Journal of Cellular Biochemistry 36:25–35 (1988)
Signal Transduction in Cytoplasmic Organization
and Cell Motility 57–67

A Domain of Synapsin I Involved With Actin Bundling Shares Immunologic Cross-Reactivity With Villin

Tamara C. Petrucci, Mark S. Mooseker, and Jon S. Morrow

Departments of Pathology (T.C.P., J.S.M.) and Biology (M.S.M.), Yale University, New Haven, Connecticut 06510

Synapsin I is a neuronal phosphoprotein that can bundle actin filaments in vitro. This activity is under phosphorylation control, and may be related to its putative in vivo role of regulating the clustering and release of small synaptic vesicles. We have compared human and bovine synapsin I by peptide mapping, and have used NTCB (2-nitro-5-thiocyano benzoic acid) cleavage to generate a series of peptide fragments from bovine synapsin I. After chymotryptic digestion, 88% of the tyrosine-containing fragments appear to be structurally identical in human and bovine synapsin I, as judged by their positions on high-resolution two-dimensional peptide maps. The alignment of the NTCB peptides within the parent protein have been determined by peptide mapping, and the ability of these fragments to precipitate with actin bundles has been measured. Only peptides that are derived from regions near the ends of the protein are active. One such 25-kDa peptide which sediments with actin also cross-reacts with antibodies to chicken villin, an actin binding and bundling protein derived from the intestinal microvillus. Since in other respects villin appears to be an unrelated protein, these results suggest the possibility that certain actin binding proteins may show immunologic cross-reactivity due to convergent evolution within the acting binding domain.

Key words: neuronal phosphoprotein, brush border, protein domains, cytoskeleton, synaptic vesicles, actin

Synapsin I is a neuronal phosphoprotein associated with small synaptic vesicles and in lesser amounts with various components of the neuronal cytoskeleton [1,2]. Recently, a cDNA clone encoding synapsin I has been characterized, and from this the complete covalent structure of the molecule has been deduced [3]. Several in-

Abbreviations used: DTT, dithiothreitol; EGTA, ethylene glycol bis(beta-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethylsulfonyl fluoride; SDS, sodium dodecyl sulfate; DFP, diisopropyl fluorophosphate; NTCB, 2 nitro-5-thiocyanobenzoic acid; LPO, lactoperoxidase.

Received March 30, 1987; accepted May 29, 1987.

vitro functions of synapsin I have also been identified, including the ability to bind synaptic vesicles [4], the neuronal plasmalemma [5], microtubules [6], neurofilaments [2,5], spectrin [7], and actin [8]. Many or all of these functions appear to be sensitive to phosphorylation of synapsin I by either cAMP or calcium and calmodulin-dependent kinases [4,5,8]. Based on these findings, it has been proposed that the primary role of synapsin I may be to mediate interactions between synaptic vesicles and various components of the cytoskeleton.

The sites of these interactions within synapsin I are poorly understood. Vesicle binding has been attributed to a collagenase-sensitive carboxyterminal region of the molecule [9], and we have previously identified by immunoblotting techniques a 25-kDA fragment generated by NTCB digestion which sedimented with bundled actin filaments, and which arose from the collagenase resistant portion of synapsin I [8]. We have now examined the ability of radiolabeled synapsin I fragments to bind directly to bundled actin, and have used iodinated peptide mapping to determine the relationship of the various NTCB synapsin I peptides to the parent molecule. These results provide additional evidence for an actin binding domain on synapsin I that is located at or near the amino terminus, and establish a conceptual framework for correllating other binding activities which are preserved in the NTCB-derived peptides with the structural topography of the molecule. We also find that polyclonal antibodies to chicken intestinal villin, another actin binding and bundling protein [10], cross-react with the 25-kDA aminoterminal fragment of synapsin I. We suggest that this cross-reactivity between synapsin I and villin may be analogous to that reported between synapsin I and protein 4.1 [7], and may result from convergent evolution of unrelated proteins to a common (eg, actin binding) function.

MATERIALS AND METHODS

Protein Purification

Synapsin I was purified after acidic extraction from frozen bovine or human brains by chromatography on CM-cellulose and hydroxylapatite [11]. Human brain material was obtained at autopsy with the consent of the family, under Yale University human investigation protocol No. 3388. Rabbit skeletal muscle actin was prepared from acetone powder extracts according to Spudich and Watt [12]. Freshly prepared actin was stored at 4°C under conditions of continuous dialysis versus G buffer (2 mM Tris-HCl, 0.2 mM ATP, 0.2 mM calcium chloride, 0.5 mm DTT, 0.2% sodium azide, ph 8.0), and then polymerized by the addition of the appropriate amount of potassium chloride immediately before use. Purified villin was prepared from isolated chicken intestinal brush borders [13].

