Structural and Functional Characterization of NP25, a Single CH Domain Protein

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Hiromi KUWATA[1),2)], Takashi YOSHIOKA[1)], Yoshinori MUTO[3)], Kazuo KUWATA[4)] and Yukio OKANO[1)]

[1)] *Department of Molecular Pathobiochemistry, Division of Disease Control, Gifu University Graduate School of Medicine*
[2)] *Department of Maternal and Pediatric Nursing, Gifu University School of Medicine*
[3)] *Department of Basic Health Science and Fundamental Nursing, Gifu University School of Medicine*
[4)] *Center for Emerging Infectious Diseases, Gifu University*

(Director: Prof. Y. OKANO)

Neuronal protein NP25 is a neuron-specific member of the calponin family proteins, which is known to interact with actin. The secondary structure of NP25 is mainly composed of the helical segments as measured by its circular dichroism (CD) spectra, and the three dimensional structure of the N-terminal half region (27 to 134) is quite homologous to the calponin homology (CH) domain as inferred from the homology modeling method.

The actin sedimentation assay using three deletion mutants of NP25 in the C-terminal region showed that the short sequence of 153-160 (RKAQQNRR) is very important for the interaction with actin, and the C-terminal 27 amino acids (173-199) also contributed to some extent. The multiple sequence alignment revealed that the short actin-binding sequence of NP25 (RKAQQNRR) is well conserved among the single CH domain proteins, but not in the actin binding domain (ABD) proteins. The NP25 sequence can be aligned with the myosin subfragment 1 with relatively low identity (15.4%). RKAQQNRR sequence can be also aligned with the F-actin binding region of myosin subfragment 1 (546–554), which forms the flexible helix-loop-helix motif projecting to the interface between F-actin and myosin. The local structure of the interaction site of myosin with actin is similar to that of the corresponding region of NP25, suggesting that NP25 interacts with F-actin like myosin.

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INTRODUCTION

The calponin homology (CH) domain is a protein module of about 100 residues that was first identified at the N-terminus of calponin, an actin-binding protein playing a major regulatory role in muscle contraction. Three major groups of CH-domain-containing proteins have been recognized on the basis of sequence analysis. The first group contains proteins with a single N-terminal CH domain (1xCH proteins) including calponin, SM22, NP25, and other signaling proteins. The second group contains those with two CH domains forming F-actin-binding domain (ABD) including spectrins, dystrophin and filamins. The third contains those with two ABDs in tandem constituting 4xCH domains such as fimbrin and plastin[1,2)].

NP25 is a member of the first group of calponin family proteins including SM22α, mp20 and calponin, and it is only observed in the highly differentiated central nervous system[3)]. The calponin family proteins except NP25 are major components of differentiated smooth muscle cells and are reported to interact with actin[4~7)]. In rats, NP25 mRNA is detected in the brain and spinal cord, but not in the liver, kidney, testis, ovary, intestine, uterus, heart, or skeletal muscle[3)]. This distribution suggests that NP25 is expressed solely in the central nervous system. When different regions of the rat brain were examined, NP25 mRNA was distributed widely, but not uniformly, in the brain, with strong signals in the hippocampus, frontal cortex, cerebellum, and midbrain[3)]. This wide and differential distribution of NP25 in the brain suggests that it may play an important role in the functioning of specific neuronal systems.

Amino acid sequence information of rat NP25 suggests that there is one casein kinase II phosphorylation site (residue number 138–142), two possible protein kinase C phosphorylation sites (180–183, 198–201) and one ATP binding sequence at the C-terminal (191–200). According to the secondary structure prediction, contents of α-helix, β-sheet and ran-

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dom structure were 36, 17 and 44%, respectively[3]. However, there has not been any experimental evidence of its conformation nor of its biological function.

Recently we demonstrated an interaction of human NP25 with filamentous actin (F-actin) by using a combination of cosedimentation assay and fluorescence resonance energy transfer (FRET)[8], and increased expressions during neural differentiation[9]. F-actin is involved in various cellular functions, such as intracellular trafficking, cell shape, coordinated cell movement, and differentiation in response to various extracellular signals[10]. Globular actin (G-actin) is involved in cell proliferation, whereas F-actin is involved in stress fiber formation and differentiation. Since dynamic changes in actin organization in cells are considered to be controlled by various actin-binding proteins[11], the actin-binding capacity of NP25 might be implicated in the regulation of the stress fiber formation or in the differentiation of neural cells. It therefore seemed of interest to investigate the interaction of NP25 with F-actin to gain further insights into the mechanism of NP25 function.

