. . *Life Science.* (2002), 12(2), 43~46

JOURNAL OF
Life Science

Interaction of a Kinesin Superfamily Protein 1A (KIF1A) with Calmodulin

Dae-Hyun Seog†

Department of Microbiology, College of Medicine, Inje University, Busan 614-735, Korea

Received: May 23, 2002

Abstract Kinesin Superfamily Protein 1A (KIF1A) is an anterograde monomeric motor transporting a subset of synaptic vesicle precursors and plays an important role in neuronal function and survival. Here, I have used the yeast two-hybrid system to identify the proteins that interacts with the tail region of KIF1A. Calmodulin was found to interact specifically with the tail region of KIF1A. Calmodulin regulates many diverse cellular functions by modulating the activity of the proteins that interact with it. KIF1A interacts with calmodulin in the yeast two-hybrid assay, which is proved by immunoprecipitation with calmodulin in brain fraction. These results indicate that KIF1A is associated with calmodulin, suggesting that calmodulin may be a key role in the regulation of anterograde transport of synaptic vesicle precursors.

Key words: Kinesin, Calmodulin, Yeast Two-hybrid, Pull down, Binding protein

Introduction

Several recent studies demonstrated the elevation of cytosolic calcium by various hormonal and physical signals [1]. Increased cytosolic calcium is believed to control biochemical and molecular processes by modulating the activity of specific protein either directly or through calmodulin [2]. Calmodulin, a highly conserved multifunctional calcium-dependent protein, is implicated in many calcium-dependent cellular processes in cells [1,2]. Calmodulin action in regulating biochemical and molecular event and ultimately physiological processes involves its interaction with calmodulin-binding proteins. The effect of this interaction usually results in regulation of enzymatic activity of the binding proteins [3,4].

Intracellular organelle transport is essential for morphogenesis and function of the cell. Cells employ microtubule motors for intracellular organelle transport. Kinesin superfamily proteins (KIFs) have been identified as the major molecular motor of microtubule-based intracellular transport [5-9]. Each member of the KIFs consists of a globular head domain and tail domain typically. The motor head domain, which is conserved among KIFs, binds to microtubules and hydrolyzes ATP to obtain the energy needed to move toward either the plus or minus end of the microtubules [6]. In contrast, the tail domains are greatly divergent among KIFs and are involved in specific association with their cargoes via adapter proteins. Among these KIFs, KIF1A is a plus-end directed monomeric neuron- specific motor protein. The gene encoding KIF1A was originally discovered as the gene underlying a paralyzed mutant (unc104) in worms [10] and later cloned from mouse [11,12]. In worms and mice, this kinesin plays a similar role in transporting synaptic vesicle precursors from the cell body to the nerve terminal. Further biochemical analysis reveals that KIF1A binds to a synaptic vesicle precursor compartment [11]. To understand how KIF1A links to particular cargoes, and deciphering the regulatory mechanisms for membrane transport remains as major unsolved research questions.

I report that KIF1A interacts with calmodulin in the yeast two-hybrid and coimmunoprecipitated with calmodulin in brain fractions. The interaction of the KIF1A and calmodulin suggest that calmodulin may role as a regulatory protein for synaptic vesicle precursors transport.

Materials and Methods

Construction of cDNA libraries for yeast two-hybrid screening

Mouse total brain RNA was prepared from 5 day-old ICR pups using the Total RNA Separator Kit (Clontech), followed by isolation of mRNA from this preparation with the aid of the mRNA Separator Kit (Clontech). Then, cDNA libraries were prepared using the SuperScript Choice System (GibcoBRL) with random hexamer primers. The cDNA library was ligated with *Eco*RI/*Not*I/*Sal*I adaptors and inserted into the pB42AD vector (Clontech). I transformed

†Corresponding author
Phone: 82-51-890-6734, Fax: 82-51-894-4500
E-mail: daehyun@ijnc.inje.ac.kr

ElectroMAX DH10B cells (GibcoBRL) with this construction and in turn obtained 6×10^6 independent clones. Finally, the plasmids were purified using the QUIAGEN Plasmid Kit. General recombinant DNA techniques were performed according to standard protocols [13].