Cysteine-specific chemical cleavage of synapsin I by S-cyanylation. Purified synapsin I was cleaved by NTCB following the procedure previously described [8]. Briefly, the protein was reacted with 2 mM NTCB in 7.5 M guanidine hydrochloride, 0.2 M Tris-HCl, 0.1 mM EDTA (pH 8.0), for 1 hr at room temperature, after which the pH was adjusted to 9.0 and the incubation continued overnight at 37°C. The guanidine-HCl was removed by dialysis against 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM 2-mercaptoethanol, pH 8.0.

Two-dimensional cellulose peptide mapping. Cellulose chymotryptic iodopeptide maps were prepared by the method of Elder [14], with the modifications as previously described [8].

Actin binding. Cosedimentation assays were performed using 150-μl aliquots of 7 μM actin and 0.7 μM of synapsin in G buffer containing 0.1M KCl, 2 mM magnesium chloride. After a 30-min incubation at room temperature, the sample was spun at 10,000g for 1 hr at 4°C in a Beckman 42.2 Ti rotor. The pellet was resuspended in the original volume of incubation buffer, and equal aliquots of supernatant and pellet were analyzed by SDS-PAGE [15] and stained with Coomassie brillant blue.

Villin and antivillin. Purified chicken villin and rabbit polyclonal antibodies to villin were prepared as described [16]. The antibody used in this study has been previously characterized [16].

Other Procedures

Electron micrographs were prepared after negative staining using 1% uranyl acetate [8]. SDS-PAGE and two-dimensional IEF/SDS-PAGE were performed by the method of Laemmli [15] and O'Farrell [17], respectively. Western immunoblots were prepared after electrophoretic transfer of SDS gels to nitrocellulose paper (Scheicher and Schuell) [18]. Protein determinations were done by the method of Lowry [19]. Collagenase digestion of synapsin I was performed according to Ueda and Greengard [11]. Cysteine determinations were performed by measuring the cysteic acid content by aminoacid analysis after performic oxidation of synapsin I [20].

RESULTS

NTCB Cleavage Defines Synapsin I in Terms of Its Cysteine Residues

Based on amino acid analysis of bovine synapsin I, it was estimated that the protein contained seven cysteine residues (data not shown). This value is in close agreement with the cysteine content (six) predicted from the rat brain cDNA clone [3]. Therefore, a complete NTCB digest would be expected to produce eight (or seven) peptide fragments, plus any additional fragments arising from the differences between synapsin Ia and synapsin Ib. The results of such a digest, analyzed by SDS-PAGE, are shown in Figure 1a. At least nine prominent cleavage fragments are evident, in addition to residual intact synapsin I. The two-dimensional ^{125}I maps of some of the more prominent fragments are shown in Figure 1b. Even though the number of peptides apparent on one-dimensional gel analysis is close to the theoretical number predicted, the maps indicate that while the 52 and the 25-kDa fragments are complementary, the maps of most of the other peptides (eg, those at 14–15 kDa) display almost the same pattern as the 52-kDa fragment. The only exception to this observation was the 23-kDa fragment, whose map was nearly identical with that of the 25-kDa fragment (data not shown). Therefore, either large regions of synapsin I did not undergo chloramine-T-mediated ^{125}I labeling, or there were many different NTCB peptides of similar molecular weight. Separate experiments in which the NTCB peptides were labeled with ^{125}I using the Boulton-Hunter reagent (which labels amino groups instead of tyrosine), also produced maps with the same degree of overlap (data not shown). Therefore, it appeared that the majority of the fragments identified by one-dimensional SDS-PAGE must actually represent more than one cleavage product. This hypothesis was confirmed by analysis of the digest using two-dimensional nonequilibrium pH gel electrophoresis followed by SDS-PAGE (eg, see Fig. 6). The alignment of the various NTCB peptides and the placement of the