There have as yet been no attempts to characterize the three-dimensional structure of NP25 or its F-actin binding mechanism. Here we first characterized its three-dimensional structure and investigated the molecular mechanism by which NP25 binds to actin, using the circular dichroism, the homology modeling, the actin sedimentation assay and the multiple sequence alignment.

MATERIALS AND METHODS

cDNA constructs and protein expression

cDNA constructs of wild type and three mutant NP25s (Figure 3) were PCR-amplified with Pyrobest DNA polymerase (TAKARA) and subcloned into pCR4Blunt-TOPO. The primer sets used were as follows; for wild type: upper (5'-AG GATCCCAGAGATGGCTAACAGGGGCCCG-3') and lower (5'-AGAATTCCTACATGATCTGCCTGGGCATCCC-3'); for Δ174–199: upper (5'-AGGATCCCAGAGATGGCTAAC AGGGGCCCG-3') and lower (5'-AGTCGACGTTCTGTCC CTGGCGA-3'); for Δ153–199: upper(5'-AGGATCCCAGA GATGGCTAACAGGGGCCCG-3') and lower (5'-ACACG TGAAACCAGGATGGCTCTCC-3'). To construct Δ153–160 fragment, the small fragment of amino acids 161–199 was first PCR-amplified using the primer sets of upper (5'-ACCC GGGTTTTCCGAGGAGCAGC-3') and lower (5'-AGAATT CCTACATGATCTGCCTGGGCATCCC-3'), then ligated with the fragment of Δ153–199. The obtained clones were sequenced and subcloned into pGEX-3X vector (Pharmacia). Using these constructs, proteins were bacterially expressed and purified with glutathione beads, and then cleaved from the GST-tag with factor Xa.

Actin sedimentation assay

Binding of NP25 to F-actin was examined using a sedimentation assay essentially as described previously[12,8]. Briefly, 2μg of purified NP25 proteins of wild type and deletion mutants were incubated for 10 min at room temperature with 10μg of actin (Sigma) in 200μℓ of buffer containing 2 mM Tris-HCl, pH7.4, 0.2mM $CaCl_2$, 0.2mM DTT, and 0.5 mM ATP. Actin polymerization was induced by adding KCl (75mM) and $MgCl_2$ (2mM) at room temperature for 1h, and the samples were centrifuged at 100,000 g for 1h at 25℃. Supernatants were carefully removed and aliquots of the supernatant and the pellets were analyzed. The samples were mixed with loading buffer and analyzed by SDS-PAGE on a 10% acrylamide gel and stained with Coomassie brilliant blue.

Circular dichroism (CD)

All the circular dichroism (CD) measurements were made using an Aviv 202 stopped-flow circular dichroism spectrometer. Far-UV CD spectra were recorded between 180 and 260 nm, using a 1-nm slit width and a 0.1-cm path length cell thermostated at 20℃. Typically, CD signals were collected for 10 sec at each wavelength. Thermal unfolding of wild type NP25 was monitored by measuring the CD signal at 218nm containing 100μM of purified NP25 in 50mM phosphate buffer (pH6.0) with 100mM Na_2SO_4 in a temperature range from 5 to 85℃ with a heating rate of 1℃/min. Secondary structure was estimated using the program, k2d[13].

Homology modeling and multiple sequence alignment

Homology modeling was conducted using ICM-3.0 software (Molsoft LLC, La Jolla, CA)[14]. Proteins with homologous sequences with NP25 were searched in the Protein Data Bank database. The most probable structure was generated according to the alignment routine in the ICM, and finally the energy-minimized structure was generated. The ICM program has the unique loop prediction routine and the identification routine of the strained parts of the design, and previously predicted conformation of the new 7 residues loop exactly matched the crystallographic data within an error of 0.5Å[14]. Multiple sequence alignment for comparing the actin binding sites was performed using CLUSTALW (http://align.genome.jp/)[15]. The loop structure of the C-terminal half of NP25 was generated by the software Insight II (MSI, Inc), and the surface potential was calculated by MOLMOL (http://www.mol.biol.ethz.ch/wuthrich/software/molmol/).

RESULTS

Secondary structure

Purified wild type NP25 was finally concentrated to 40 μmol at pH7.0. The obtained CD spectrum is shown by ● in Fig. 1.

