect of altered pH upon the stiffness–pCa relationships of soleus and SVL fibres at 10 °C. Values are scaled to the pCa 4·5, pH 7·00 value in each fibre, and are shown as means ± standard error of the mean; n is 5 for soleus and 3 for SVL. In some instances the error bar was smaller than the symbol size. An asterisk at any pCa indicates that the mean value at pH 7·00 is significantly greater then the mean value at pH 6·20, $P < 0·05$.

maximum tensions were 0·79 ± 0·01 P_0 ($n = 5$) in soleus fibres and 0·54 ± 0·02 P_0 ($n = 5$) in SVL fibres. The effect of low pH to depress tension was completely reversed by returning the fibres to solutions of pH 7·00, indicating that there was no long-term effect of pH on contractile function.

Fast time base records of tension and sarcomere length show the effect of altered pH on the stiffness of soleus and SVL fibres (Fig. 2). Small-amplitude changes in sarcomere length were measured in the centre of the fibre (encompassing nearly 300 sarcomeres) by laser diffraction. In soleus fibres during maximal Ca^{2+} activation, stiffness was unaffected when pH was lowered to pH 6·20; however, the stiffness of rat SVL and rabbit psoas fibres was markedly depressed at low pH. For the soleus fibre shown in Fig. 2, stiffness was 34·5 N m^{-1} at pH 7·00 and at pH 6·20, whereas

isometric tension decreased from 67·2 kN m^{-2} to 56·1 kN m^{-2} when pH was lowered. For the SVL fibre shown in Fig. 2, stiffness decreased from 20·0 N m^{-1} to 15·0 N m^{-1} and tension from 67·5 kN m^{-2} to 35·8 kN m^{-2} when pH was lowered from 7·00 to 6·20. At pH 6·20 and pCa 4·5, mean stiffness of soleus fibres was 1·06 ± 0·03 (n = 5) of the

