Monoclonal Antibody Register

Monoclonal Antibody KN-01 against the Heavy Chain of Kinesin

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Background

Kinesin belongs to the group of motor proteins known to convert chemical energy released from ATP into mechanical energy. It is a microtubule-associated molecular motor whose ATPase activity is strongly promoted by microtubules. Within the last decade numerous kinesin isoforms and related proteins sharing a common motor domain were described in eukaryotic organisms (Hirokawa, 1998). One prominent member of the kinesin superfamily comprising more than 100 proteins is the conventional kinesin, which essentially contributes to the anterograde vesicle transport in neuronal cells (Schnapp et al., 1992). Conventional kinesin purified from mammalian brain homogenates is a heterotetramer consisting of two heavy (120 to 130 kDa) and two light chains (60 to 70 kDa), resulting in a molecular mass about 400 kDa. Each heavy chain contains an N-terminal globular motor domain with both a microtubule-binding site and an ATPase active centre, a stalk region which is responsible for heavy chain dimerization, as well as a C-terminal globular tail domain which is implicated in cargo binding. Light chains seem to have a regulatory function (Verhey et al., 1998). In immunofluorescence kinesin appears to be localized on vesicles, but anti-kinesin antibodies differing in their epitope specificity label various cellular components (Lin et al., 1996). It was shown that kinesin was also involved in the interaction of microtubules with intermediate filaments of the vimentin type (Kreitzer et al., 1999).

Description of the antibody KN-01

Production

Kinesin was purified from fresh porcine brain homogenates by a combined procedure of ion exchange chromatography, microtubule affinity binding in the presence of 5'-adenylylimidodiphosphate (AMP-PNP), and gel filtration according to the protocol of Kuznetsov and Gelfand (1986). Throughout the preparation, 50 mM imidazole buffer (pH 6.8), supplemented with 50 mM KCl, 0.5 mM $MgCl_2$, 0.5 mM EGTA, 0.1 mM EDTA and 0.5 mM dithiothreitol, was used. The purified kinesin was frozen in aliquots and stored in liquid nitrogen. The hybridoma cell lines producing antibodies were obtained after immunization of a Balb/c mouse with the obtained kinesin and subsequent fusion of the spleen cells with Sp2/0 myeloma cells. Details of the fusion, screening by ELISA, cloning and production of ascitic fluid have been described previously (Viklický et al., 1982; Dráber et al., 1988).

Specificity

Immunoblotting analysis performed with the enriched fraction of porcine brain kinesin showed that the KN-01 antibody reacted moderately strongly with the heavy chain of the molecule. No cross-reaction was observed with the light chain of kinesin nor with high-molecular-weight proteins in the preparation (Fig. 1A, lane 2). A negative control monoclonal antibody of the same immunoglobulin class against α-tubulin, the TU-16 (IgM), (Dráberová and Dráber, 1998), gave no staining (Fig. 1A, lane 3), whereas a positive control antibody against kinesin (Sigma, Cat. No. K 1005) confirmed that the stained protein band corresponded to the heavy chain of kinesin (Fig. 1A, lane 4). After chymotryptic cleavage of purified kinesin followed by immunoblotting, the KN-01 antibody reacted with a different set of polypeptides in comparison with commercial antibody (Fig. 1B). This indicates that KN-01 recognizes different antigenic determinants on the kinesin molecule. The KN-01 antibody was also applied in immunoprecipitation experiments using the enriched fraction of porcine brain kinesin. In immunoprecipitated material the commercial anti-kinesin antibody stained the protein band with the mobility corresponding to the heavy chain of kinesin (Fig. 2, lane 2). Immunoblotting performed with freshly prepared high-speed extract

Received November 7, 2002. Accepted January 2, 2002.

This work was supported by the grant OE 68/1 from the Ministry of Education of the Czech Republic and by the grant 312/96/K205 from the Grant Agency of the Czech Republic.

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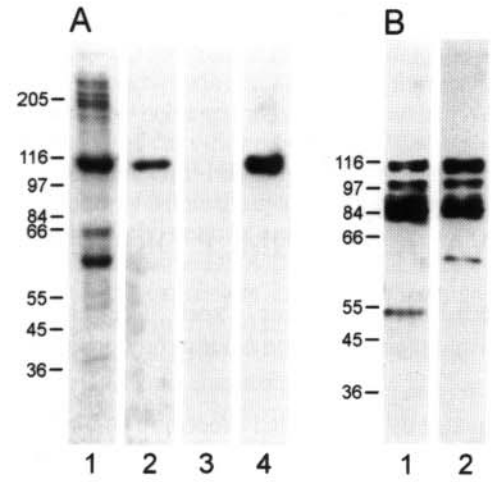

Fig. 1. Specificity of antibody KN-01 to kinesin as determined by immunoblotting. A, enriched preparation of kinesin separated on 7.5% SDS-PAGE. Lane 1, Coomassie blue staining. Lanes 2-4, immunoreactivity tests: antibody KN-01 (lane 2), antibody against tubulin TU-16 (lane 3), commercial antibody against kinesin (lane 4). B, chymotryptic fragments of kinesin separated on 10% SDS-PAGE. Immunostaining with KN-01 antibody (lane 1) and commercial antibody against kinesin (lane 2). Numbers on the left margin indicate the position of specific molecular-weight markers in kDa.

