Atomic Force Microscopy of the Myosin Molecule

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ABSTRACT Atomic force microscopy (AFM) has been used to study the structure of rabbit skeletal muscle myosin deposited onto a mica substrate from glycerol solution. Images of the myosin molecule have been obtained using contact mode AFM with the sample immersed in propanol. The molecules have two heads at one end of a long tail and have an appearance similar to those prepared by glycerol deposition techniques for electron microscopy, except that the separation of the two heads is not so well defined. The average length of the tail (155 ± 5 nm) agrees well with previous studies. Bends in the myosin tail have been observed at locations similar to those observed in the electron microscope. By raising the applied force, it has been possible locally to separate the two strands of the α-helical coiled-coil tail. We conclude that the glycerol-mica technique is a useful tool for the preparation of fibrous proteins for examination by scanning probe microscopy.

INTRODUCTION

The recent development of scanning probe microscopy (SPM), and in particular atomic force microscopy (AFM) (Binnig et al., 1986), has provided a new tool with which to investigate biological structures at high resolution. One of the major advantages of SPM is the ability to image surfaces under liquid. Under certain conditions it has therefore been possible to perform the microscopy of biological specimens under near-native conditions of pH and ionic strength (Marti and Amrein, 1993). This ability to image in aqueous environment combined with no requirement for staining or coating and no electron-beam damage means that biological processes can be followed (Drake et al., 1989; Häberle et al., 1992; Thomson et al., 1994). In applying SPM to biological specimens, the possibility of the scanning probe disrupting the surface must however be considered, particularly for cases in which the specimen-probe interaction is stronger than the specimen-substrate interaction. Also, the biomolecule must be spread evenly over large areas of the substrate so that the search time for a suitable region is not prohibitive. Thus, there is a need to establish reliable methods for the preparation of macromolecules for scanning probe microscopy. The substrate on which the molecules are spread needs to be molecularly flat, but for atomic force microscopy, unlike scanning tunnelling microscopy (STM), there is no requirement that it be electrically conducting. It is therefore possible to use mica, the same substrate that is routinely used in the preparation of a wide range of protein molecules for electron microscopy. One of the methods that has been developed for electron microscopy is to deposit the molecules from a solution containing 50% glycerol (Elliott et al., 1976; Tyler and Branton, 1980; Mould et al., 1985). The presence of the glycerol reduces the wetting of the mica so that the film retracts carrying the low molecular weight solutes with it, leaving the macromolecules bound to the mica.

The protein molecule that was principally used to develop this technique was myosin, because of its characteristic shape. The myosin molecule has two heads flexibly attached at one end of a long tail (Slayter and Lowey, 1967; Elliott and Offer, 1978; Walker et al., 1985). The heads, which are about 19 nm long, are curved and wider at their ends than near the junction with the tail. They have ATPase and actin-binding activity and act as cross-bridges to generate tension in muscle contraction (for a review see Trinick and Knight, 1987). The atomic structure of the proteolytically cleaved heads, known as subfragment 1 (S1), has recently been reported (Rayment et al., 1993). The tails, 156 nm long and 2 nm wide, are made up of two polypeptide chains wound in an α-helical coiled-coil. Smooth-muscle myosin, adsorbed on highly oriented pyrolytic graphite (HOPG), has been imaged by scanning tunnelling microscopy in a preliminary study by Faruqi et al. (1993). However, obtaining fields of individual protein molecules on HOPG is difficult to achieve and interpretation may also be difficult because the contrast seen in STM images is not always topographical in nature but can be due to electronic artifacts generated by the substrate (Clemmer and Beebe, 1991). Atomic force microscopy is largely free of these kinds of artifacts. In this paper we have used myosin as a test molecule to determine whether the glycerol-mica technique could be used in the preparation of protein molecules for atomic force microscopy.