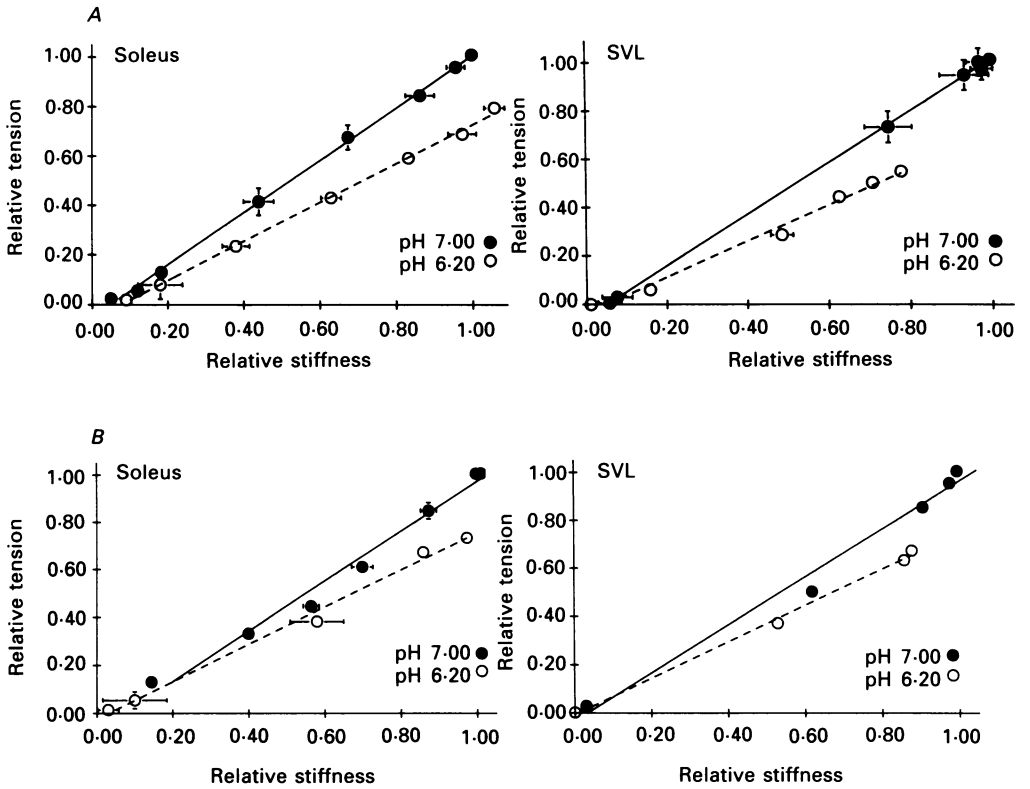

Fig. 5. *A*, plots of relative tension *versus* relative stiffness at pH 7·00 and pH 6·20 in soleus and SVL fibres at 10 °C. Values are scaled relative to the value obtained at pCa 4·5 and pH 7·00 in each fibre. Values are means ± standard error of the mean; n is 5 for soleus and 3 for SVL. In some instances the error bar was smaller than the symbol size. Linear regression lines are shown for the data at pH 7·00 and the data at pH 6·20. Correlation coefficients were greater than 0·99 for each data set. *B*, plots of relative tension *versus* relative stiffness in soleus and SVL fibres at 15 °C and pH 7·00 or pH 6·20. Values are scaled to the values obtained at pCa 4·5 and pH 7·00 in each fibre. Values are means ± standard error of the mean, except for the SVL, for which the results from a single fibre are shown. In some instances for soleus data (n of 2) the error bar was smaller than the symbol size. Linear regression lines were fitted to the data at pH 7·00 and to the data at pH 6·20. Correlation coefficients were greater than 0·99 for each data set.

value obtained at pCa 4·5 and pH 7·00, while in SVL fibres the value was 0·78 ± 0·02 (n = 5).

A summary of the effects of altered pH on the tension–pCa relationships from soleus and SVL fibres is shown in Fig. 3. The effect of low pH to shift the tension–pCa relationship to the right (i.e. to higher $[Ca^{2+}]$) seen previously at 15 °C in soleus and SVL fibres (Metzger & Moss, 1987) was less apparent in soleus fibres at 10 °C, with pCa_{50} (pCa necessary for half-maximal tension or stiffness) values of 5·62 at pH 7·00

and 5·54 at pH 6·20. Similarly, there was no effect of reduced pH on the stiffness–pCa relationship in soleus fibres (Fig. 4); however, at each pCa in SVL fibres, stiffness was significantly depressed at pH 6·20 compared to pH 7·00. The pCa_{50} values for SVL fibres were 5·85 at pH 7·00 and 5·62 at pH 6·20. A summary of the effects of altered pH on pCa_{50} values obtained from tension and stiffness data is presented in Table 2.

TABLE 2. Summary of the effects of altered pH on pCa_{50} values obtained from the cumulative tension–pCa and stiffness–pCa relationships

	Soleus			SVL		
	pH 7·00	pH 6·20	(Difference)	pH 7·00	pH 6·20	(Difference)
			10 °C			
Tension	5·62	5·54	(−0·08)	5·84	5·56	(−0·28)
Stiffness	5·63	5·57	(−0·06)	5·85	5·62	(−0·23)
			15 °C			
Tension	6·10	5·75	(−0·35)	6·00	5·68	(−0·32)
Stiffness	6·23	5·88	(−0·35)	6·08	5·80	(−0·28)

Values were obtained from Hill plots of the cumulative data at 10 and 15 °C; n for 10 °C values are given in Table 1; n for 15 °C data is 2 for soleus while the SVL value is from one fibre. The difference value was obtained by subtracting the pCa_{50} at pH 7·00 from the pCa_{50} at pH 6·20; thus, a negative difference indicates a rightward shift of the relationship at pH 6·20.

Assuming that stiffness is a measure of the number of cross-bridge attachments to actin (Huxley & Simmons, 1971), plots of relative tension *versus* relative stiffness yield information about the force per cross-bridge attachment. In Fig. 5*A*, relative tension has been plotted as a function of relative stiffness for soleus and SVL fibres at pH 7·00 and pH 6·20. At pH 7·00, the slope of the tension–stiffness relationship was 1·05 for both soleus and SVL fibres. At pH 6·20, the slope of the tension–stiffness relationship markedly decreased to values of 0·78 for soleus fibres and to 0·74 for SVL fibres. These results indicate that the force per cross-bridge attachment is similarly depressed in fast- and slow-twitch muscles at low pH. In all instances, the relationship of tension *versus* stiffness was linear, with correlation coefficients (r) greater than 0·99. This suggests that the force per cross-bridge attachment at constant pH is unaffected by changes in $[Ca^{2+}]$; however, our results do not exclude the possibility that Ca^{2+} has opposite effects on the force per cross-bridge attachment and the number of attachments so that linearity of the relationship is maintained as $[Ca^{2+}]$ is varied. However, results of the effect on pH on rigor tension (below) argue against this possibility. Results obtained from rabbit psoas fibres were qualitatively similar to those from SVL fibres (data not shown).