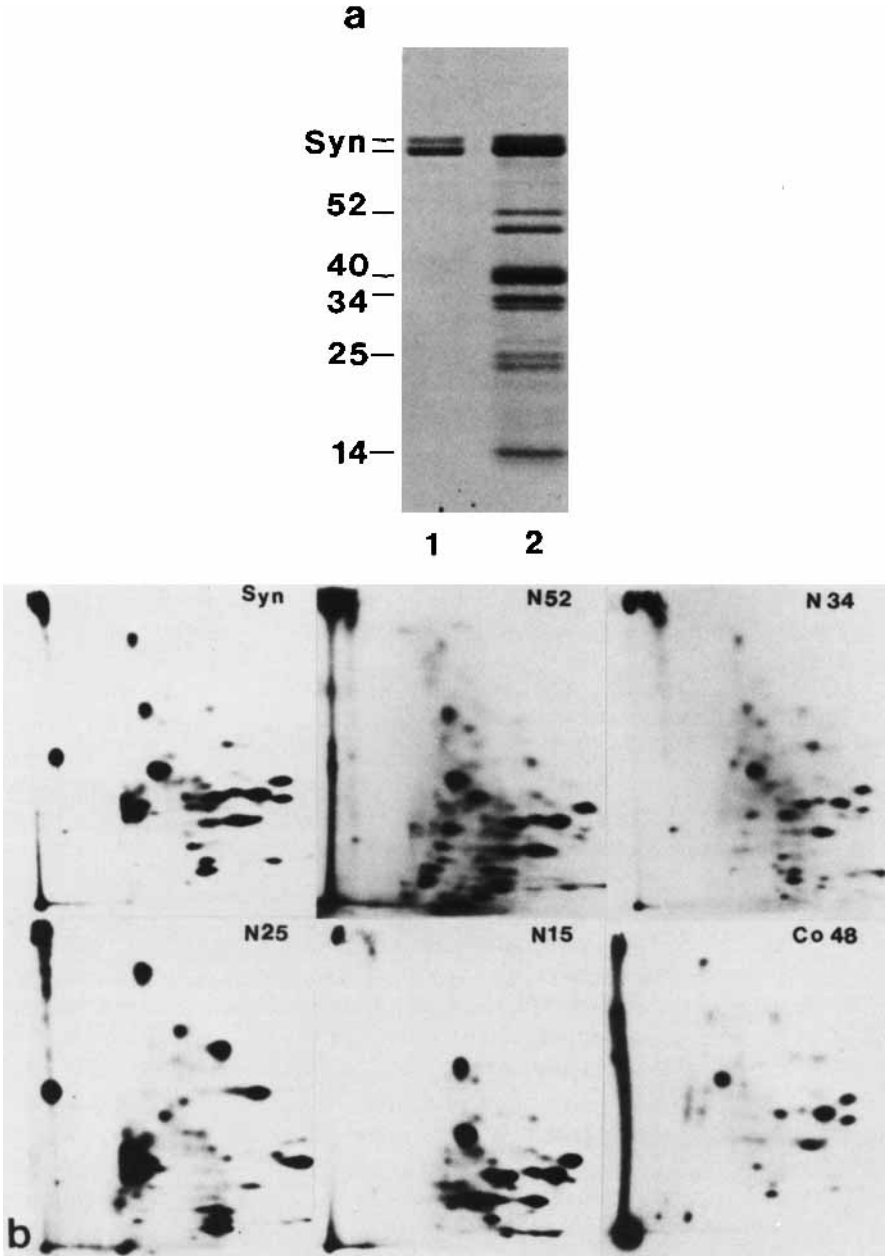

Fig. 1. Cleavage of synapsin I by NTCB generates multiple-intermediate sized peptides. **a:** Coomassie-blue-stained SDS-PAGE of purified bovine synapsin (**lane 1**) and the same protein after cleavage at its cysteine residues by NTCB (**lane 2**). The larger fragments result from incomplete cleavages. **b:** Autoradiograms of the two-dimensional ^{125}I-labeled peptide maps generated by chymotryptic digestion of selected NTCB peptides (labeled with the prefix "N") or of the collagenase resistant domain (labeled with the prefix "Co"). The molecular weights in kDa of each peptide are also indicated. Note that the N52 and N25 peptides derive from complimentary halves of the molecule, since their composite map accounts for all of the peptides present in the map of intact synapsin (Syn).

