Flexibility within the heads of muscle myosin-2 molecules

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Introduction

The heads of muscle myosin molecules generate the force and movement for muscle contraction, but their mechanical properties are controversial. Myosin forms the thick filaments of muscle, generating an axial force through the heads binding to actin subunits of the thin filaments and producing sliding of the filaments past one another. The heads comprise a motor domain which hydrolyses ATP to power movement, and the changes in motor conformation are amplified by a lever that extends from the motor and connects to the thick filament backbone. This lever is an α-helix that is stabilised by the attachment of two calmodulin-like light chains that wrap around it. X-ray crystallography has shown variation in lever shape and more strikingly a diversity of angles of attachment of the lever to the motor that arise from differences in the conformation of the polypeptide at the junction between motor and lever. However, crystallography is not the best way to determine the origins of this diversity, since flexibility in solution has to be suppressed when crystals form. Electron microscopy of individual molecules offers an alternative approach, because diversity within a large number of images of molecules can represent the diversity of conformations adopted by each molecule over time through thermal excitation. Quantitative analysis of this diversity can then yield estimates of mechanical properties.

Results

Atomic structures of chicken skeletal and scallop cross-striated muscle myosin heads isolated by proteolysis have been deduced previously by X-ray crystallography, so we collected images of these heads by negative stain electron microscopy and also from rabbit skeletal muscle myosin which is commonly studied. In addition to native chicken myosin heads we studied the product of reductive methylation, since this procedure was used to induce the chicken protein to crystallise, and we wanted to test whether this had modified its structural characteristics.

Aligning and averaging thousands of images of individual heads allows consistent features to be reinforced while noise is suppressed (Fig. 1). The heads are usually oriented in a specific way so we see a particular outline for the motor domain that fits well with a view of the crystal structure. In addition the lever shows detail that is recognisable as the light chains. Further classification of the images based on just the pixels in the lever region of the images, shows the diversity of lever position relative to the fixed motor domain (Fig. 1). The lever appears flexible, mainly about a fulcrum situated at the junction between the motor and lever domains. This coincides with a site at which the head can flex in forming a specific, shut down conformation when muscle myosin molecules are enzymatically inactive.

A quantitative assessment of the characteristics of the lever flexibility was obtained by measuring lever angles in a large dataset of head images classified into many lever classes. For all four different myosin head species, the data are well fitted by a Gaussian distribution Fig. 1). This strongly suggests that the variation in lever angle seen in the data derives from thermal excitation of an elastic protein conformation, acting as a torsion spring, as goverened by the Equipartition Theorem. The variance value obtained from the fitted Gaussian distribution is thereby related to the mechanical compliance of the elastic element. The torsional stiffnesses of the four types of heads is similar and averages about 25 pN.nm/rad^2. For a muscle myosin head working as part of the thick filament, this torsional stiffness is manifested in the resistance of the tip of the lever to axial movements along the muscle fibre, *i.e.* as if the lever was a bending cantilever. This cantilever stiffness is estimated at about 0.4 pN/nm. This value is lower than recent estimates for

the stiffness of crossbridges in muscle, and we therefore suggest that attachment of the head to actin increases the stiffness of the motor-lever junction.

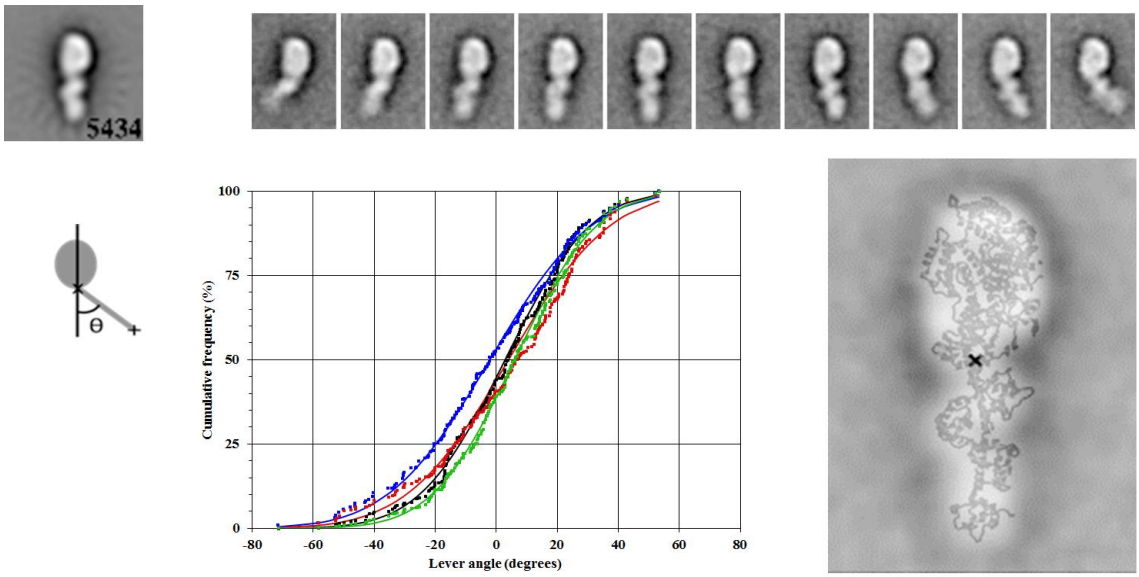

Figure 1: Flexibility in myosin heads. Top left global average of 5,434 motor-aligned head images. Top right shows classification of images into 10 classes based on lever angle. Lower left shows lever angle definition. Centre shows the distribution of lever angle for 4 types of head, together with fitted Gaussians. Blue denotes scallop heads, black is methylated chicken, green is chicken and red is rabbit. Right shows scallop head crystal structure backbone ribbon superposed on an image average from the centre of the distribution, with the flexion fulcrum as an **x**.

All four types of myosin head examined showed similar shapes, including the median (unstrained) angle between the lever and motor domains. Thus reductive methylation of the chicken heads prior to crystallisation has no obvious effect on the head shape. The good fit of that shape to the scallop crystal structure for all the types of head indicates that the scallop structure more closely resembles the structure of the myosin head in solution, and the more bent shape of the chicken crystal structure suggests that the forces between molecules during crystallisation of the chicken heads has favoured a conformer containing some internal strain.

Publications

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Collaborators

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