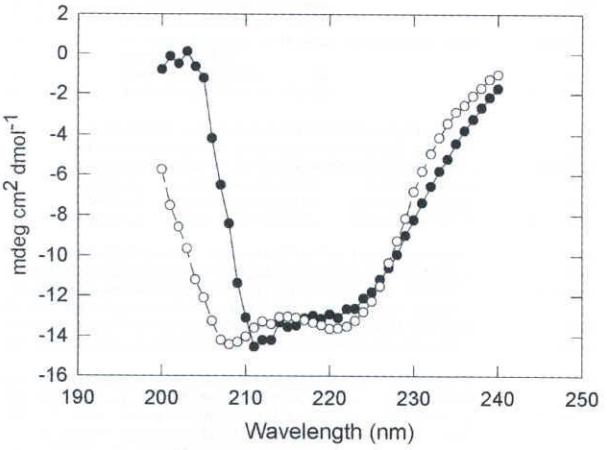

Fig. 1 CD spectrum of NP25 (●) (100 μM, pH6.0, 50mM phosphate buffer with 100mM Na_2SO_4). Contents of α-helix, β-sheet and random structure were calculated to be 27%, 22% and 51%, respectively. Theoretically generated curve was also plotted (○) using the program, k2d[13].

The spectrum apparently shows the helical characteristics with relatively high helical content. To quantify the contents of the secondary structure, we used the software k2d[13]. The theoretically predicted CD curve is shown by ○ in Fig. 1. Obtained contents of the α-helix, β-sheet and random structures were 27%, 22% and 51%, respectively, which are essentially consistent with the results of the secondary structure prediction[3].

Structure of the N-terminal half

Homology modeling of NP25 was conducted using the homology modeling tool in ICM 3.0[14]. Although more than 10 proteins were initially selected as candidates in the homology modeling procedure, most of the generated structures were composed of random coil or small segments of β-sheets, and were apparently incompatible with the CD spectrum of NP25. However, among the candidates only calponin[16] and myosin[17] have high helical content. In fact, the NP25 sequence was quite homologous to the CH domain (identity = 45.5%). The three dimensional structure of NP25 was modeled according to the CH domain structure[17] and the tentatively generated structure was further optimized by the energy minimization procedure. The finally obtained structure includes only the region corresponding to the amino acid residues from 27 to 134, the N-terminal half, and does not include the C-terminal half of NP25, i. e. from 135 to 199.

As shown in Fig. 2a, the final model structure is compact and also globular, consisting of a core of 4 helix bundles (I, III, IV and VI), which are connected by long loops with two short helical structures (II and V). Three of the core helices of (III, IV and VI) form a triple-helix bundle in which helices III and VI are approximately parallel with helix IV. The N-terminal helix I lies perpendicular to helices III and VI and the

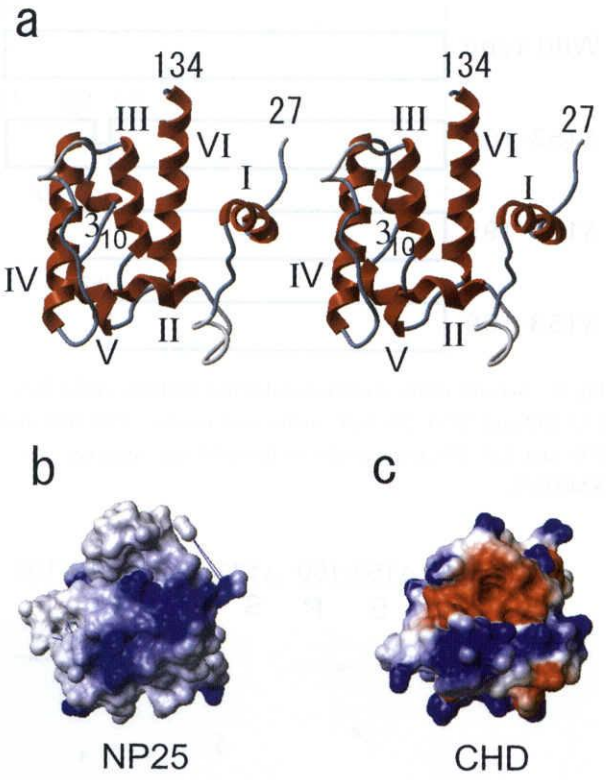

Fig. 2 (a) A stereoview of the model structure of NP25 constructed by the homology modelling based on information on the solution structure of the CH domain[16]. (b) Surface electrical potential of NP25 calculated using MOLMOL. Blue and red colours indicate positive and negative charges, respectively. (c) Surface electrical potential of CH domain of calponin calculated by MOLMOL. Blue and red colors indicate positive and negative charges, respectively.