Screening of KIF1A-binding proteins by yeast two-hybrid assay

A Matchmaker LexA two-hybrid system was used for the screening according to the Manufacturer,s manual (Clontech). In brief, a part of the KIF1A gene [amino acid (a.a.) 400-1696] was fused to the DNA-BD region of the pLexA vector (Clontech) by the PCR method. Then the plasmid was cotransfected with brain cDNA library plasmids into the yeast strain EGY48 carrying the p8op-lacZ gene, grown on an SD/-His/-Trp/-Ura plate supplemented with glucose. Selection of positive clones was performed on a SD/-His/-Trp/-Ura/-Leu plate containing galactose/raffinose/X-gal/BU salts. Subsequent to these procedures, the bait plasmids were recovered from the positive clones to confirm whether the observed interaction was genuine, by retransformation into the yeast. I repeated these procedures several times and analyzed their sequences for which interaction was reproducibly ascertained.

β-galactosidase activity in liquid cultures of yeast

The β-galactosidase activity in liquid cultures of yeast was assayed as described previously [12]. In brief, mid-log phase yeast cells were collected and permeabilized with 0.1% sodium dodecyl sulphate (SDS) and chloroform. The chromogenic substrate o-nitrophenyl-D-galactoside was added in excess to this lysate, and after incubation at 30℃, the reaction was stopped by increasing the pH to 11, by the addition of 1 M Na_2CO_3. The OD at 420 nm, which corresponds to the absorbance by the reaction product, o-nitrophenol, was determined by spectrophotometry and normalized to the reaction time and the cell density.

Subcellular Fractionstion and Coimmunoprecipitation

Subcellular fractionation was performed as described previously [14]. A mouse brain was homogenized in ice-cold homogenization buffer (0.32 M sucrose, 4 mM HEPES, pH 7.3) supplemented with protease inhibitors. The homogenate was classified to P1 fraction by using centrifugation once at 900 ×g and once at 1000 ×g for 10 min, respectively. The supernatant was centrifuged at 12,000 ×g for 15 min, the resulting supernatant was saved (S2). For immunoprecipitation experiment on the S2 fraction, samples were diluted in the same volume of 2 ×binding buffer (50 mM HEPES, 240 mM KCl, 2 mg/ml BSA, 0.2% TX-100, pH 7.4) and incubated with anti-calmodulin antibody, or guinea pig IgG for 2 hrs at 4℃, followed by precipitation with protein A Sepharose (Amersham Pharmacia). Binding buffer (1 ×) was used to washing the beads five times. The precipitates were analyzed by immunoblotting with antibodies against KIF1A antibody.

GST Pull Down with Calmodulin

GST-calmodulin was expressed in *E. coli* BL21 GOLD (Stratagen). GST pull down was performed as earlier described [14].

Results and Discussion

Identification of KIF1A interacting proteins by yeast two-hybrid screening

KIF1A is a plus-end-directed monomeric neuron-specific motor protein and is used for anterograde transport of synaptic vesicle precursors [11]. To identify binding partners of the KIF1A tail, we have screened mouse brain cDNA library using the KIF1A as bait. Out of screen of 6×10^6 colonies, we obtained 30 positive clones which were cDNA fragments containing full length of calmodulin (27 identical clones) and 14-3-3 (1 clone). Analysis of the other colonies is under way to determine the *in vivo* relevance of their affinity for KIF1A.

KIF1A motor interacts with the calmodulin molecule

The molecular structures of KIF1A have been extensively studied [7,9,15]. KIF1A is composed of three domains: motor, putative coiled-coil, PH domains. The motor domain interacts with microtubule. The PH domain can interact with native vesicles and PtdIns(4,5)P2-containing liposomes. To identify the binding domain of calmodulin with KIF1A tail domain, I constructed a series of deletion mutants of the KIF1A to determine the minimal binding domain to the calmodulin molecule (Fig. 1). The minimal domain required for binding was located in the coiled-coil region of KIF1A. In addition, I quantified the binding affinity of calmodulin to KIF1A by measuring β-galactosidase activity in liquid cultures of the yeast. The interaction of KIF1A with calmodulin was the 180 U. This binding was sufficiently strong to mediate sorting *in vivo*. Thus, the coiled-coil domain (aa, 400-700) of KIF1A is responsible for the interaction with calmodulin. Furthermore, the tails of KIF1B (830 to end), KIF5A (806 and end), KIF5B (808 to end), KIF5C (930 to and) and KIF17 (939 to end) were tested for calmodulin binding (Fig. 2). Only that of KIF1A bound with calmodulin.

