Muscle contraction on the molecular level. Actin-myosin interaction studied in an in vitro motility assay
Hamelink, W.

Citation for published version (APA):
Summary and Conclusions
This thesis started with an outline of muscle research on the molecular level. Step size is an important parameter, although literature up till now does not give an unambiguous answer about myosin’s power stroke being loosely or tightly coupled to ATP hydrolysis. The overall question to be solved remains “How is the ATP hydrolysis coupled to mechanical events?” In vitro motility assays are supposed to provide a tool to gain knowledge about the mechanism of energy conversion in muscle into work. The ‘basic’ in vitro motility assay, as introduced by Kron et al. [1986], in which actin filaments slide over myosin(fragment)-coated surfaces, allows measurement of unloaded velocity [Chapter 1]. In this thesis we describe the successful implementation of an in vitro motility assay in our lab, by means of optimisation of several already existing methods (e.g. isolation and labelling of proteins) [Chapter 2]. For the analysis of unloaded and loaded actin filament velocity we considered different known methods and we concluded that for our measurements we had to develop a new method. This new method, which we called the path reconstruction method, enables us to analyse speed of uniformly sliding actin filaments. The distributions of motile and non-motile filament speed can easily be divided in two distinct speed populations, at any frame rate. In addition we are able to detect and sort out non-uniformly sliding filaments [Chapter 3].

The calcium regulation mediated by troponin-tropomyosin complexes on actin filaments was restored by reconstitution of thin filaments [Chapter 4 and 5]. To test the applicability of our in vitro motility assay and the path reconstruction method we studied the effect of several drugs that allegedly have a direct action on the contractile apparatus.

Verapamil, diltiazem and nifedipine affect the actin-myosin interaction directly, as seen from the decrease in the percentage of motile actin filaments. These calcium antagonists also affect the interaction of thin filaments with myosin, as shown from the increase in speed of non-motile thin filaments. Verapamil increased speed and percentage of motile thin filaments by increase of the number of active cross-bridges and increases calcium sensitivity, which results in an increase in force exerted by the filaments. Diltiazem and nifedipine show a significant decrease in speed of motile thin filaments at maximum activation (pCa 4). The further decrease of the speed by diltiazem with decreasing calcium concentration was explained by a decrease in driving force. In the case of nifedipine, the mechanism behind the stepwise calcium dependence of motile thin filaments was thought to be an increase in cooperativity of the troponin-tropomyosin complex [Chapter 4].
Next we studied the mechanism of the positive inotropic effect of doxorubicin on rat heart muscle and rabbit skeletal muscle. The positive inotropic effect on actin filaments moving over rat heart muscle HMM can be explained as a consequence of a direct effect on the actin-myosin interaction itself. The positive inotropic effect of doxorubicin on thin filaments moving over rabbit skeletal muscle HMM seems to be related to a reduced friction of the troponin-tropomyosin complex or an increase in cooperativity between these troponin-tropomyosin complexes. In contrast, we found a negative inotropic effect from acute exposure to doxorubicin of pure actin filaments moving over rabbit skeletal muscle HMM [Chapter 5].

We succeeded in developing a technique to apply a magnetic load to bead-tailed actin filaments. Orienting measurements of speed on bead-tailed actin filaments without a load applied showed that this speed is indistinguishable from unloaded speed of actin filaments without a bead. This indicated that there is no viscous friction caused by the bead, despite the fact that the beads are rather large (2.8 μm in diameter). We were able to measure velocity of bead-tailed actin under load. Apparently the bead-tailed actin filaments are not lifted from the myosin-coated surface by this ‘huge’ bead. The shape of the v/l (=v/F)-curve shows the hyperbolic appearance known from force-velocity curves derived from experiments with muscle fibres, unlike the S-shaped curves measured with the centrifugal method [Chapter 6].

In conclusion, we can state that although calibration and measurements need refinement, we did develop a technique in which the bead-tailed actin filaments can be put to a halt. This means that a loading force can be applied to the extent that driving force developed by actin-myosin interactions can be matched. More experiments will be needed to relate results obtained with this method with the characteristics of contracting muscle. However, it seems a promising method to explore for this purpose, not only for use in fundamental muscle research but also for other cell biological questions concerning a quantitative approach to mechanisms of motility.
Reference