cysteine residues within the overall topography of the molecule was determined based on the following observations and strategy (using "N" to signify that the peptide is generated by NTCB cleavage, and expressing the molecular weight of the fragment(s) in kilodaltons). These alignments are summarized in Figure 2. 1) The N23 and N25 peptides are nearly identical, and are complementary to the N52. No 50- or 54-kDa peptides with a map identical with the N52 were present. Therefore, a cysteine residue must be present within 2 kDa from a terminus, and the N23/25 peptide must be at that terminus. Alternatively, the N23 and N25 peptides may arise from the differences in synapsin Ia and Ib. While this possibility cannot be rigorously excluded, it was deemed less likely because the map of N23 lacks a prominent spot that is present on the maps of synapsin Ia, Ib, and N25. 2) The map of N50 is similar to the N52, but contains some spots from N23/25 (data not shown). Therefore, it is an overlap peptide between the N23/25 and the N52, and there must be a cysteine within N23. There must also be another cysteine residue near the opposite end of N52. 3) The maps of the N40 and N38 (data not shown) are identical with the map of intact synapsin I. Separate observations (eg, Fig. 5) indicate that two peptides are present in this band. Therefore, at least one cysteine is present at the approximate center of the molecule. 4) The maps of N32 and N34 are identical, and also match closely the map of N50. Therefore, they must arise from the center of synapsin I. The fact that there are fragments at 32 and 34 kDa also suggests that there are two closely spaced cysteine residues near the center of the molecule. 5) The map of the approximately 14-kDa band (N15 in Fig. 1b) contains most of the spots in N52 and the N34. This band must therefore contain fragments from several portions of the molecule. Three peptides of this size predicted from the alignment of the larger fragments are shown in Figure 2. The observed map for this band (Fig. 1b) is consistent with the predicted composite map for three peptides positioned as indicated in Figure 2. While separate experiments involving the mapping of these fragments after they have been separated

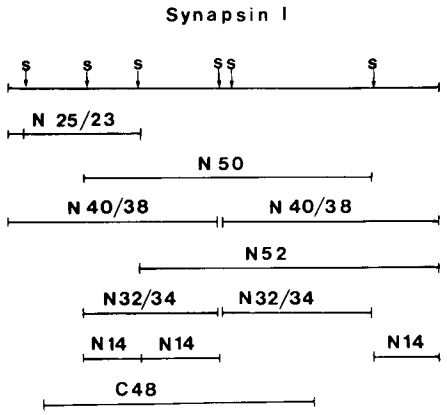

Fig. 2. Summary of the covalent structure of synapsin I as determined by mapping of NTCB peptides. Additional maps beyond those shown in Figure 1 and maps labeled with [^{125}I]-Boulton-Hunter reagent were required to complete this alignment (data not shown). Seven cysteine residues were determined by amino acid analysis; six of these are accounted for in this analysis. Similar-sized fragments arise from different portions of the molecule. The approximate position of the collagenase-resistant core is shown at the bottom (C48).

by two-dimensional NEPHGEF/SDS-PAGE will be required to confirm these alignments, the degree of internal consistency in the present experiments lends confidence to the proposed assignments.

After the determination of these alignments, the position of the collagenase-resistant core of synapsin I was determined by a comparison of its map (Co48, Fig. 1b) with the maps of the NTCB fragments. It contains many of the spots of the N52, and some from N23/25, placing it as shown in Figure 2.

Human and Bovine Synapsin I are Very Similar

The peptide maps of human and bovine synapsin I were compared (Fig. 3). Of the 81 spots clearly discernible on a composite map of the two proteins, 71 spots were in the identical positions on either map. Therefore, since these maps are extremely sensitive to changes in either the charge or hydrophobicity of a peptide, the sequences of these 71 tyrosine-containing chymotryptic fragments must be identical or nearly so. Thus, the two proteins appear to share at least 88% sequence identity, and probably more.

NTCB Peptides From the Ends of Synapsin Sediment With Actin

Synapsin I bundles actin filments in vitro in a phosphorylation-dependent fashion [8]. An example of this interaction is shown in Figure 4. At 10,000g, a solution of 7 μM F-actin will not sediment in 1 hr [8]. However, in the presence of unphosphorylated synapsin I, the low-speed sedimentation of actin is enhanced, as well as that of the synapsin I (Fig. 4a). The actin bundles induced by synapsin I can be visualized after negative staining (Fig. 4b).