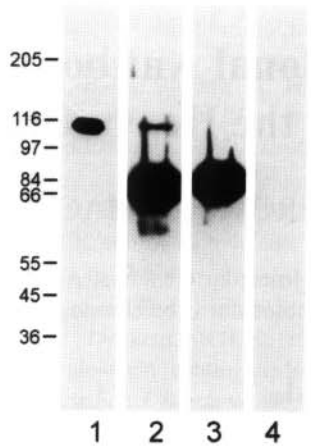

Fig. 2. Specificity of antibody KN-01 as determined by immunoprecipitation of the enriched preparation of porcine brain kinesin. Samples were precipitated with the KN-01 antibody bound to immobilized sheep anti-mouse antibody. Proteins remaining after precipitation (lane 1), immunoprecipitated proteins (lane 2), KN-01 antibody bound to immobilized anti-mouse antibody (lane 3) and proteins bound to immobilized anti-mouse antibody without KN-01 antibody (lane 4) were separated on 7.5% SDS-PAGE. Immunostaining with commercial antibody against kinesin. Numbers on the left margin indicate the position of specific molecular-weight markers in kDa. Immunoprecipitation experiments were performed as described (Dráberová and Dráber, 1993).

of porcine brain, where the level of soluble kinesin was substantially lower than in the enriched preparation, gave no immunostaining either in the position of kinesin or in the position of αβ-tubulin dimers and microtubule-associated proteins. To rule out that the corresponding epitope in freshly prepared extracts was masked by phosphorylation, the extracts were dephosphorylated before blotting using the standard procedure with alkaline phosphatase from *Escherichia coli* (Shaw et al., 1986). The control antibody NF-01, directed against a phosphorylated epitope on the heavy chain of neurofilament triplet protein (Lukáš et al., 1993), did not react with phosphatase-treated samples. However, the reactivity of KN-01 in immunoblotting of phosphatase-treated samples was not enhanced. This indicates that the antibody has a low affinity to SDS-denaturated protein and that the relevant epitope is probably not masked by phosphorylation. Alternatively, the epitope could be masked by other modifications. An immunofluorescence test was designed to determine whether the KN-01 antibody was capable of reacting with the microtubule-bound kinesin. Taxol-stabilized microtubules (without microtubule-associated proteins) were mixed in solution with purified kinesin and AMP-PNP to promote the kinesin binding. Aliquots of the mix-

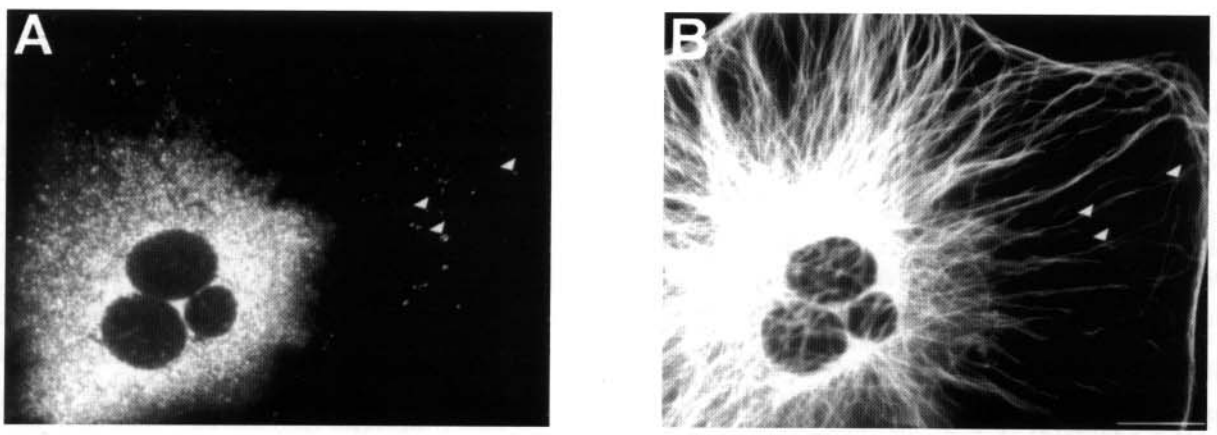

Fig. 3. The binding specificity of the KN-01 antibody as determined by double-label immunofluorescence of 3T3 cells. A – staining with KN-01 antibody. B – staining with anti-tubulin antibody. The cells were fixed by formaldehyde and extracted by Triton X-100 as described (Dráberová and Dráber, 1993). Arrows indicate the same positions. Bar – 10 μm.

ture were then adsorbed on coverslips, fixed with glutaraldehyde and after $NaBH_4$ treatment stained with antibody. A bright staining of microtubules was obtained. Microtubules lacking kinesin were stained only with the anti-tubulin antibody, but not with KN-01. Immunofluorescence experiments with fixed cell lines showed that the antibody gave a typical dot-like staining characteristic for localization of kinesin in vesicles (Fig. 3A). Double-label fluorescence with polyclonal affinity-purified anti-tubulin antibody (Dráber et al., 1991) (Fig. 3B) revealed a co-distribution of stained vesicles with microtubules on the cell periphery. The KN-01 antibody also stained vesicles in embryonal mouse fibroblasts 3T3 as well as in rat basophilic leukaemia cells RBL. The staining pattern was dependent on the fixation protocol. The best decoration was observed in samples fixed with formaldehyde followed by Triton X-100 extraction. In comparison to a commercial antibody from Sigma, the KN-01 antibody provided more discrete dots in immunofluorescence of cell lines.

Properties

The KN-01 antibody (IgM, kappa) can bind porcine brain kinesin and detects the heavy chain of kinesin in ELISA and immunfluorescence tests. It can be used in immunoblotting of enriched kinesin preparations.

Acknowledgement

We thank I. Macháčková for skillful technical assistance.

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