Results obtained at 15 °C

The effect of reduced pH to depress maximum tension in soleus and SVL fibres was similar at 15 and 10 °C. A summary of the effects of altered pH on the Ca^{2+} sensitivity of isometric tension is shown for soleus and SVL fibres in Table 2. In contrast to results from soleus fibres at 10 °C, altered pH had a pronounced effect upon the tension–pCa relationship obtained from these fibres at 15 °C, with pCa_{50} values of 6·10 at pH 7·00 and 5·75 at pH 6·20. Similarly, in soleus fibres at 15 °C, the

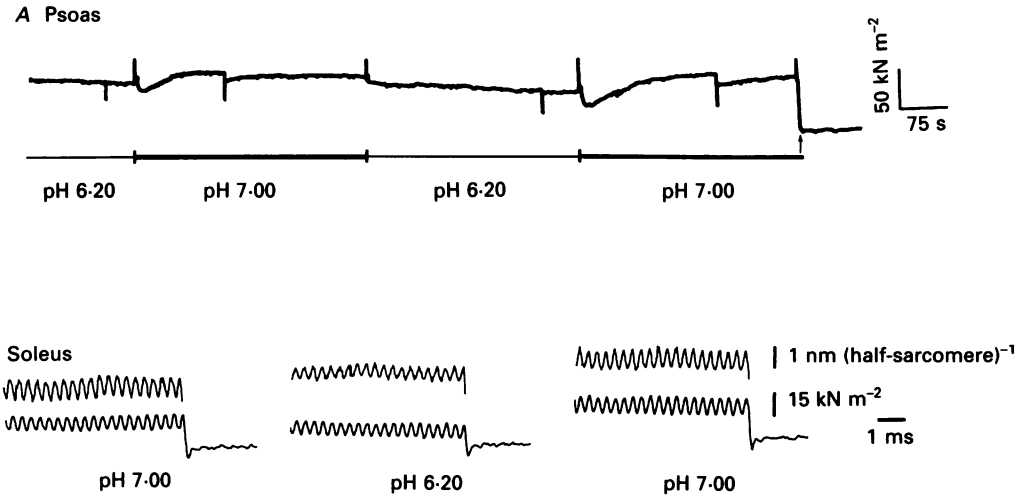

Fig. 6. *A*, slow time base recording of rigor tension (no ATP or CP added; pCa 9·0) in a rabbit psoas fibre. The fibre was transferred from relaxing solution to a rigor solution of pH 7·00 and steady tension was allowed to develop. The fibre was then transferred directly to rigor solution of pH 6·20 (leftmost part of trace). The fibre was transferred several times between the rigor solutions of differing pH and then returned to relaxing solution (arrow at rightmost part of trace). Note the increase in steady rigor tension when the fibre was transferred from the pH 6·20 rigor solution to the pH 7·00 rigor solution. The tension baseline was not fixed during this recording due to small thermal drift of the force transducer output; however, thermal drift occurred on a slower time scale compared to the response of tension to changes in pH. In this fibre rigor tension at pH 6·20 averaged 46·2 kN m^{-2}, whereas rigor tension at pH 7·00 averaged 62·7 kN m^{-2}. Temperature was 15 °C. Fibre length was 2·55 mm. Downward tension deflections at each pH resulted from the rapid length release (followed by a restretch) to determine total tension. Resting tension, measured in pCa 9·0 relaxing solution, was subtracted from total tension to obtain the rigor tension value. Upward tension deflections resulted from transferring the fibre through the solution–air interface. Sarcomere length averaged 2·62 μm in relaxing solution and 2·63 μm in pCa 4·5 activating solution. *B*, fast time base recordings of tension (lower traces) and sarcomere length (upper traces) from a soleus fibre in rigor (no added ATP or creatine phosphate; pCa 9·0) at pH 7·00 (left traces), pH 6·20 (middle traces) and again at pH 7·00 (right traces). Temperature was 15 °C. In this fibre the mean amplitude of the change in sarcomere length was 0·99 nm (half-sarcomere)$^{-1}$. In this experiment the fibre was placed in relaxing solutions between the measurements at pH 7·00 and pH 6·20. P_0 in this fibre was 82·5 kN m^{-2}. End-to-end fibre length was 2·52 mm; sarcomere length was 2·49 μm at pCa 9·0 and 2·45 μm at pCa 4·5. The drop of tension during the later part of each trace resulted from a rapid release in muscle length as described in Fig. 1.