To determine which portions of synapsin I were participating in the bundling interaction, an NTCB digest of the protein was labeled with ^{125}I using lactoperoxidase and glucose oxidase, after which it was incubated with F-actin and sedimented at 10,000g for 1 hr. These results are shown in Figure 5. Note that all fragments which contain either end of the synapsin I molecule sediment, including both of the N40s and one of the N12–N14 fragments (cf Fig. 2). Note also the relatively poor labeling of the N52, since over half of its mass is composed of the tyrosine-poor collagenase-sensitive domain of synapsin I (cf Fig. 2). None of the N32–N34 fragments sediment with the actin.

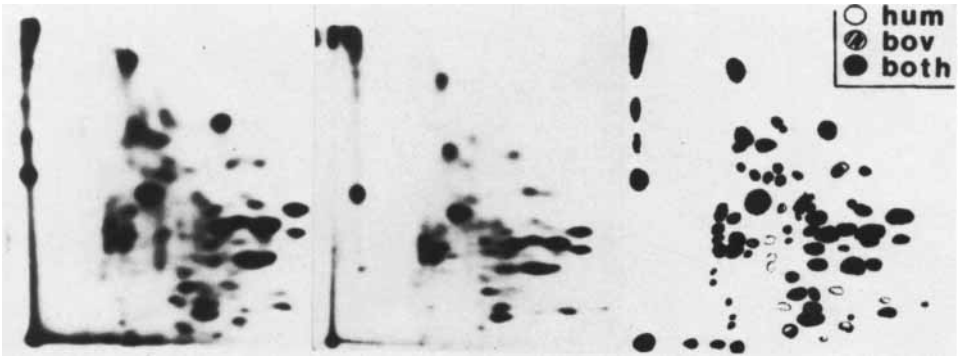

Fig. 3. Human and bovine synapsin I share extensive sequence homology, as determined by radioiodinated peptide mapping. Autoradiograms of human synapsin I (**left**) and bovine synapsin I (**center**) are shown. A comparison of the two maps (**right**) indicates that 71/81 (88%) of the tyrosine containing peptides generated by chymotryptic cleavage appear to be identical in the two species.

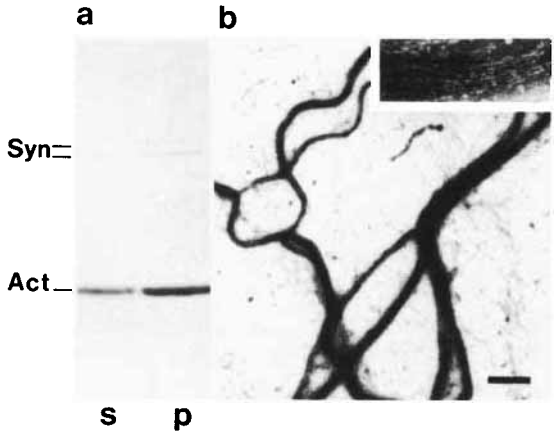

Fig. 4. Intact synapsin I bundles actin filaments in vitro. **a:** Synapsin I enhances the sedimentation of solutions of F-actin. Under these conditions (7 μM actin, 100 μM KCl, 2 mM magnesium, 2 mM Tris-HCl, 0.2 mM calcium, 0.2 mM DTT, 0.2 mM ATP, pH 8.0) actin alone will not sediment at 10,000g for 1 hr at 4°C (data not shown). In the presence of synapsin I (0.7 μM), the precipitation of the actin is enhanced. The supernatant (s) and pellet (p) fractions after analysis by SDS-PAGE and staining with Coomassie blue are shown. **b:** Actin bundles are formed when synapsin I is present, as shown in this negative-stained electron micrograph. The bar = 0.9 μM. **Inset:** Higher-power view (8 × that of Fig. 4b) demonstrates the parallel arrays of bundled actin filaments. The bound synapsin I may be identified in these views.

