6. ATP-Binding Site in Dynein β-Heavy Chain: Identification by Molecular Cloning

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Abstract: The cDNA clone for sea urchin dynein β-heavy chain was isolated and sequenced. The predicted amino acid sequence contained the sequence of authentic β-heavy chain and the ATP-binding consensus motif, which is located in the midregion of the molecule.

Key words: Dynein; β-heavy chain; cloning; ATP-binding site.

Introduction. Dynein and kinesin are microtubule-motor proteins that use the energy of ATP-hydrolysis to move in opposite directions along a microtubule. The striking difference between the electron microscopic structures of dynein and of kinesin is accompanied by a difference between the position of the ATP-binding site on their heavy chains, which is located at the N terminus of the heavy chain in kinesin (Yang et al. 1989) and in the midregion of the β-heavy chain in dynein (Mocz et al. 1988). The claret gene product, whose motor domain is located at the C terminus of the molecule, belongs to the kinesin-related family (Endow et al. 1990) but moves toward the microtubule’s minus-end, a direction characteristic of dynein (Walker et al. 1990). The position of the ATP-binding site on a motor molecule may relate to the directionality of movement along a microtubule. Since claret protein possesses the ATP-binding consensus sequence in the midregion of the molecule, it is necessary to identify the position of such motif on dynein β-heavy chain by molecular cloning. The identification of immunoreactive clones from expression libraries by Garber et al. (1989), and Foltz and Asai (1990) can not be taken as proof that the cDNA clone for the β-heavy chain has been isolated. This paper describes the first established cDNA clone for dynein β-heavy chain.

Methods. A cDNA library to the unfertilized egg poly(A)⁺RNA from the sea urchin Anthocidaris crassispina, in λgt11 vector, was screened by affinity-purified antibody to sperm dynein heavy chains (Ogawa et al. 1990). Six positive recombinants (λJ292, λJ296, λA101, λA102, λA103, and λA104) were obtained (Ogawa 1990). The λA103 insert DNA was subcloned into phagemid pTZ19R. Two transformant DNAs with reversed orientation were obtained and a number of nested deletions were generated in SphI/BamHI digested DNAs. Nucleotide sequence was determined by Sanger's dideoxynucleotide chain-termination method using Sequenase Ver.2 (Kraft et al. 1988).

Results. The nucleotide and deduced amino acid sequences of λA103 insert DNA are shown in Fig. 1. The cDNA insert has a reading frame identical to that of the β-galactosidase in λgt11.

Axonemal dynein β-heavy chain gives rise to the ATPase-containing fragment A (Ogawa and Mohri 1975) and outer doublet subfiber A microtubule-binding fragment B by trypsin digestion (Ow et al. 1987). In a preceding paper, we have shown that epitopes for peptide encoded by λA103 passed f1 of fragment B into f2 peptide of fragment A in the β-heavy chain molecule, as shown in Fig. 2A (Ogawa 1990). We prepared fragment A and
sequenced the N terminus of f2 peptide by the method of Matsudaira (1987). The amino acid sequence was QQVAPLQANEVAI. This sequence was found at the arbitrary residue 429-441 of Fig. 1 (first open box). Therefore, this clone, which contains a 3,865 base-pair insert, including EcoRI/NotI adaptor sequence, was named ADf3HCA103.

In view of the nucleotide-binding ability of f2 peptide (Mocz et al. 1988), we inspected the predicted amino acid sequence for common features found in other nucleotide-binding proteins. The consensus sequence element indicative of ATP-binding is GXXXXGKT (or S) (X is any amino acid) (Walker et al. 1982). This was found in the ADf3HCA103 sequence, beginning at Gly 1089, showing that the ATP-binding site is separated by 661 residues from the N terminus of f2 peptide to the C terminus. According to Gibbons et al. (1987),
As shown in Table I, the amino acid sequence within the ATP-binding 'segment I' of the β-heavy chain (second open box of Fig. 1) possesses higher homologies with myosin and claret protein than with kinesin and dynamin. Myosin, kinesin, and dynamin have the consensus sequence in the N terminus of the molecule. We confirm that the β-heavy chain (Fig. 2A) and claret protein possess it in the midregion of the molecule.

**Discussion.** The location of tryptic fragments in the β-heavy chain molecule proposed in this paper was different from that suggested by Mocz *et al.* (1988) (Fig. 2). According to their model, fragment A is located at the N terminal side of the β-heavy chain against fragment B, and the γ-P, binding (ATP-binding) site is separated by $M_r$, 120,000 from the N
terminus of f2 peptide to the C terminus. DNA sequencing shows that this distance should be equivalent to 661 amino acid residues. In the present model with reversed polarity (Fig. 2A), fragment A is located at the C terminal side of the β-heavy chain against fragment B and the γ-β; binding site is separated by Mr 70,000 from the N terminus of f2 peptide to the C terminus. This is in good agreement with the DNA sequencing data.

Molecular cloning of kinesin and of claret protein indicates that the motor domain also contains the microtubule-binding sequence extending from the ATP-binding site into the C terminal side of the molecule (Yang et al. 1989; Endow et al. 1990). Axonemal dynein and fragment A can be activated by tubulin or microtubule (possibly by outer doublet fiber B-microtubule) under physiological conditions (Ogawa 1973). Since the present work shows that the β-heavy chain shares the amino acid sequence with claret protein in the functionally important domain, the above mentioned microtubule-binding sequence may be found in the fragment A region of the β-heavy chain by sequencing the full size cDNA clone.

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References