two short helices (II and V) are located close together at the N-termini of helices III and VI, respectively. A 3_{10} helix is also present in the loop between helices IV and V. These configurations of various segments are quite comparable to those of the CH domain determined by solution NMR[16]. Thus, the three dimensional structure of the N-terminal half of NP25 is essentially the same as that of the CH domain.

To compare the structural characteristics of the NP-25 N-terminal half and the CH domain, we calculated their surface potential profiles. Although the global shape of the model structure of NP25 is quite similar to that of the CH domain as described above, the molecular surface of NP25 is almost uniformly positively charged as shown in Fig. 2b, and this feature is quite different than that of the CH domain, which has both positive and negative charges, as shown in Fig. 2c.

Actin sedimentation assay

Three kinds of deletion mutants in their C-terminal halves were constructed, as illustrated in Fig. 3.

Using these mutants, the actin sedimentation assay was performed to map the binding region of NP25 with actin. As shown in Fig. 4, the band of wild type NP25 (as shown * 1)

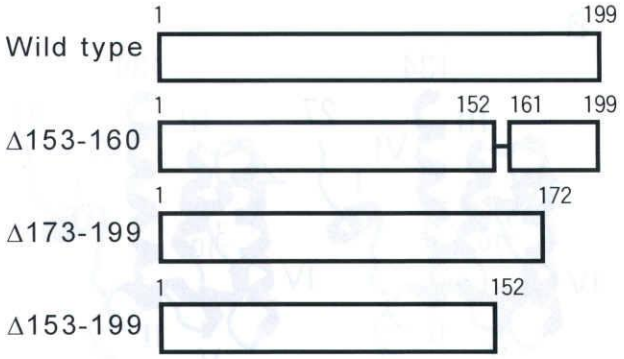

Fig. 3 Scheme of the constructed deletion mutants. Δ153–160, Δ173–199 and Δ153–199 lack amino acid residues 153–160, 173–199, and 153–199, respectively, of the wild type sequence (Acc # XM002852).

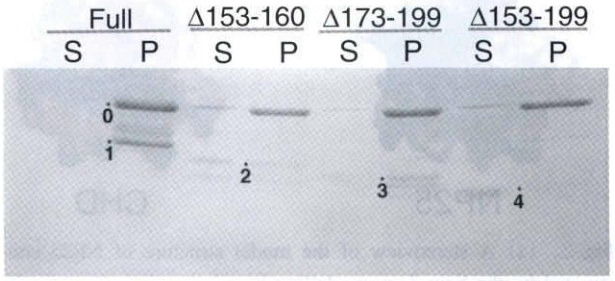

Fig. 4 Profile of the actin sedimentation assay on SDS-PAGE using a 10% acrylamide gel stained with Coomassie brilliant blue. The expected sizes of NP25s of wild type (＊1) and various mutants, Δ153–160 (＊2), Δ173–199 (＊3) and Δ153–199 (＊4) are indicated. The band of actin is indicated as ＊0. S and P indicate the supernatant and the pellet, respectively.

was observed in the pellet, indicating strong interaction with actin.

Compared with wild type, weak bands of Δ153–160 (＊2) or Δ173–199 (＊3) were observed in the pellet, and also in the supernatant, which indicated that these mutants could still bind to actin, though their interactions were relatively weak. Between these two mutants, Δ173–199 bound more strongly than Δ153–160. On the other hand, Δ153–199 (＊4) was observed only in the supernatant, showing that it failed to interact with actin. These results indicate that the interaction site with actin exists between 153–199. Lower affinity of Δ153–160 than Δ173–199 mutant suggests that the sequence between 153–160 (RKAQQNRR) of NP25 is the major site of interaction with actin.

Structure of the C-terminal half and actin binding site

Amino acid sequences of NP25, actin binding domain (ABD) proteins, and single CH domain proteins were compared using multiple sequence alignments[18,19]. In general, single CH domain proteins have the characteristic sequence corresponding to residues from 153 to 160 in NP25 sequence, as shown in red characters in Fig. 5.