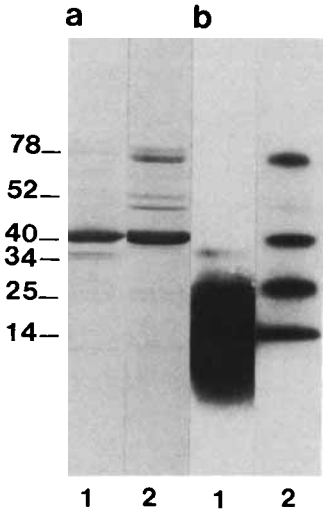

Fig. 5. Only NTCB fragments that include the ends of the synapsin I molecule participate in its actin bundling activity. **a:** Coomassie blue-stained SDS-PAGE of synapsin I that was labeled by LPO with ^{125}I, digested with NTCB, and then incubated with F-actin and sedimented at 10,000g for 1 hr. The presence of intact synapsin in these digests assures that bundling will occur. **Lane 1** is the supernatant fraction; **lane 2** the pellet. Note that peptides of 32–34 kDa are not associated with the actin bundles. **b:** Autoradiogram of the gels shown in panel a. Note the absence of the 32–34 kDa peptides as well as smaller fragments in the actin bundles. Separate mapping experiments establish that the prominent nonbinding fragments (32–34 kDa) arise from the central portions of synapsin I (cf. Fig. 2).

Antibodies to Villin React With the N25 Peptide of Synapsin I

Because of the actin binding/bundling ability of synapsin I, its similarity to other actin binding proteins was of interest. Immunoblotting experiments using intact synapsin I indicated that there was a limited degree of cross-reactivity between bovine synapsin I and highly specific antibodies directed against villin from chicken intestinal brush borders (data not shown). The portions of synapsin I responsible for this cross-reactivity was determined by immunoblotting a NTCB digest of synapsin I after its separation by two-dimensional isoelectric focusing/SDS-PAGE. This result is shown in Figure 6. The only reactive fragments were the N23 and N25, or fragments which would be expected to contain portions of N23 and N25 (cf Fig. 2). A summary of this result, in the context of the other known functions of synapsin I, is shown in Figure 7.

DISCUSSION

Comparison of the topographic model of synapsin I depicted here with the predicted sequence derived from cDNA studies [3], indicates that the N25 peptide

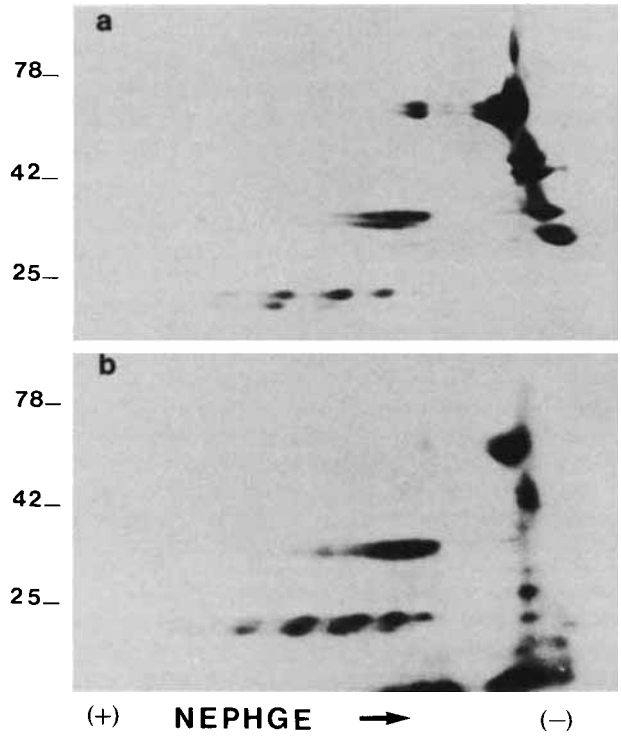

Fig. 6. Antibodies to chicken brush border villin react selectively with the 38–40 and the 23–25 kDa NTCB peptides of synapsin I. **a:** Nonequilibrium two-dimensional pH gel-SDS-PAGE of an NTCB digest of synapsin I, stained with Coomassie blue. Note that the 23–25 peptide which sediments with the actin bundles (cf. Fig. 5) is the most acidic fragment in the protein. **b:** Autoradiogram of Western blot of gel shown in panel a, stained with antibodies to villin. Note that the intact synapsin, the 38–40 kDa, and the 23–25 kDa fragments react strongly.

