presence of rigor or rigor-like myosin heads AM can not, however, be
detected by X-ray diffraction studies on contracting muscle. To give information
about the state of myosin heads after the end of powerstroke, we studied changes in
the state of myosin heads when single Ca-activated skinned muscle fibers were transferred into high-Ca rigor solution (pCa,4), by applying quick releases (0.5-1% of Lo, complete in 1-2ms followed by restretch) at various times after the transfer of the fiber into high-Ca rigor solution. Unexpectedly, the fibers exhibited distinct tension recovery Pr following quick release. The value of Pr relative to the maximum Ca-activated isometric tension Po (Pr/Po) was about 0.4 at 10s after the transfer of the fiber into rigor solution, and decreased with time to completely disappear in 10-20min. In the presence of 5-10mM EDTA, chelating Mg, the amplitude of Pr was markedly reduced, and disappeared in 5-10min. After disappearance of Pr, the fiber only showed tension drop coincident with quick release. These results suggest that AM-ADP myosin heads are responsible for the tension recovery following quick release. Pr, on the other hand, the intrinsic strength of rigor solution was reduced from 170 to 50mM by totally removing KCl, the amplitude of Pr was increased appreciably, and did not disappear for 20-30min. Concerning the cyclic actin-myosin interaction, it seems possible that, after the end of power-
stroke, myosin heads take the form of AM-ADP having a long average lifetime.

801-Pos Board B556
Non-Linear Cross-Bridge Elasticity, ATP-Independent Attachment and ATP-Velocity Relationships for Skeletal Muscle Actomyosin
Malin Persson, Elina Bengtsson, Lasse ten Siethoff, Alf Månsson.
Department of Chemistry and Biomedical Sciences, Linnaeus University, Kalmar, Sweden.
The idea that contraction of skeletal muscle and heart results from ATP-driven actomyosin cross-bridge cycles is generally accepted. However, operational details remain controversial. For instance, in conflict with most accepted views, evidence was recently presented [1] for appreciably non-linear elasticity with low stiffness for post-power-stroke cross-bridges. Moreover, a non-
ymphobic relationship was observed [2] between MgATP concentration and sliding velocity for actin filaments propelled in vitro by myosin subfragment 1 or full length myosin. Here we present convincing evidence for a hyperbolic [MgATP]-velocity relationship \( r^2=0.998 \); Michaelis-Menten constants, \( \nu_{max}=15.28 \pm 0.28 \, \mu m/s \) (mean \pm SEM) and \( K_{M}=0.389 \pm 0.023 \, mM \) when actin filaments are propelled by heavy meromyosin from rabbit fast skeletal muscle myosin (28-29°C; 3 independent experiments). Because the hyperbolic [MgATP]-velocity relationship is not readily consistent with inter-head cooperativity the results were interpreted using a cross-bridge model with independent myosin heads. The inter-state transition rates were strain-dependent and the model had one detached state and five attached actomyosin (AM) states with either MgATP (AMATP) or MgADP and/or inorganic phosphate \( (P_i) \) or no nucleotide at the active site. The AMADPP state was a strongly bound pre-power-stroke state whereas the remaining states without \( P_i \) were post-power-stroke states required to account for strain-dependent MgADP-release on the one hand and MgATP-dependence of velocity and competitive inhibition of MgATP binding by MgADP (AM, AMADP, AMATP) on the other. The MgATP induced detachment was supplemented by MgATP independent, but strain-dependent, detachment from the rigor (AM) state. This model predicts a hyperbolic [MgATP]-velocity relationship if the crossbridge elasticity is non-linear but a non-hyperbolic [MgATP]-velocity relationship (cf. [2]) if cross-bridge elasticity is non-linear but a non-hyperbolic [MgATP]-velocity relationship was observed [2] between MgATP concentration and ATP-Velocity Relationships for Skeletal Muscle Actomyosin (Kaya et al., Science 329:686-688) and our theoretical investigation demonstrated that energy landscapes are asymmetric because the crossbridges are much more compliant in compression than in tension due to a combination of buckling and bending of S2. Thus, actin-myosin transition rates are also significantly altered too. In order to assess the effect of nonlinear crossbridge compliance on sarcomere contraction we implemented the observed nonlinear crossbridge compliance in the computational platform MUSICO(MUScle Stimulation COde). In addition, this platform includes explicit 3-D sarcomere structure, extensible actin and myosin filaments, various models for the acto-
myosin cycles and the thin filament regulation via continuous flexible chain (CFC). We compared the model predictions between linear and nonlinear crossbridge compliances for classical experiments in muscle, such as force development, isotonic shortening or lengthening and T1-T2 transitions. The predictions from the nonlinear crossbridge sarcomeric model deviated largely from the experiments, mainly because the small resistance of compressed crossbridges during shortening and distorted profile power stroke transition rates. Simple adjustments of actin-myosin transition rates provided some improvements but the model predictions are still far from the observations. Thus, these findings invite re-examination of models of muscle contraction and revision of basic understanding of actomyosin cycle in the 3-D sarcomere lattice.

Supported by: NIH R01 AR048776 and R01 DC 011528, and Serbian Ministry of Science grants III41007 and OI174028.

804-Pos Board B559
Flexibility within the Heads of Muscle Myosin-2 Molecules
Neil Billington1, Derek J. Revill1, Stan A. Burgess1, Peter D. Chantler2, Peter J. Knight3
1Biological Sciences, University of Leeds, Leeds, United Kingdom, 2Molecular and Cellular Biology, Royal Veterinary College, London, United Kingdom.
We show that negative stain electron microscopy and image processing of nucleotide-free (apo) strain myosin 2 molecule and one of both light chains, is capable of resolving significant amounts of struc-
tural detail. The overall appearance of the motor and the lever is similar in rabbit,
scallop and chicken S1. Projection matching of class averages of the different S1 types to projection views of two different crystal structures of apo S1 shows that all types most commonly closely resemble the appearance of the scallop S1 structure rather than the methylated chicken S1 structure. Methylation of chicken S1 has no effect on the structure of the molecule at this resolution: it too most commonly resembles the scallop S1 crystal struc-
ture. The lever is found to vary in its angle of attachment to the motor domain, with a hinge point located in the so-called plant region between the converter