KIF1A is associated with calmodulin in vitro and in vivo

To demonstrate the interaction between KIF1A and calmodulin, I did pull down assay using a GST fusion protein containing the tail region of KIF1A. The result was that KIF1A was bound to calmodulin. However, KIF17, known to interact with mLin-2 and NMDA receptor was not bound

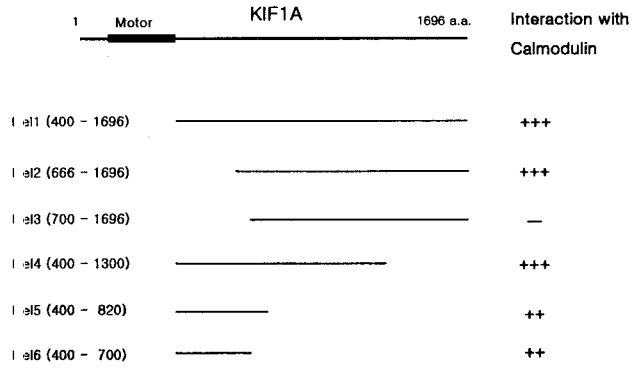

Fig. 1. Identification of KIF1A interacting protein by yeast two-hybrid screening.
Six different deletion constructs of KIF1A cDNA were tested for their interaction ability with calmodulin. The number in the bracket of each deletion mutant indicates the amino acid number of KIF1A. Calmodulin and KIF1A deletion constructs were co-transformed into yeasts. The minimal required domain (400-700) showed strongest binding affinity (indicated by +++, bound to KIF1A and calmodulin; -, not bound to KIF1A and calmodulin).

Bait Calmodulin

Prey	
KIF1A	++
KIF1B	-
KIF2	-
KIF3A	-
KIF3B	-
KIF5A	-
KIF5B	-
KIF5C	-
KIF17	-

Fig. 2. Interaction between KIFs and calmodulin.
The amino acid residues of KIFs were fused to the pLexA DNA binding domain. Calmodulin specifically interacted with KIF1A but not KIF1B, KIF2, KIF3A, KIF3B, KIF5A, KIF5B, KIF5C and KIF17 (indicated by ++, bound to calmodulin; -, not bound to calmodulin).

to calmodulin (Fig. 3). The result is also confirmed by the yeast two-hybrid assay. I further investigated the specificity of the binding between KIF1A and calmodulin in neurons. Calmodulin was coimmunoprecipitated with KIF1A antibody, but not with the IgG (Fig. 4). Thus, suggesting that a calmodulin is the specific binding partner of KIF1A *in vivo*.

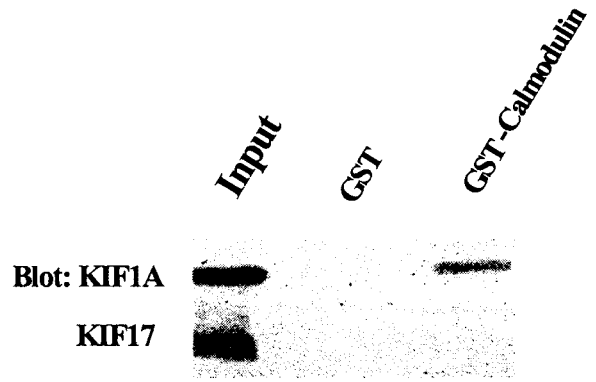

Fig. 3. Association of KIF1A with calmodulin in pull down assay.
Proteins in the mouse brain lysate were pulled down by GST fusion protein containing the calmodulin. Elution fractions were resolved by SDS-PAGE, and Western blotting was performed using KIF1A antibody. Camodulin specifically pulled down with KIF1A, but not with KIF17.

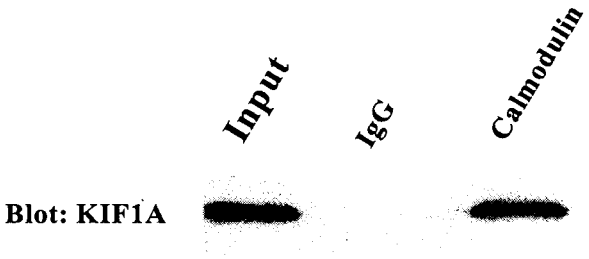

Fig. 4. Coimmunoprecipitation with KIF1A and calmodulin *in vivo*.
Lysates of the S2 fraction was immunoprecipitated with calmodulin antibody or guinea pig IgG (control), and the precipitates were immunoblotted for KIF1A.