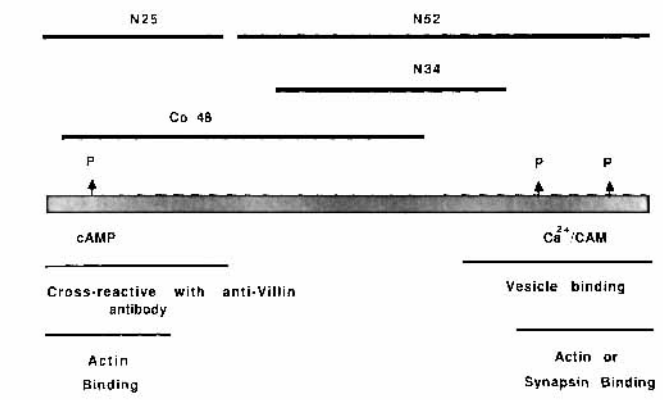

Fig. 7. Hypothetical structural and functional topography of synapsin I. The alignment of prominent NTCB peptides is indicated, as well as the location of the collagenase-resistant core (Co 48), the vesicle binding site, and the sites of phosphorylation [2,3]. Separate preliminary evidence (Petrucci and Morrow, unpublished observations) indicates that the 23-25 kDa fragment is able to bind to actin directly, hence its assignment here as an actin binding site. This region of synapsin I also cross-reacts with antibodies to villin, another actin binding protein. Additional experiments will be necessary to determine if the NTCB fragments derived from the other end of the synapsin molecule near the vesicle binding site also bind actin directly.

must be from the amino terminal portion of the protein. In addition, the location of the cysteine residues as determined here agrees closely with their predicted location based on the cDNA sequence [3], except for two discrepancies. The predicted sequence indicates that there are two adjacent cysteine residues at positions 102 and 103, which would be within N25. The NTCB mapping technique cannot distinguish two so closely spaced cysteine residues; however, the presence of a similar pair of cysteines in bovine synapsin I would account for the missing cysteine in the NTCB analysis compared with the cysteine content determined by amino acid analysis. The second discrepancy is the identification of a cysteine near the aminoterminus of the protein, which accounts for the N25 to N23 conversion. No such cysteine is predicted from the cDNA studies, which were done on rat synapsin. An alternative explanation for the origin of the N23 from the N25 would attribute this difference to the two isoforms of synapsin, Ia and Ib. We do not favor this interpretation, for the reasons cited above. However, it cannot rigorously be excluded at this time. The alternative explanation is that bovine synapsin I contains an additional cysteine residue compared to rat synapsin I, as suggested by the cysteic acid content of the protein after oxidation.

These experiments also demonstrate that it is the ends of the synapsin I molecule that are involved with its actin binding or bundling activity. Because intact synapsin I is always present in the NTCB digests, these experiments cannot determine whether the active peptide fragments are binding directly to actin or to the synapsin I that is bound to the actin. In order to bundle actin filaments, synapsin I must be capable of cross-linking adjacent filaments. Thus, synapsin I either contains one actin binding site and forms dimers (or multimers), or each synapsin I contains at least two actin binding sites. The active fragments examined here are thus binding either to the actin directly, or to the synapsin I directly, which then is bound to actin. Other experiments using purified peptides will be required to determine the affinity of each of the fragments for actin or synapsin I. However, because N25 cross-reacts with antibodies

```
SYNAPSIN   24 AASPGATPGSAAASAERASTAAPVASPAAPSPGSSGGGGFFS 65
              | | |||    **    |     **    ||       |  *|
GELSOLIN  570 ANSAGATRAVEVLPKAGALNSNDAFVLKTPSAAYLWVGTGAS 611

SYNAPSIN   66 SLSNAVKQTTAVAAATSSEQVGGGSGGAGAGAPPPGCCWSSTS 108
               *     |  * **     ||  ||   |*     |     ||
GELSOLIN  612 EAEKTGAQELLRVLRAQPVQVAEGSEPDGFWEALGGKAAYRTS 654
```

Fig. 8. Comparison of sequence homologies in synapsin I and plasma gelsolin. The amino acid sequences predicted from the sequence of cDNA clones encoding for synapsin I [3] or plasma gelsolin [22] were compared using the program BESTFIT [23]. No gaps were allowed. Bars represent identical residue matches. The asterisks represent conservative replacements. A region encompassing residues 24–108 of synapsin aligned with residues 570–654 of gelsolin. The degree of sequence identity was 22%. Allowing for conservative amino acid substitutions, the degree of homology is 34%. Residues 24–108 of synapsin I are predicted to fall within N25 (cf Fig. 2).

to villin, another actin binding protein, this peptide is presently favored as the site most likely to be directly involved with the actin filament.