stiffness–pCa relationship was shifted to the right at reduced pH, with pCa_{50} values of 6·23 at pH 7·00 and 5·88 at pH 6·20 (Table 2). However, as was the case for soleus fibres at 10 °C, stiffness during maximal activation by Ca^{2+} was unaffected by pH.

Results from SVL fibres obtained at 15 °C were qualitatively similar to those obtained at 10 °C (Table 2).

Plots of the relationship between relative tension and relative stiffness in fast and slow muscles at 15 °C are presented in Fig. 5*B*. The slope of the tension–stiffness relationship at pH 7·00 was 1·04 for soleus fibres and 0·99 for SVL fibres. At pH 6·20,

the slopes decreased to 0·77 for soleus fibres and to 0·75 for SVL fibres. The stiffness results are in good agreement with those obtained at 10 °C, and suggest that the effect of lowered pH to reduce force per cross-bridge attachment is not dependent upon temperature, at least over the range studied here. The results further support our conclusion above that the effect of pH to modulate force per cross-bridge attachment is fibre-type independent.

TABLE 3. Summary of the effect of altered pH on relative tension and stiffness of soleus and SVL fibres in rigor

	Tension	Stiffness
SVL	0·67 ± 0·12	0·89 ± 0·06
Soleus	0·62 ± 0·08	0·88 ± 0·06

Values were calculated by dividing the value at pH 6·20 by the value obtained at pH 7·00 in the same fibre. Each value represents the mean ± standard error of the mean, with five to six observations per group. In some experiments the fibres were relaxed between rigor measurements, while in others the fibres were directly transferred between rigor solutions of different pH. These two protocols gave similar results and the data were pooled.

Rigor experiments

Another aim of this study was to investigate the possibility that pH may specifically alter the chemical bond formed between myosin and actin. To this end, we examined the effects of pH on tension and stiffness of fibres in rigor since cross-bridge cycling is completely inhibited in the absence of substrate. Slow and fast time base records of the effects of pH on rigor tension and stiffness are shown in Fig. 6. In the fast time base records shown, rigor tension was 19·8 kN m^{-2} at pH 7·00 and 8·5 kN m^{-2} at pH 6·20. The return to pH 7·00 resulted in recovery of tension to 23·9 kN m^{-2} indicating that the effect of low pH to reduce rigor tension is reversible. In this fibre, stiffness was 23·7 N m^{-1} at pH 7·00, 26·8 N m^{-1} at pH 6·20, and 29·5 N m^{-1} upon returning pH to 7·00. Mean values of the effects of altered pH on tension and stiffness are summarized in Table 3. The slow time base record clearly shows the increase in steady rigor tension upon transferring the fibre from a pH 6·20 to a pH 7·00 solution. The time course of the tension rise may in part be due to the diffusion of the pH 7·00 solution into the fibre.

DISCUSSION

The mechanism by which altered pH modulates force in striated muscle fibres is unknown. Previous findings from skinned fibres showed that the depression of maximum isometric force due to reduced pH is not reversed by increasing the concentration of free Ca^{2+} (Fabiato & Fabiato, 1978). Recently, we showed that in fibres from which whole troponin was partially removed from the thin filament, to produce a steady submaximal tension in the absence of Ca^{2+}, the effect of pH to modulate *Ca^{2+}-insensitive* tension was similar to the effect observed in *Ca^{2+}-activated* control fibres (Metzger & Moss, 1988). These findings suggest that the basis for pH modulation of force involves (1) a reduction in the number of cross-bridge attachments to actin, and/or (2) a reduction in the force per cross-bridge attachment.

A primary finding of this study is that at maximal levels of Ca^{2+} activation, stiffness was reduced at low pH in fast-twitch but not in slow-twitch fibres. Assuming that stiffness provides an index of the number of cross-bridge attachments to actin (Huxley & Simmons, 1971), these results suggest that at maximal Ca^{2+} concentration, decreased pH causes a decrease in number of cross-bridge attachments to actin in fast-twitch fibres only. This result establishes a basis for our earlier observation of a greater H^+-induced depression of maximum isometric force in fibres containing the fast isoforms of contractile and regulatory proteins as compared to those containing the slow isoforms (Metzger & Moss, 1987, 1988).