NP25
SM22
KIAA0120
h1
mp20
myophilin
MANRGPSYGLSREVQEKIEQKYDADLEN
MANKGPSYGMSREVQSKIEKKYDEELEE
VSALLSSPLERSPQPPPPIGMANRGPAYGLSREVQQKIEKQYDADLEQ
EVKNKLAQKYDHQREQ
MS LERAVRAKIASKRNPEMDK
MSNVPPPSGLSYQVKKKLEGKRDKDQEN
LVDWIILQCAEDIEHPPPGRAHFQKWLMDGTVLCKLINSLYPPGQEPIPKISES
LVEWIIVQCGPDVGRPDRGRLGFQVWLKNGVILSKLVNSLYPDGSKPV-KVPENP
LIQWITTQCRKDVGRPQPGRENFQNWLKDGTVLCELINALYPEGQAPVKKIQAS
LREWIEGVTGRRIGN NFMDGLKDSIILCEFINKLQP GSVKKINES
AQEWIEAIIAEKFPAGQ SYEDVLKDGQVLCKLINVLSP NAVPKVNSS
ALEWIEALTGLKLDRSK LYEDILKDGTVLCKLMNSIKP GCIKKINEN
NP25
SM22
KIAA0120
h1
mp20
myophilin
--KMAFKQMEQISQFLKAAETYGVRTTDIFQTVDLWEGKDMAAVQRTLMALGSVAVTKDD
-PSMVFKQMEQVAQFLKAAEDYGVIKTDMFQTVDLFEGKDMAAVQRTLMALGSLAVTKND
--TMAFKQMEQISQFLQAAERYGINTTDIFQTVDLWEGKNMACVQRTLMNLGGLAVARDD
--TQNWHQLENIGNFIKAITKYGVKPHDIFEANDLFENTNHTQVQSTLLALASMAKTKGN
-GGQ-FKFMENINNFQKALKEYGVPDIDVFQTVDLYEKKDIANVTNTIFALGRATYKHAD
-ATMPFKIMENISAFLEAMKGYGVPVADLFQTVDLFEKKDIAQVTRTLFALGRTCQTHPE
GCYRGEPSWFHRKAQQNRRGFSEEQLRQGQNVIGLQMGSNKGASQAGMTGYGMPRQIM
GHYRGDPNWFMKKAQEHKREFTESQLQEGKHVIGLQMGSNRGASQAGMTGYGRPRQIIS
GLFSGDPNWFPKKSKENPRNFSDNQLQEGKNVIGLQMGTNRGASQAGMTGYGMPRQIL
KVNVG VKYAEKQGRKFEPGKLREGRNIIGLQMGTNKFASQQGMTAYGTRRHLYDP
--FKG-PFLGPKPADECKRDFTEEQLKAGQTIVGLQAGSNKGATQAGQN-LGAGRKILLG
--YSG-PVLGPKLATENKREFTEQQLREGQNVVSLQYGSNKGASQAGIN-MGKQRMIMD-
NP25
SM22
KIAA0120
h1
mp20
myophilin

Fig. 5 Multiple sequence alignment of NP25 with single CH domain proteins. NP25, SM22, KIAA0120, h1 calponin, mp20 and myophilin are used for the alignment.

This region includes a relatively high content of positively charged residues lysine (K) and arginine (R). On the other hand, actin binding domain (ABD) proteins do not possess such characteristic motif around their corresponding regions.

To gain further insight into the structural characteristics of the F-actin binding region of NP25 (153 to 160), we conducted the homology modelling analysis down to much lower similarity (less than 20%). We found that the NP25 is also homologous with the S1 domain of myosin but with low identity (15.6%). Alignment results displayed in Fig. 6 indicated