MATERIALS AND METHODS

Myosin was prepared from the back and hind leg muscles of rabbits by the method of Perry (1955). The samples were prepared using the mica sandwich technique (Mould et al., 1985) using a solution of 5 μg/ml myosin, 50% (v/v) glycerol with a final concentration of either 0.3 M KCl, 0.3 M KCl with 20 mM potassium phosphate, or 0.5 M ammonium acetate. A 2-cm^2 piece of mica was freshly cleaved and a 2- to 4-μl drop of the myosin solution placed between the two halves. These were reapposed for ~60 s to allow the myosin to adsorb to the mica. The two halves of the sandwich

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were then peeled apart carefully and dried in vacuo for 1 h to remove excess glycerol and water. No shadowing or coating of the molecules was performed and the samples were stored in a covered Petri dish until used. Imaging was performed at constant force, using a Nanoscope III AFM (Digital Instruments, Santa Barbara, CA), with 200-μm silicon nitride cantilevers with a nominal spring constant of 0.06 Nm^{-1} (also supplied by Digital Instruments). Samples were immersed in 1-propanol to reduce the "pull off" force and permit more stable forces for imaging (Hansma et al., 1992; Weisenhorn et al., 1992). The best images were obtained using a force normal to the surface of between 1 and 2 nN, with a scan rate of between 5 and 10 Hz, for a 256 × 256 pixel image.

RESULTS AND DISCUSSION

Myosin molecules could be successfully imaged when deposited on a mica surface from 50% glycerol solution containing either 0.5 M ammonium acetate (Fig. 1 *A*) or 0.3 M KCl (Fig. 1 *B*). Some searching of the sample was required to find a region where recognizable myosin molecules could be observed. The resolution obtained in these regions is similar to that of electron microscope images of myosin prepared by metal shadowing. Apart from certain differences in the appearance of the heads, which are discussed below, the molecules have an appearance similar to that seen in the electron microscope. The average length of the tail of intact myosin molecules was 155 ± 5 nm as measured from the head-tail junction to the free end of the tail. Under similar preparation conditions to those presented here, Elliott and Offer (1978) and Walzthöny and Eppenberger (1986) have observed, using electron microscopy, that the length of the tail in intact rabbit myosin is 156 nm and 155.9 ± 4.8 nm, respectively. A small number of molecules appeared to have truncated tails ~120 nm in length, presumably due to mechanical breakage by the probe. The average width of the tails varied between 10 and 16 nm, which is substantially larger than the value of 2 to 3 nm obtained from negatively stained samples in the electron microscope (Walker et al., 1985). This broadening is typical of AFM images of adsorbed biomolecules and is mainly due to the convolution of the AFM probe with the molecule (Murray et al., 1993).

The most significant difference observed between the images presented here and those previously obtained using electron microscopy (Elliott and Offer, 1978; Walker et al., 1985) is in the appearance of the two heads of the molecule. The separation of the heads observed in this study does seem to depend at least partly on the preparation conditions. For samples prepared with 0.5 M ammonium acetate two heads were observed in about one-third of the molecules (Figs. 1 *A* and 2). However, almost all of the molecules observed from samples prepared with 0.3 M KCl showed a single head region in which it was rarely possible to distinguish any substructure (Fig. 1 *B*). While association of the heads at low pH is a likely explanation for the appearance of the molecules in the case of samples prepared using KCl with no buffer, the reason for only partial separation of the heads when using 0.5 M ammonium acetate (at pH 7) has not yet been determined. One possible explanation is that the propanol environment, combined with the effects of the lateral force exerted by the AFM probe, may be driving this association. A contributing cause may also be that the shape of the heads observed in AFM images (Fig. 2) is much flatter and broader than observed using electron microscopy. This is thought to be due to the force exerted by the probe on the sample deforming the protein structure in these regions.

Some of the molecules observed have sharp bends in the tail at distances from the C-terminus in positions similar to those seen in the electron microscope (Elliott and Offer, 1978). However, the number of molecules observed with sharp bends has so far been limited. Hence, the significance of these correlations cannot be fully assessed until a large enough data set has been obtained.

Attempts to adjust the pH using phosphate buffer when preparing samples with 0.3 M KCl produced noisy images in which it was impossible to unambiguously identify myosin molecules. This was attributed to extensive contamination of the sample by phosphate during sample preparation (Tyler and Branton, 1980).