Another conclusion of the present study is that there is a direct effect of increased $[H^+]$ to reduce the force per cross-bridge attachment, an effect that was found to be similar in both slow-twitch and fast-twitch fibres. This conclusion is derived from results in which the slope of the relationship between relative tension and relative stiffness decreased markedly as pH was lowered. It must be made clear, however, that our interpretation that this decrease in slope reflects a decrease in the force per attachment is based upon an assumption that cross-bridge stiffness is unaffected by changes in pH. This assumption was made in part since it provides a more straightforward interpretation of the data. If pH were to affect stiffness per attachment, then, for example, to account for the results obtained from soleus fibres at pCa 4·5, the number of cross-bridge attachments would have to decline and the stiffness of each attachment would have to increase to maintain stiffness constant while varying pH.

An effect of low pH to reduce force per cross-bridge attachment may also be inferred from the measurements of rigor tension and stiffness. In rigor, stiffness was depressed proportionately less than tension at low pH. The observed effects of pH to modulate rigor tension agree with the results of an earlier study on skinned cardiac myocytes and skeletal muscle fibres (Fabiato & Fabiato, 1978). In that study [ATP] was approximately 3 μmol l^{-1}, a concentration subsequently shown to be sufficient to support the cyclic attachment and detachment of cross-bridges (Moss & Haworth, 1984). Since altered pH appears to have direct effects on cross-bridges that are cycling, we wished to test whether there are effects upon cross-bridges that are inhibited from cycling due to the absence of substrate. The effect of reduced pH to depress rigor tension was similar in slow- and fast-twitch fibres, a result which fits well with our finding that at low pH the slope of the tension–stiffness relationship is depressed to the same extent in slow- and fast-twitch fibres. The finding that pH modulates tension in fibres in rigor suggests that H^+ ion has a direct effect on the strength of the chemical bond between actin and myosin.

The molecular basis for the pH dependence of force per cross-bridge attachment is not known. Discussion of this point is limited due to the lack of information about the nature of the bond formed between myosin and actin during contraction. Previous studies showing a marked effect of ionic strength on force production (Gordon, Godt, Donaldson & Harris, 1973) suggest that ionic bonds are involved in the formation of the myosin–actin complex. Recently, there has been considerable progress toward defining the specific sites on myosin and actin that interact during contraction. Suzuki *et al.* (1987) have synthesized the heptapeptide Ile-Arg-Ile-Cys-Arg-Lys-Gly, which is the amino acid sequence around the SH1 cysteine residue of

myosin subfragment 1. The heptapeptide was found to bind actin with high affinity and to competitively inhibit the formation of the acto-S1 rigor complex in solution. The peptide has also been shown to markedly depress Ca^{2+}-activated force in skinned skeletal muscle fibres (Chase & Kushmerick, 1989). It is not clear how altered pH may affect this peptide, and thus influence its interaction with actin, since the pK values of the R groups (pK_R) of the basic amino acids Arg and Lys contained in the sequence are greater than 10. In the physiological range of pH, ionization of these amino acids would not be affected. Alternatively, sequencing studies of the myosin binding site in the primary structure of actin indicate the presence of a number of acidic amino acid residues (Asp and Glu) having pK_R values around 4·0–4·5 (Sutoh, 1982; Méjean, Boyer, Labbé, Derancourt, Benyamin & Roustan, 1986). In our experiments, the ionization of these amino acids would be markedly altered only if the pH in the immediate vicinity of actin is lower than in the bulk solution when pH is lowered from 7·00 to 6·20. If this were the case, the acidic amino acids of the putative myosin binding site in the actin sequence would become less negatively charged at the lower pH. Assuming that the bond formed between actin and myosin results from an electrostatic attraction between specific basic amino acids in the myosin sequence and acidic amino acids in actin (Sutoh, 1982), then at low pH the electrostatic force of attraction between these amino acids would be reduced. In this working model, force per cross-bridge attachment might be expected to decrease as the electrostatic attractive force is reduced at low pH.