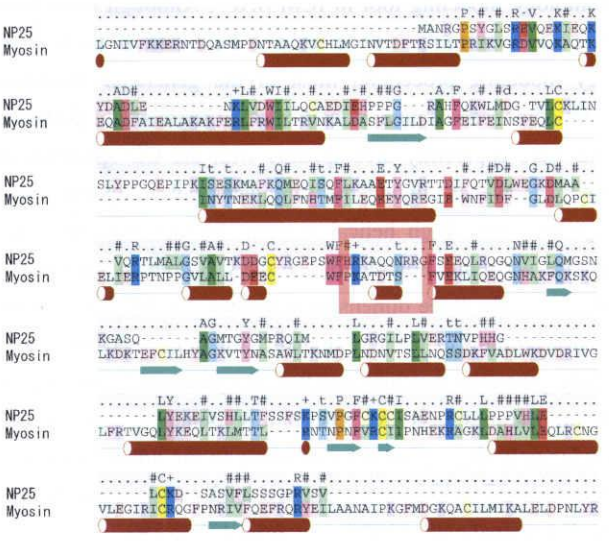

Fig. 6 Sequence alignment of NP25 and myosin. In the top line, amino acid symbol, +, − and # indicate the identical, positively charged, negatively charged and hydrophobic residues, respectively. In the bottom line, red bar and green arrow indicate the α helix and β sheet, respectively. NP25 structure is predicted to be mainly helical. Major F-actin binding site of NP25, RKAQQNRR is marked with a square. Furthermore, this region is predicted to be homologous to myosin S1 Domain.

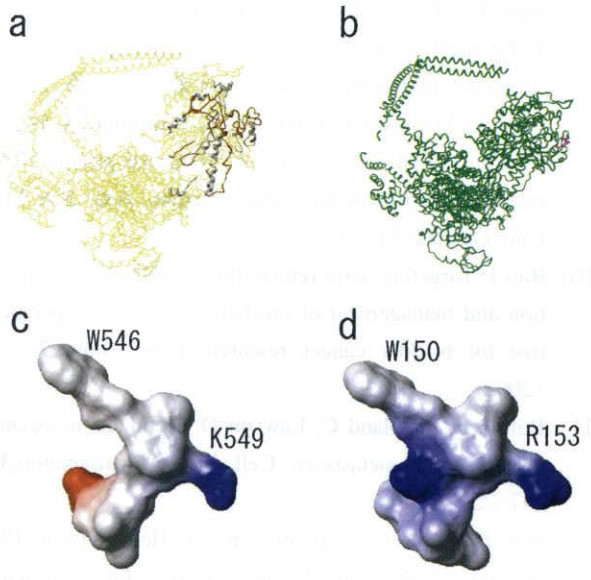

Fig. 7 (a) Superimposition of NP25 (grey and red) on the S1 domain of myosin (yellow). Grey and red indicate the helix and loop, respectively. Superimposition of NP25 and F-actin was performed using ICM-3.0. (b) Location of the F-actin binding site in myosin (magenta). This structure mimics the F-actin binding region of NP 25, residues 153–160. The corresponding region of myosin S1 domain is the residue from 546 to 554, which constructs the actual actin binding site, that corresponds to the C-terminal part of helix-loop-helix structure (helix from 516 to 542, loop from 543 to 546, and helix from 547 to 558). (c) Surface potential of the loop region (546 to 554 of S1, WFPKATDTS) of myosin calculated by MOLMOL. Blue and red colours indicate positive and negative charges, respectively. Lysine 546 forms a kink extruding the positive charge to the actin side. (d) Surface potential of the loop region (153 to 160, WFHRKAQQN) of NP25 calculated by MOLMOL. Blue and red colours indicate positive and negative charges, respectively. Arginine 156 forms a kink similarly to myosin projecting the positive charge to the actin side.

that the F-actin binding region of NP25 could be assigned to the C-terminal part of the helix-loop-helix structure.

The model structure of NP25 was constructed by the homology modeling based on the reported structure of myosin. Then, superimposition of NP25 to whole myosin is displayed in Fig. 7a, though some portions that are not homologous are disrupted in the model. Intriguingly, NP25 corresponds as a whole to the S1 domain of myosin.

The F-actin binding helix-loop-helix motif of myosin is displayed specifically in magenta in Fig. 7b.

To get closer insight into the actin binding loop, we calculated the surface potential of that loop in myosin. As shown in Fig. 7c, the region projecting into the actin is composed of lysine 549 with a positive charge. On the other hand, the corresponding loop of NP25 is also characterized by a positively charged residue, arginine 153, as shown in Fig. 7d.

DISCUSSION

The N-terminal half of NP25 is essentially homologous to CH domains of single CH domain proteins, and is compact and also globular, consisting of a core of 4 helix bundles (I, III, IV and VI) (see Fig. 2a). They are connected by long loops with two short helical structures (II and V). Three of the core helices of (III, IV and VI) form a triple-helix bundle in which helices III and VI are approximately parallel to helix IV. The N-terminal helix I lies perpendicular to helices III and VI and the two short helices (II and V) are located close together at the N-termini of helices III and VI, respectively. A 3_{10} helix is also present in the loop between helices IV and V. In spite of the above common structural features between NP 25 and single CH domain proteins, their surface potentials are quite contrastive, as shown in Figs. 2b and 2c. The surface of NP25 is essentially surrounded by positive charges, while the CH domain of calponin has both positive and negative charges. This characteristic feature of NP25 may provide the structural foundation for the difference of biological functions between NP25 and calponins[18].