In this study, I have isolated KIF1A binding protein, calmodulin. The interaction appears to occur not only *in vitro* but also *in vivo*. The presence of a calmodulin binding domain and a motor domain in KIF1A suggests a role for calcium and calmodulin in at least some of the microtubule-based motor functions [16]. Work in the future to elucidate the regulation of KIF1A motor activity for calmodulin will help to answer some of the questions raised by the presence of these complexes in cells.

Acknowledgment

This work was supported by the 2001 Inje University Research Grant.

References

1. Roberts, D. M. and A. C. Harmon. 1992. Calcium-modulated proteins: Targets of Intracellular calcium signals in higher plants. *Annu. Rev. Plant Phys. Plant Mol. Biol.* **43**, 375-414.
2. Pooviah, B. W. and A. S. N. Reddy. 1993. Calcium and signal

transduction in plants. *Crit. Rev. Plants Sci.* **12**, 185-211.
3. Corneliussen, B., M. Holm, Y. Waltersson, J. Onions, B. Hallberg, A. Thornell and T. Grundstrom. 1994. Calcium/calmodulin inhibition of basic-helix-loop-helix transcription factor domains. *Nature* **368**, 760-764.
4. Deavours, B. E., A. S. Reddy and R. A. Walker. 1998. Ca2+/calmodulin regulation of the Arabidopsis kinesin-like calmodulin-binding protein. *Cell Motil. Cytoskeleton.* **40**, 408-416.
5. Brendza, R. P., L. R. Serbus, J. B. Duffy and W. M. Saxton. 2000. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science* **289**, 2120-2122.
6. Hirokawa, N. 1996. The molecular mechanism of organelle transport along microtubules: the identification and characterization of KIFs (kinesin superfamily proteins). *Cell Struct. Funct.* **21**, 357-367.
7. Hirokawa, N. 1998. Kinesin and dynein superfamily proteins and the mechanism of organelle transport. *Science* **279**, 519-526.
8. Okada, Y. and N. Hirokawa. 2000. Mechanism of the single-headed processivity: diffusional anchoring between the K-loop of kinesin and the C terminus of tubulin. *Proc. Natl. Acad. Sci. U S A.* **97**, 640-645.
9. Miki, H., M. Setou, K. Kaneshiro and N. Hirokawa. 2001. All kinesin superfamily protein, KIF, genes in mouse and human. *Proc. Natl. Acad. Sci. U S A.* **98**, 7004-7011.
10. Otsuka, A. J., A. Jeyaprakash, J. Garcia-Anoveros, L. Z. Tang, G. Fisk, T. Hartshorne, R. Franco and T. Born. 1991. The C. elegans unc-104 gene encodes a putative kinesin heavy chain-like protein. *Neuron* **6**, 113-122.
11. Okada, Y., H. Yamazaki, Y. Sekine-Aizawa and N. Hirokawa. 1995. The neuron-specific kinesin superfamily protein KIF1A is a unique monomeric motor for anterograde axonal transport of synaptic vesicle precursors. *Cell* **81**, 769-780.
12. Yonekawa, Y., A. Harada, Y. Okada, T. Funakoshi, Y. Kanai, Y. Takei, S. Terada, T. Noda and N. Hirokawa. 1998. Defect in synaptic vesicle precursor transport and neuronal cell death in KIF1A motor protein-deficient mice. *J. Cell Biol.* **141**, 431-441.
13. Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. Molecular cloning: a laboratory manual. *Cold Spring Habor Laboratory, Cold Spring Habor, New York.*
14. Takeda, S., H. Yamazaki, D.H. Seog, Y. Kanai, S. Terada and N. Hirokawa. 2000. Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. *J. Cell Biol.* **148**, 1255-1265.
15. Setou, M., T. Nakagawa, D.H. Seog and N. Hirokawa. 2000. Kinesin superfamily motor protein KIF17 and mLin-10 in NMDA receptor-containing vesicle transport. *Science* **288**, 1796-1802.
16. Kikkawa, M., Y. Okada and N. Hirokawa. 2000. 15 A resolution model of the monomeric kinesin motor, KIF1A. *Cell* **100**, 241-252.