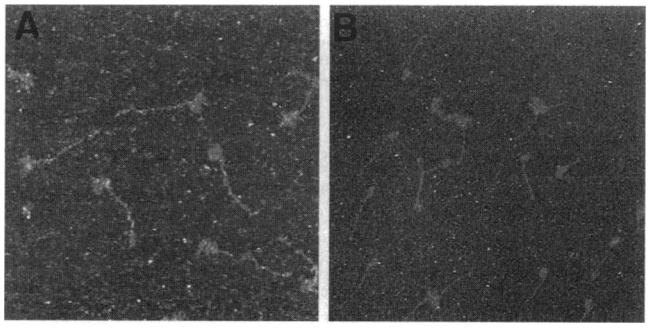

FIGURE 1 Contact mode AFM images showing fields of myosin molecules adsorbed onto a mica substrate using the mica sandwich technique and imaged under propanol. (*A*) Preparation using 0.5 M ammonium acetate in 50% glycerol solution. Image dimensions are 791 × 791 nm. (*B*) Preparation using 0.3 M KCl in 50% glycerol solution. Image dimensions are 1131 × 1131 nm.

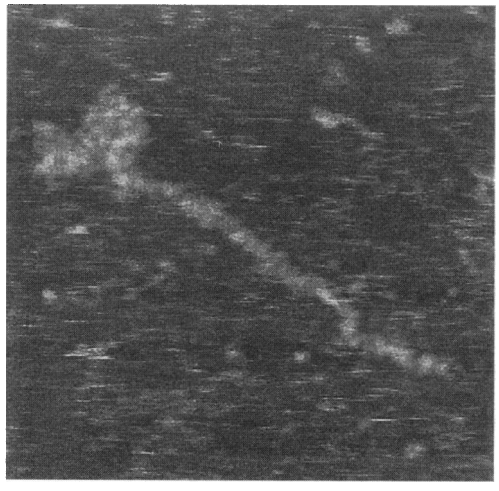

FIGURE 2 Contact mode AFM image showing a single myosin molecule prepared using 0.5 M ammonium acetate in 50% glycerol solution. The two heads are clearly visible, although they appear flatter and broader than similarly prepared electron microscope images. Imaged under propanol. Image dimensions are 207 × 207 nm.

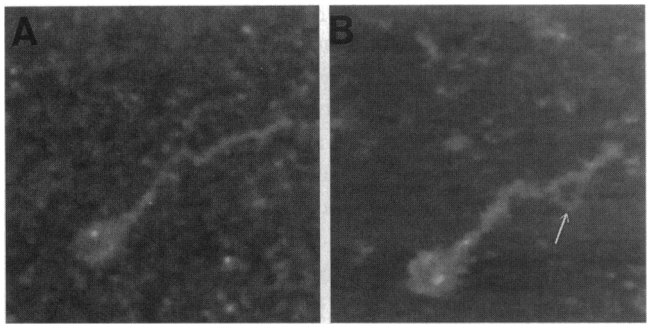

FIGURE 3 (*A*) Contact mode AFM image showing a myosin molecule imaged at a normal force of 1 nN. (*B*) The same molecule after the force has been increased to 4 nN. The arrow indicates a loop of material which is thought to be a strand of α-helix that has been teased out of the tail by the AFM probe. Image dimensions are 244 × 244 nm.

Attempts to modify individual myosin molecules by increasing the applied force produced three different effects. Typically the molecule in question was swept from the scan area by the AFM probe. However, in some cases the α-helical coiled-coil tail was severed by the action of the probe with the section of the tail not connected to the heads being swept out of the scan area, also presumably by the action of the probe. The cutting of a biomolecule into sections has been demonstrated before in the case of DNA (Vesenka et al., 1992), where a small section of the molecule is "erased" by high force imaging of the section to be removed. What is interesting in our case is that in one instance a more subtle alteration of the molecule occurred. Upon increasing the normal applied force from 1 nN (Fig. 3 *A*) to 4 nN (Fig. 3 *B*), a small loop of material was seen to emerge from the myosin tail. It is possible that this loop is one of the α-helical strands of the coiled-coil that has been pulled out from the rest of the tail by the probe. If this is the correct interpretation, the contour length suggests that this strand has been stretched during this process.

AFM has thus been successful in imaging intact untreated myosin molecules. The resolution is equivalent to that obtained in electron microscopy of shadowed molecules for the tail section of the molecule. However, the two heads of the molecule are often observed associated, and this is thought to be due to a combination of effects due to the preparative buffer, the propanol environment, and the interaction of the AFM probe with the sample. Our results indicate that the glycerol-mica procedure widely used for the preparation of protein molecules for the electron microscope is suitable also for the preparation of such specimens for the atomic force microscope.

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