At 15 °C in both fibre types and at 10 °C in fast-twitch fibres we observed the characteristic effect of low pH to reduce the Ca^{2+} sensitivity of steady-state isometric tension. However, in soleus fibres at 10 °C we found only a small rightward shift of the tension–pCa relationship as pH was reduced from 7·00 to 6·20. The basis for pH modulation of the Ca^{2+} sensitivity of tension is unknown (see Metzger & Moss, 1987, 1988). Part of the loss in Ca^{2+} sensitivity could be the result of a reduction in co-operative activation of the thin filament by attached cross-bridges. There is evidence to suggest that cycling cross-bridges serve to activate, in a co-operative manner, regions of the thin filament by enhancing the binding affinity for Ca^{2+} (Güth & Potter, 1987). Our finding that the Ca^{2+} sensitivity of the number of cross-bridge attachments is reduced at low pH may provide a mechanism for the decrease in co-operative activation of the thin filament (Moss, Giulian & Greaser, 1985). Consistent with this hypothesis, in fibres in which the number of cross-bridge attachments was relatively unaffected by the change in pH (i.e. soleus fibres at 10 °C) a marked decrease in the Ca^{2+} sensitivity of tension was not observed at low pH. While such a mechanism could account for the reduction in the Ca^{2+} sensitivity of tension due to low pH it is likely that other factors are involved as well (Godt, 1981; Blanchard, Pan & Solaro, 1984).

Our findings suggest that the depression of maximum Ca^{2+}-activated tension due to low pH may involve two processes: (1) an effect on the force per cross-bridge attachment, which is fibre-type independent and (2) an effect to reduce the number of cross-bridge attachments to actin in fast- but not in slow-twitch fibres. These results support the hypothesis that during contractile activity of sufficient intensity to reduce intracellular pH, part of the decrement in the force can be attributed to increased $[H^+]$. However, it is also clear that other factors such as the effects of

inorganic phosphate on the cross-bridge (Hibberd, Dantzig, Trentham & Goldman, 1985; Cooke *et al.* 1988; Godt & Nosek, 1989) as well as possible disturbances in excitation–contraction coupling (Eberstein & Sandow, 1963; Gonzales-Serratos, Somlyo, McClellan, Shuman, Borrero & Somlyo, 1978) have important roles in fatigue.

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REFERENCES

ALLEN, J. D. & MOSS, R. L. (1987). Factors influencing the ascending limb of the sarcomere length–tension relationship in rabbit skinned muscle fibres. *Journal of Physiology* **390**, 119–136.

BLANCHARD, E. M., PAN, B. & SOLARO, R. J. (1984). The effect of acidic pH on the ATPase activity and troponin Ca^{2+} binding of rabbit skeletal myofilaments. *Journal of Biological Chemistry* **259**, 3181–3186.

CHASE, P. B. & KUSHMERICK, M. J. (1988). Effects of pH on contraction of rabbit fast and slow skeletal muscle fibers. *Biophysical Journal* **53**, 935–946.

CHASE, P. B. & KUSHMERICK, M. J. (1989). Ca-dependence of a myosin heavy chain (HC) peptide binding to and dissociating from skinned fibres from rabbit psoas muscle. *Biophysical Journal* **55**, 406a (abstract).

COOKE, R., FRANKS, K., LUCIANI, G. B. & PATE, E. (1988). The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *Journal of Physiology* **395**, 77–97.

DONALDSON, S. K. B. & HERMANSEN, L. (1978). Differential, direct effects of H^+ on Ca^{2+}-activated force of skinned fibers from soleus, cardiac and adductor magnus muscles of rabbit. *Pflügers Archiv* **376**, 55–65.

EBERSTEIN, A. & SANDOW, A. (1963). Fatigue mechanisms in muscle fibres. In *The Effect of Use and Disuse on Neuromuscular Function*, ed. GUTMANN, E. & HNÍK, P., pp. 515–526. Elsevier, Amsterdam.

FABIATO, A. & FABIATO, F. (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *Journal of Physiology* **276**, 233–255.

FABIATO, A. & FABIATO, F. (1979). Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *Journal de physiologie* **75**, 463–505.

FITTS, R. H. & METZGER, J. M. (1988). Mechanisms of muscular fatigue. In *Principles of Exercise Biochemistry*, ed. POORTMANS, J. R., pp. 212–229. Karger, Basel.