It has been argued that multiple CH domains in ABD proteins have strong F-actin binding capacity, but the CH domain in single CH domain proteins such as calponin is insufficient for F-actin binding[5,18]. Thus, single CH domain proteins are considered to possess the actin-binding site outside of the CH domain. In the present study, actin sedimentation assay demonstrated that the actin-binding site of NP25 located between 153–199, especially at residues 153–160, which is different from those of the CH domain of this protein. The conserved domain search analysis also revealed the presence of a calponin-like repeat (CLR) sequence at 174–197 of NP25 as well as the N-terminal CH domain. The CLR is a short conserved sequence consisting of 23 amino acids, which is observed in the C-terminal region of calponin and SM22. Family members of single CH domain proteins have tandem repeats of CLR, which regulate stabilization of actin filaments[20]. Our results of cosedimentation assay showed stronger interaction of the 153–160 sequence with F-actin, and weak association of the 173–199 sequence corresponding to the CLR with F-actin was also observed.

The 153–160 sequence is notably conserved in single CH domain proteins when analyzed using the multiple sequence analysis. This motif is characterized by tryptophan and phenylalanine followed by characteristic positively charged residues, such as lysine or arginine (see Figs. 5, 7c & d). As shown in Figs. 7c & d, this loop forms a kink extruding the residues with positive charge onto the actin side, thus forming the actin-binding site. The preceding tryptophan moiety may work as a switch, since NP25 also has an ATP binding site at the C-terminal region.

In the homology modeling analysis, the corresponding region of the 153–160 in NP25 is assigned as the residue from 546 to 554 in the myosin subfragment S1, which constitutes

the actin-binding site clarified by x-ray crystallography, which is shown in magenta in Fig. 7b. This region corresponds to the C-terminal part of helix-loop-helix structure (helix from 516 to 542, loop from 543 to 546, and helix from 547 to 558). This region also changes its conformation upon binding with actin, while binding of ATP to S1 disrupts the actin-binding site and changes its conformation again. Thus, this region is quite flexible and actually the three dimensional structure by x-ray crystallography could not have been determined by the ordinary method, but by the special computer simulation technique[17]. The conformational flexibility may be essential in this region in order to conduct the biological function, i. e. binding to the F-actin. Although the physiological function of NP25 is not yet clarified, the above findings will help the understanding of the structural and local functional features. Also, this tendency may be somewhat persistent in other single CH proteins such as SM22.

