Native Structure and Physical Properties of Bovine Brain Kinesin and Identification of the ATP-Binding Subunit Polypeptide†

George S. Bloom,* Mark C. Wagner, K. Kevin Pfister, and Scott T. Brady

Department of Cell Biology and Anatomy, University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Received June 2, 1987; Revised Manuscript Received October 29, 1987

ABSTRACT: Kinesin was extensively purified from bovine brain cytosol by a microtubule-binding step in the presence of 5′-adenylyl imidodiphosphate (AMP-PNP), followed by gel filtration chromatography and sucrose gradient ultracentrifugation. The products consistently contained 124 000 (124K) and 64 000 (64K) dalton polypeptides. These two polypeptides appear to represent heavy and light chains of kinesin, respectively, because they copurified on sucrose gradients to a constant and equimolar stoichiometry and bound stably to microtubules in the presence of AMP-PNP but not ATP. The mobilities of 124K and 64K in sodium dodecyl sulfate–polyacrylamide gels under reducing conditions were the same as under nonreducing conditions. A diffusion coefficient of $(2.24 \pm 0.21) \times 10^{-7}$ cm^2 s^{-1} and a sedimentation coefficient of $(9.56 \pm 0.34) \times 10^{-13}$ s were determined for native kinesin by gel filtration and sucrose gradient ultracentrifugation, respectively. These values were used to calculate a native molecular weight of about 379 000 and suggest that kinesin has an axial ratio of approximately 20. Extensively purified kinesin exhibited microtubule-activated ATPase activity, and only the 124K subunit incorporated ATP in photoaffinity labeling experiments using [^{32}P]ATP. Collectively, these data favor the interpretation that bovine brain kinesin is a highly elongated, microtubule-activated ATPase comprising two subunits each of 124 000 and 64 000 daltons, that the subunits are not linked to one another by disulfide bonds, and that the heavy chains are the ATP-binding subunits.

Because the axon lacks the molecular machinery to synthesize proteins, this region of the neuronal cell must import its resident proteins from the perikaryon. Many of these proteins are packaged in membrane-bounded compartments, such as mitochondria, small vesicles, and tubulovesicular structures, that travel toward the axon terminal at rates of 0.5–5 μm/s (Grafstein & Forman, 1980; Weiss, 1982; Lasek & Brady, 1982). Organelle traffic in the axon also occurs in the retrograde direction but is confined largely to prelysosomal structures (Smith, 1980; Tsukita & Ishikawa, 1980; Fahim et al., 1985). Together, the anterograde and retrograde organelle movements are known as "fast axonal transport". Similar organelle motility has been observed in the cytoplasm of cell types as diverse as HeLa cells (Freed & Lebowitz, 1970), frog keratocytes (Hayden et al., 1983; Hayden & Allen, 1984), and giant amoebae (Koonce & Schliwa, 1985), implying that the process is of widespread biological significance. Recently, the use of video enhanced light microscopy in conjunction with correlative immunofluorescence or electron microscopy has established that microtubules serve as tracks along which organelles travel and that individual microtubules can support bidirectional vesicle transport (Hayden et al., 1983; Hayden & Allen, 1984; Koonce & Schliwa, 1985; Schnapp et al., 1985).

One of the leading challenges to emerge from these findings has been to identify mechanochemical proteins responsible for translocating membrane-bounded organelles along microtubules. Important clues to the identity of a motor for fast axonal transport were gained from studies of axoplasm extruded from the squid giant axon. Video-enhanced light microscopy revealed that these preparations support microtubule-directed organelle movements indistinguishable from those observed in intact pieces of giant axon (Allen et al., 1982; Brady et al., 1982, 1985). Because isolated axoplasm lacks a plasma membrane, it has been possible to test systematically the solution requirements for fast axonal transport. By use of this and related approaches, the presence of ATP was shown to be necessary for organelles to translocate along microtubules (Brady et al., 1982, 1985; Sabri & Ochs, 1972; Adams, 1982; Forman et al., 1984), and a nonhydrolyzable ATP analogue, 5′-adenylyl imidodiphosphate (AMP-PNP), was found to inhibit this process (Brady et al., 1983, 1985; Lasek & Brady, 1985). Even in the presence of nearly stoichiometric levels of ATP, AMP-PNP blocks organelle movements completely, while promoting the formation of stable complexes of organelles, microtubules, and, apparently, the motors for organelle transport (Lasek & Brady, 1985). By contast, in other motility systems, such as the axoneme (Satir et al., 1981; Penningroth et al., 1982) and actomyosin (Greene & Eisenberg, 1980; Biosca et al., 1986), AMP-PNP promotes dissociation of the motor (dynein or myosin) from the structure it moves (microtubules or actin filaments). The effects of AMP-PNP on organelle motility in the axon indicated, therefore, that fast axonal transport is driven by a new class of mechnochemical protein that is biochemically and pharmacologically distinct from dynein and myosin (Lasek & Brady, 1985).

The evidence that AMP-PNP stabilizes binding of a fast axonal transport motor to microtubules in situ suggested that this nuleotide might be able to serve as a selective probe for the biochemical identification of the motor. Accordingly, Brady (1985) purified microtubules from chick brain extracts in the presence and absence of AMP-PNP. The polypeptide compositions of the two preparations were very similar, but one polypeptide, with a molecular weight by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of

†Supported by NIH Grants GM-35364 (G.S.B.), NS-23868 (S.T.B. and G.S.B.), and NS-23320 (S.T.B.), NSF Grant BNS-8511764 (S.T.B.), Welch Foundation Grant I-1077 (G.S.B. and S.T.B.), and NIH Postdoctoral Fellowship Award GM-10143 (K.K.P.).

0006-2960/88/0427-3409$01.50/0