The cross-reactivity of villin antibodies with synapsin I is reminiscent of the previously reported cross-reactivity of protein 4.1 antibodies with synapsin I [7]. Although this observation initially suggested that perhaps protein 4.1 and synapsin I were related proteins, the sequences of the cDNA clones for these two proteins do not display significant homologies [3,21]. However, a limited homology between portions of synapsin I and other actin binding proteins, such as villin and profilin, has been noted [3]. A comparison of the carboxyterminal domain of chicken villin and synapsin I identified a region of 37 residues that displayed 24% identity and 49% homology (conservative amino acid substitutions) between the two proteins. The same region showed 21% identity and 43% homology to profilin, another actin binding protein. Paradoxically, we find that this region of synapsin I (residues 399–435, which would fall in N32–N34) does not sediment with the bundled actin filaments, and does not cross-react with our antivillin antibody. However, a comparison of synapsin I with the sequence of gelsolin [22], using the program BESTFIT [23], identified another region of synapsin I with limited homology to an actin binding protein. This alignment is shown in Figure 8. Over a stretch of 85 residues (synapsin residues 24–108), there was 22% identity and 34% homology with residues 570–654 of gelsolin. This region of gelsolin has been implicated in actin binding [22], and residues 24–108 of synapsin I fall within N25, the fragment of synapsin I that sediments with bundled actin and which cross-reacts with the antivillin antibodies. Since sequence information for this region of villin is not yet available, the degree of homology of this region with synapsin I cannot be determined. However, given the limited sequence identity that has so far been demonstrated between synapsin I and the various actin binding proteins, it seems unlikely that they have evolved from a common ancestral gene. A more plausible explanation is that these proteins now share weak homologies (and limited antibody cross-reactivity) as a result of convergent evolution. In the case of synapsin I and other actin binding proteins, such as protein 4.1, villin, profilin, and gelsolin, the common structural motif guiding their convergence may be the need to bind actin.

ACKNOWLEDGMENTS

We thank Dr. Thomas Leto for assistance in operating the fitting program and Dr. Alan Harris for assistance with the preparation of human synapsin I. This work

was supported by basic research grants 1-924 (M.S.M.) and 5-377 (J.S.M.) from the March of Dimes Foundation; by NIH grant AM25387 (M.S.M.); and by a special collaborative "Oncology" project of the Italian Research Council and the National Cancer Institute (USA) (T.C.P.).

REFERENCES

1. De Camilli P, Cameron R, Greengard P: J Cell Biol 96:1337, 1983.
2. Goldenring JR, Lasher RS, Vollano ML, Ueda T, Saito S, Sternberg NH, Sternberg LA, De Lorenzo RJ: J Biol Chem 261:8495, 1986.
3. McCaffery CL, DeGennaro LJ: EMBO J 5:3167, 1986.
4. Schiebler WR, Jahn R. Doucet JP, Rothlein J, Greengard P: J Biol Chem 261:8383, 1986.
5. Steiner JP, Ling E, Bennett V: J Biol Chem 262:905, 1987.
6. Baines AJ, Bennett V: Nature 319:145, 1986.
7. Baines AJ, Bennett V: Nature 315:410, 1985.
8. Petrucci TC, Morrow JS: J Cell Biol 105:1355, 1987.
9. Ueda T: J Neurochem 1:297, 1981.
10. Bretscher A, Weber K: Proc Natl Acad Sci 76:2321, 1979.
11. Ueda T, Greengard P: J Biol Chem 252:5155, 1977.
12. Spudich JA, Watt S: J Biol Chem 256:4866, 1971.
13. Coleman TR, Mooseker MS: J Cell Biol 101:1850, 1985.
14. Elder JH, Pickett RA, Hampton J, Lerner RA: J Biol Chem 252:6510, 1970.
15. Laemli UK: Nature 227:680, 1970.
16. Shibayama T, Carboni J, Mooseker MS: J Cell Biol 105:335, 1987.
17. O'Farrell PH: J Biol Chem 250:4007, 1975.
18. Towbin H, Stachelin T, Gordon J: Proc Natl Acad Sci USA 76:4350, 1979.
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: J Biol Chem 193:265, 1951.
20. Moore S: J Biol Chem 238:235, 1962.
21. Conboy J, Kan YW, Shohet SB, Mohandas N: Proc Natl Acad Sci USA 83:9512, 1986.
22. Kwiatkowski DJ, Stossel TP, Orkin SH, Mole JE, Colten HR, Yin HI: Nature 323:455, 1986.
23. BESTFIT is a program supplied by the University of Wisconsin Genetics Computer Group, Madison, WI.