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REFERENCES

1) Korenbaum E, Rivero F: Calponin homology domains at a glance. J Cell Sci **115**, 3543–3545 (2002)
2) Gimona M, Djinovic-Carugo K, Kranewitter WJ, Winder SJ: Functional plasticity of CH domains. FEBS Lett **513**, 98–106 (2002)
3) Ren WZ, Ng GYK, Wang R-X, Wu PH, O'Dowd BF, Osmond DH, George SR, Liew C-C: The identification of NP25 : a novel protein that is differentially expressed by neuronal subpopulations. Mol Brain Res **22**, 173–185 (1994)
4) Prinjha RK, Shapland CE, Hsuan JJ, Totty NF, Mason IJ, Lawson D: Cloning and sequencing of cDNAs encoding the actin cross-linking protein transgelin defines a new family of actin-associated proteins. Cell Motil Cytoskeleton **28**, 243–255 (1994)
5) Gimona M, Mital R: The single CH domain of calponin is neither sufficient nor necessary for F-actin binding. J Cell Sci **111**, 1813–1821 (1998)
6) Fu Y, Liu HW, Forsythe SM, Kogut P, McConville JF, Halayko AJ, Camoretti-Mercado B, Solway J: Mutagenesis analysis of human SM22 : characterization of actin binding. J Appl Physiol **89**, 1985–1990 (2000)
7) Morgan KG, Gangopadhyay SS: Cross-bridge regulation by thin filament-associated proteins. J Appl Physiol **91**, 953–962 (2001)
8) Mori K, Muto Y, Kokuzawa J, Yoshioka T, Yoshimura S, Iwama T, Okano Y, Sakai N: Neuronal protein NP25 interacts with F-actin. Neurosci Res **48**, 439–446 (2004)
9) Mori K, Yoshioka T, Kokuzawa J, Yoshimura S, Iwama T, Muto Y, Okano Y, Sakai N: Neuronal protein NP25 increases during neural differentiation. Acta Sch Med Univ Gifu **52**, 14–19 (2004)
10) Rao J: Targeting actin remodeling profiles for the detection and management of urothelial cancers – a perspective for bladder cancer research. Front Biosci **7**, 1–8 (2002)
11) Button E, Shapland C, Lawson D: Actin, its associated proteins and metastasis. Cell Motil Cytoskeleton **30**, 247–251 (1995)
12) den Hartigh JC, van Bergen en Henegouwen PM, Verkleij AJ, Boonstra J: The EGF receptor is an actin-binding protein. J Cell Biol **119**, 349–355 (1992)
13) Andrade MA, Chacon P, Merelo JJ, Moran F: Evaluation of secondary structure of proteins from UV circular dichroism spectra using an unsupervised learning neural network. Protein Eng **6**, 383–390 (1993)
14) Schapira M, Raaka BM, Samuels HH, Abagyan R: Rational discovery of novel nuclear hormone receptor antagonists. Proc Natl Acad Sci. USA **97**, 1008–1013 (2000)
15) Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22**, 4673–4680 (1994)
16) Bramham J, Hodgkinson JL, Smith BO, Uhrin D, Barlow PD, Winder SJ: Solution structure of the calponin CH domain and fitting to the 3D-helical reconstruction of F-actin:calponin. Structure **10**, 249–258 (2002)
17) Mendelson R, Morris EP: The structure of the actomyosin subfragment 1 complex: results of searches using data from electron microscopy and x-ray crystallography. Proc Natl Acad Sci USA **94**, 8533–8538 (1997)
18) Stradal T, Kranewitter W, Winder SJ, Gioma M: CH domains revisited. FEBS Lett **431**, 134–137 (1998)
19) Peruzzi D, Calabrese G, Faenza I, Manzoli L, Matteucci A, Gianfrancesco F, Billi AM, Stuppia L, Palka G, Cocco L: Identification and chromosomal localisation by fluorescence in situ hybridization of human gene of phosphoinositide-specific phospholipase C beta(1). Biochim Biophys Acta **1484**, 175–182 (2000)
20) Gimona M, Kaverina I, Resch GP, Vignal E, Gurgstaller G: Calponin Repeats Regulate Actin Filament Stability and Formation of Podosomes in Smooth Muscle Cells. Mol Biol Cell **14**, 2482–2491 (2003)

単一カルポニンホモロジードメインタンパク質 NP25の構造機能解析

Neuronal protein25（NP25）は，神経系に特異的な，アクチンと相互作用することが知られているカルポニンファミリータンパク質の1つである。円偏光二色性（CD）スペクトルで調べたNP25の2次構造はヘリックス構造が主であり，ホモロジーモデリングで調べたN末側（アミノ酸27から134）の3次構造は，カルポニンのカルポニンホモロジー（CH）ドメインとよく似ている。

3種類のC末側部分欠失変異体を用いたアクチン共沈法によって以下のことが明らかとなった。すなわち，アミノ酸153–160（RKAQQNRR）の短い配列がアクチンとの相互作用に非常に重要であり，C末側27アミノ酸（173–199）もある程度相互作用に関与している。NP25の短いアクチン結合配列が，単一CHドメインタンパク質の間でよく保存されているが，アクチン結合ドメイン（ABD）タンパク質の間ではそれほどではないことが多重配列アラインメント法によって明らかとなった。また，NP25はミオシンサブフラグメント1と低いながらもホモロジー（15.4%）があり，RKAQQNRR配列は，ミオシンサブフラグメント1のF-アクチン結合領域（546–554）と対応していることが分かった。ミオシンのF-アクチン結合領域（546–554）は，F-アクチンとミオシンの境界部に突出した柔軟な（折れ曲がった）ヘリックス–ループ–ヘリックス構造を形成している。このようにミオシンのF-アクチン結合部位と，NP25のF-アクチン結合部位の局所立体構造は類似しているため，NP25はミオシンのようにF-アクチンと相互作用している可能性がある。