GODT, R. E. (1981). A simple electrostatic model can explain the effect of pH upon the force–pCa relation on skinned frog skeletal muscle fibres. *Biophysical Journal* **35**, 385–392.

GODT, R. E. & LINDLEY, B. D. (1982). Influence of temperature upon contractile activation and isometric force production in mechanically skinned muscle fibres of the frog. *Journal of General Physiology* **80**, 279–297.

GODT, R. E. & NOSEK, T. M. (1989). Changes of intracellular milieu with fatigue or hypoxia depress contraction of skinned rabbit skeletal and cardiac muscle. *Journal of Physiology* **412**, 155–180.

GONZALES-SERRATOS, H., SOMLYO, A. V., MCCLELLAN, G., SHUMAN, H., BORRERO, L. M. & SOMLYO, A. P. (1978). Composition of vacuoles and sarcoplasmic reticulum in fatigued muscle: electron probe analysis. *Proceedings of the National Academy of Sciences of the USA* **75**, 1329–1333.

GORDON, A. M., GODT, R. E., DONALDSON, S. K. B. & HARRIS, C. E. (1973). Tension in skinned frog muscle fibers in solution of varying ionic strength and neutral salt composition. *Journal of General Physiology* **62**, 550–574.

GÜTH, K. & POTTER, J. D. (1987). Effect of rigor and cycling cross-bridges on the structure of troponin C and on the Ca^{2+} affinity of the Ca^{2+}-specific regulatory sites in skinned rabbit psoas fibres. *Journal of Biological Chemistry* **262**, 13627–13635.

HIBBERD, M. G., DANTZIG, J. A., TRENTHAM, D. R. & GOLDMAN, Y. E. (1985). Phosphate release and force generation in skeletal muscle fibers. *Science* **228**, 1317–1319.

HOFMANN, P. A., METZGER, J. M., GREASER, M. L. & MOSS, R. L. (1990). The effects of partial extraction of light chain 2 on the Ca^{2+} sensitivities of isometric tension, stiffness and velocity of shortening in skinned skeletal muscle fibers. *Journal of General Physiology* **95**, 477–498.

HUXLEY, A. F. & SIMMONS, R. M. (1971). Proposed mechanism of force generation in striated muscle. *Nature* **233**, 533–538.

MÉJEAN, C., BOYER, M., LABBÉ, J. P., DERANCOURT, J., BENYAMIN, Y. & ROUSTAN, C. (1986). Antigenic probes locate the myosin subfragment 1 interaction site on the N-terminal part of actin. *Bioscience Reports* **6**, 493–499.

METZGER, J. M., GREASER, M. L. & MOSS, R. L. (1989). Variations in cross-bridge attachment rate and tension with phosphorylation of myosin in mammalian skinned skeletal muscle fibers. *Journal of General Physiology* **93**, 855–883.

METZGER, J. M. & MOSS, R. L. (1987). Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles. *Journal of Physiology* **393**, 727–742.

METZGER, J. M. & MOSS, R. L. (1988). Depression of Ca^{2+} insensitive tension due to reduced pH in partially troponin extracted skinned skeletal muscle fibers. *Biophysical Journal* **54**, 1169–1173.

MOSS, R. L. (1979). Sarcomere length–tension relation of frog skinned muscle fibres during calcium activation at short lengths. *Journal of Physiology* **292**, 177–192.

MOSS, R. L. & HAWORTH, R. A. (1984). Contraction of rabbit skinned skeletal muscle fibers at low levels of magnesium adenosine triphosphate. *Biophysical Journal* **45**, 733–742.

MOSS, R. L., GIULIAN, G. G. & GREASER, M. L. (1985). The effects of partial extraction of TnC upon the tension–pCa relationship in rabbit skinned skeletal muscle fibers. *Journal of General Physiology* **86**, 585–600.

ROBERTSON, S. P. & KERRICK, W. G. L. (1979). The effects of pH on Ca^{2+}-activated force in frog skeletal muscle fibers. *Pflügers Archiv* **380**, 41–45.

SUTOH, K. (1982). Identification of myosin-binding sites on the actin sequence. *Biochemistry* **21**, 3654–3661.

SUZUKI, R., NISHI, N., TOKURA, S. & MORITA, F. (1987). F-actin-binding synthetic heptapeptide having the amino acid sequence around the SH1 cysteinyl residue of myosin. *Journal of Biological Chemistry* **262**, 11410–11412.