X-ray Crystallographic Study of the Unconventional Molecular Motor Myosin V

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Background and Significance

Myosins perform a wide and ever-expanding range of cellular functions. In muscles, "conventional" myosins, referred to as class II myosins because they contain two heavy chains, form the sarcomeric lattice necessary for efficient force transduction. This class of myosins is also essential for cytokinesis within nonmuscle cells. Phylogenetic analyses indicate that, in addition to the well-studied muscle myosins of class II, at least 15 new classes of "unconventional" myosins can be identified.[1] Unconventional myosins have been implicated in a variety of cellular functions, including organelle transport, translocation, cell division, and the maintenance of structures within the cells. All myosins have a relatively well-conserved 80 kDa motor domain, which is almost exclusively located towards the N-terminus. This part of the molecule is responsible for both ATPase and actin binding functions. Following the motor domain is a more variable region, the regulatory domain (also known as lever-arm or neck region). This part of the molecule generally holds from one to seven ~24 amino acids long repeats known as IQ motifs. The canonical core region of each IQ repeat is represented by the consensus sequence IqxxxRGxxxR. The IQ motifs represent the target recognition sequence for a variety of ~17 kDa molecules, known as myosin light chains (MLCs). The MLCs are members of the calmodulin-like superfamily of EF-hand proteins. Notice, however, that, in contrast to calmodulin, many of the MLCs have lost their ability to bind Ca^{2+}.

The *Saccharomyces cerevisiae* myosin V (Myo2p) has been implicated in the transport of organelles within the cells and in polarized growth.[2] A notable feature of this myosin is that its heavy chain C-terminal end (about 500 amino acids) folds into a globular domain, the tail globular domain (TGD), that appears to have a direct role in targeting cellular organelles such as vesicles or vacuoles.

The regulatory domain of this myosin holds six IQ motifs. These IQ repeats bind either calmodulin or a myosin light chain known as MLC1. MLC1 has also been found to make up part of the actomyosin contractile ring during cytokinesis, where it interacts with both a conventional class II myosin (Myo1p) and a non-myosin IQ domain containing protein (Iqg1p) in late mitosis.[3] MLC1 is essential and haploinsufficient. Thus, *mlc*1Δ cells require a plasmid carrying *mlc*1 for viability and normal cytokinesis.[2] In an effort to understand the function and regulation of the myosin V molecular motor and MLC1 in particular, we have succeeded in crystallizing complexes of IQ2, IQ3, IQ4, and IQ2-IQ3 with MLC1 (see Table I).

Since molecular replacement, using calmodulin or other myosin light chains as search models, failed to produce a solution for any of the MLC1-IQx structures, we embarked on a multiple-wavelength anomalous dispersion (MAD) approach based on crystals of a Seleno-methionine (Se-Met) derivative of MLC1. Because MLC1 contains a single Met residue in 148 amino acids, which together with the IQ fragment, brings the total number of amino acids in the asymmetric unit to 172 (for any of the MLC1 crystals), we decided to introduce an additional Met into MLC1 by site-directed mutagenesis. Among the various mutants tested, a single one (I64M) could be both expressed and crystallized. Moreover, crystals of this mutant could be obtained only for the complex with IQ4 of the heavy chain.

Methods and Materials

MLC1 mutant I64M (containing two methionines in 148 amino acids) was expressed in *Escherichia coli* strain BL21 (DE3) pLysS. Bacteria were grown in minimal media, supplemented with Se-Met, where the endogenous Met biosynthesis was also inhibited by the addition of Lys, Thr, Phe, Leu, and Val. When the culture reached an OD_{600nm} of 0.5, 1 mM IPTG was added and the expression was allowed to proceed for three hours. Se-Met MLC1-I64M was purified on a DE52 column. The presence of the Se-Met substitution was confirmed by mass spectrometry.

As mentioned above, crystals of Se-Met MLC1-I64M could be obtained only in complex with the synthetic peptide (24 amino

TABLE I. Summary of crystals of MLC1 complexes with the myosin V IQ repeats.

