

MOLECULAR MOTOR

PRIORITY CLAIM

[0001] This application is a continuation-in-part, and claims benefit of PCT Application PCT/US00/20925 filed Jul. 31, 2000, which was published in English under PCT Article 21(2), and designating the U.S., which claims the benefit of U.S. Provisional Application No. 60/146,975 filed Aug. 3, 1999.

FIELD OF THE DISCLOSURE

[0002] This disclosure relates to molecular motors, and particularly such motors that are powered by proteins.

BACKGROUND OF THE DISCLOSURE

[0003] One of the fundamental properties of biological organisms is the ability to move, or to at least transport cellular components, even on a molecular scale. The biological structure that permits macroscopic movement in animals is muscle, which can be either striated (skeletal), smooth, or cardiac. The molecular structure and function of muscle has been the subject of scientific fascination and research for over a century. As early as the 1840s, William Bowman had suggested that striations in skeletal muscle represented bands of intracellular material with differing refractive indices. These intracellular materials were eventually identified as actin and myosin.

[0004] The contractile unit in skeletal muscle is known as a myofibril, which consists of a series of Z-disks to which are attached thin filaments of actin. The Z-disks divide each myofibril into repeating units called sarcomeres, and within each sarcomere is a thick filament of myosin which has heads that can form crossbridges to the actin. In the presence of ATP, the myosin heads undergo a conformational change that causes the cross bridges to link to the actin, and the myosin heads move the actin filaments relative to the myosin filaments. This movement brings the Z-disks closer together, which on a macroscopic level contracts (shortens) the muscle, and implements musculoskeletal function. Although cardiac and smooth muscle differ in their cellular architecture from skeletal muscle, they too rely on the interaction of myosin and actin to contract.

[0005] The myosin molecule consists of six polypeptide subunits: two identical heavy chains with a molecular weight of about 200,000 kDa each, and four light chains of about 20 kDa each. In electron micrographs, purified myosin looks like a long thin rod containing two globular heads protruding at one end. This two-headed type of myosin is called myosin II to distinguish it from the smaller, single headed myosin I molecule (having a shorter tail) that is involved in cytoplasmic movements in some nonmuscle cells. The functions of portions of the myosin molecule have been investigated by using the protease trypsin to cleave the myosin II molecule into two fragments called light meromyosin (a coiled tail portion) and heavy meromyosin (which contains the globular heads of the molecule, and a portion of the coiled tail). The function of actin and myosin, and their molecular structure, are more fully described in Kendrew, *The Encyclopedia of Molecular Biology*, 1994, pages 688-691; and Kleinsmith and Kish, *Principles of Cell and Molecular Biology*, second edition, 1995, chapter 13, which are incorporated by reference.

[0006] A variety of motor proteins other than actin and myosin are also known. The motor protein kinesin, for example, was discovered in 1985 in squid axoplasm. Vale et al., *Cell* 42:39-50, 1985. Kinesin is just one member of a very large family of motor proteins. Endow, *Trends Biochem. Sci.* 16:221-225, 1991; Goldstein, *Trends Cell Biol.* 1:93, 1991; Stewart et al., *Proc. Natl. Acad. Sci. USA* 88:8470-8474, 1991. Another such motor protein is dynein. Li et al., *J Cell Biol.* 126:1475-1493, 1994. Kinesin, dynein, and related proteins move along microtubules, whereas myosin moves along actin filaments. Like myosin, kinesin is activated by ATP.

[0007] Kinesin is composed of two heavy chains (each about 120 kDa) and two light chains (each about 60 kDa). The kinesin heavy chains include three structural domains: (a) an amino-terminal head domain, which contains the sites for ATP and microtubule binding and for motor activity; (b) a middle or stalk domain, which may form an α -helical coiled coil that entwines two heavy chains to form a dimer; and (c) a carboxyl-terminal domain, which probably forms a globular tail that interacts with the light chains and possibly with vesicles and organelles. Kinesin and kinesin-like proteins are all related by sequence similarity within an approximately 340-amino acid region of the head domain, but outside of this conserved region they show no sequence similarity.

[0008] Purified motor proteins are capable of generating movement even outside biological organisms. The motility activity of purified kinesin on microtubules has, for example, been demonstrated in vitro. Vale et al., *Cell* 42:39-50, 1985. Full-length kinesin heavy chain and several types of truncated kinesin heavy chain molecules produced in *E. coli* are also capable of generating in vitro microtubule motility. Yang et al., *Science* 249:42-47, 1990; Stewart et al., *Proc. Natl. Acad. Sci. USA* 90:5209-5213, 1993. The kinesin motor domain has also been shown to retain motor activity in vitro after genetic fusion to several other proteins including spectrin (Yang et al.), glutathione S-transferase (Stewart et al.), and biotin carboxyl carrier protein (Berliner, *269 J Biol. Chem.* 269:8610-8615, 1994).

[0009] Similarly, methods have been developed for purification or recombinant production and manipulation of motor proteins, and methods of attaching actin to non-biological substrates are also known. Ishima et al., *Cell* 92:161-171, 1998. Microtubules can be routinely reassembled in vitro from tubulin purified from bovine brains. The nucleation, assembly, and disassembly reactions of microtubules have been well characterized. Cassimeris et al., *Bioessays* 7:149-154, 1987. More recently, recombinant tubulin has been produced in yeast. Davis et al., *Biochemistry* 32:8823-8835, 1993.

[0010] Efforts have been made in the past to harness the molecular activity of motor proteins for useful work outside of biological organisms. U.S. Pat. No. 5,830,659, for example, disclosed a system for purifying a molecule of interest from a mixture by aligning microtubules in a separation channel leading out of a liquid reservoir. A kinesin-ligand complex was then added to the liquid reservoir, in the presence of ATP, and the ligand was selected to bind to the molecule of interest in the liquid. When the kinesin came into contact with the microtubules in the channel, the kinesin-ligand (and its bound molecule of interest) were