Crystal	Symmetry	Unit cell parameters	Data collected
MLC1+IQ2	$P2_12_12_1$	a=43.6 Å, b=56.5 Å, c=56.93 Å, α=β=γ=90°	1.7 Å, BioCARS 14-BM-D
MLC1+IQ3	$P4_32_12$	a=b=52.79 Å, c=118.74 Å, α=β=γ=90°	3.1 Å, BioCARS 14-BM-D
MLC1+IQ4	$P2_12_12_1$	a=49.0, b=121.08 Å, c=29.0 Å, α=β=γ=90°	2.1 Å, in house
Se-Met MLC1-I64M+IQ4	$P2_12_12_1$	a=46.73, b=115.7 Å, c=56.3 Å α=β=γ=90°	2.4 Å, BioCARS 14-BM-D
2xMLC1 + IQ2 + IQ4		Not characterized yet	

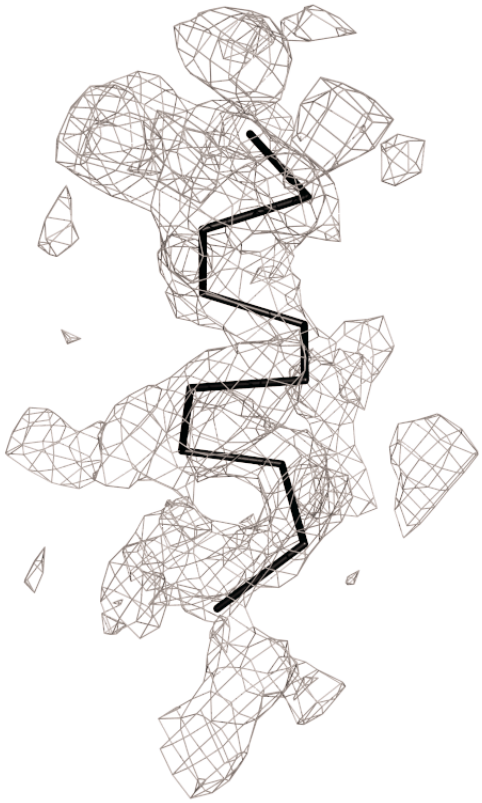

FIG. 1. Section of the Se-Met MLC1-I64M IQ4 electron density map calculated with DM-refined experimental phases, which were derived from three Se sites found and refined with the program SOLVE. The map was calculated at 2.9 Å resolution and is contoured at 1.2σ

acids long) corresponding to the myosin V IQ4. Before crystallization, MLC1-I64M was mixed with IQ4 in a 1:1.5 ratio. Crystals of the complex were grown using the vapor diffusion method and under the same conditions as those of wild type MLC1+IQ4. The crystals were transferred into a cryoprotectant solution containing, in addition to the crystallization buffer, 18% glycerol. Notice that although data collection from crystals of MLC1-IQ4 works significantly better at room temperature, freezing of the crystals was necessary in order to conduct a complete MAD experiment on a single crystal. Based on the fluorescence spectrum collected around the Se absorption edge at the BioCARS beamline 14-BM-D, four different energies were selected for the MAD experiment. Data were measured at the absorption peak (λ = 0.9779 Å), inflexion point (λ = 0.9781 Å), and two (lower and upper) remote (λ_1 = 1.0332 Å, λ_2 = 0.9407 Å) wavelength using an ADSC Quantum-4 detector. Data were processed using DENZO and SALEPACK.

Results and Discussion

Crystals of MLC1+IQ4 tend to suffer major damage on freezing under a broad range of cryo-cooling conditions, displaying among other defects a significant increase of mosaicity. As a consequence, most of the frozen crystals of Se-Met MLC1-I64M IQ4 could not be collected. The best candidate for our MAD experiment was a crystal with the same symmetry as wild-type MLC1+IQ4 but with different unit cell parameters: a = 46.73, b = 115.7 Å, c = 56.3 Å vs. a = 49.0, b = 121.08 Å, c = 29.0 Å (see Table 1). The changes in the unit cell include a duplication of one of the unit cell dimensions and, as a consequence, two molecules in the asymmetric unit (instead of one, as in the wild-type crystals). Three of the four expected Se sites were found and refined with the program SOLVE.[5] The experimental phases from SOLVE were further refined by solvent flattening and histogram matching with the CCP4 program DM.[4] The DM-modified electron density map clearly displays helices and many of the side chains in the structure (Fig. 1). Since this experiment was conducted very recently (March 15, 2001), we have not yet finish ed tracing the molecules. Finally, it is important to mention that we expect that solutions for the four remaining MLC1 complexes will be found using this first structure as a search model for molecular replacement.

Acknowledgments

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