Homo- and Heterodimerization of Synapsins*

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In vertebrates, synapsins constitute a family of synaptic vesicle proteins encoded by three genes. Synapsins contain a central ATP-binding domain, the C-domain, that is highly homologous between synapsins and evolutionarily conserved in invertebrates. The crystal structure of the C-domain from synapsin I revealed that it constitutes a large (>300 amino acids), independently folded domain that forms a tight dimer with or without bound ATP. We now show that the C-domains of all synapsins form homodimers, and that in addition, C-domains from different synapsins associate into heterodimers. This conclusion is based on four findings: 1) in yeast two-hybrid screens with full-length synapsin IIa as a bait, the most frequently isolated prey cDNAs encoded the C-domain of synapsins; 2) quantitative yeast two-hybrid protein-protein binding assays demonstrated pairwise strong interactions between all synapsins; 3) immunoprecipitations from transfected COS cells confirmed that synapsin II heteromultimerizes with synapsins I and III in intact cells, and similar results were obtained with bacterial expression systems; and 4) quantification of the synapsin III level in synapses I/II double knockout mice showed that the level of synapsin III is decreased by 50%, indicating that heteromultimerization of synapsin III with synapsins I or II occurs in vivo and is required for protein stabilization. These data suggest that synapsins coat the surface of synaptic vesicles as homo- and heterodimers in which the C-domains of the various subunits have distinct regulatory properties and are flanked by variable C-terminal sequences. The data also imply that synapsin III does not compensate for the loss of synapsins I and II in the double knockout mice.

Synapsins constitute a family of abundant synaptic vesicle proteins that are peripheral membrane proteins, and they are attached to the vesicle by an unknown mechanism (1, 2). There are at least three synapsin genes. Of these, the genes encoding synapsins Ia, Ib, IIA, and IIb were characterized first (3), whereas the gene encoding synapsin IIIa (no synapsin IIIb has been found) was identified more recently (4, 5). Synapsins Ia and Ib are the most abundant synapsin isoforms, and synapsin IIIa is the least abundant synapsin isoform. All synapsins share a conserved N-terminal phosphorylation site of unknown function that is a substrate for Ca-dependent protein kinase A and Ca\(^{2+}\), calmodulin-dependent protein kinase I. In addition, synapsins Ia and Ib and possibly other synapsins contain multiple phosphorylation sites for a large number of kinases including Ca\(^{2+}\), calmodulin-dependent protein kinase II and proline-directed kinases (6–9).

Sequence analyses revealed that synapsins are composed of conserved N-terminal and central domains that are shared between all synapsins and variable C-terminal domains that differ between synapsins (3). The largest domain of synapsins is the central C-domain, which measures more than 300 residues and is the only domain that is conserved in invertebrate synapsin (10). The C-domain is flanked on the N terminus by short A- and B-domains; the A-domain contains the only phosphorylation site that is present in all synapsins. On the C terminus, the C-domain is followed by two to three variable domains that differ between synapsins except for the E-domain, a shared domain found at the very C-terminal domain of synapsins Ia, IIA, and IIIA but not synapsin Ib or IIb (3–5).

Although synapsins bind to a number of proteins in vitro, including all elements of the cytoskeleton (actin, microtubules, neurofilaments, and spectrin) and multiple Ca\(^{2+}\)-binding proteins (calmodulin and annexin VI), their functions have remained obscure (reviewed in Refs. 11 and 12). Even the detailed analysis of knockout mice lacking both synapsins I and II showed only that synapsins perform essential functions in regulating exocytosis of synaptic vesicles and that synaptic vesicles are destabilized without synapsins (13–15). These functions agree well with the vesicular localization and stoichiometric phosphorylation of synapsins but do not tell us what synapsins actually do. A clue to the functions of synapsins was obtained recently from an unexpected direction: the crystal structure of the C-domain of synapsin I revealed that it is highly homologous to bacterial ATP-dependent synthetases, indicating an enzyme function (16). In support of an enzyme function of synapsins, biochemical studies demonstrated that the C-domains of all synapsins bind ATP with high affinity (3, 17). Interestingly, ATP-binding to the three synapsin C-domains is differentially regulated. Ca\(^{2+}\) is required for ATP binding to synapsin I but inhibits ATP binding to synapsin III and has no effect on ATP binding to synapsin II (4, 17). In synapsins I and II, the difference in Ca\(^{2+}\) regulation was found to be due to a single, evolutionarily conserved amino acid residue that differs between the two synapsins (17). The biochemical and crystallographic studies established that synapsins are evolutionarily conserved ATP-binding proteins in which ATP binding is differentially regulated, providing a rationale for the existence of multiple synapsins. However, the studies have not yet identified an enzyme activity associated with synapsins, which remains to be demonstrated.

In addition to revealing that synapsin C-domains are ATP-binding proteins, the crystal structure also demonstrated that the C-domain of synapsin I forms a dimer in the presence or absence of ATP (18). The contact surface between the two subunits in the dimer is very large, indicating a stable interaction. Because the C-domains of different synapsins are homologous to each other, questions arise as to whether other
synapsins also form homodimers and whether the three synapsins associate into heterodimers. Such heterodimers would link together synapsin isoforms that presumably perform similar functions via their C-domains but that perform these functions in the context of distinct regulatory properties. In the current study, we performed yeast two-hybrid screens with the original intention of identifying possible substrates for the C-domain ATPase. Upon analysis, however, we found that the large majority of the specific prey clones isolated encode synapsins, suggesting that synapsins are their own major binding partners. We then identified the mechanism of multimerization and showed that it occurs in vivo. Finally, we analyzed synapsin I and II double knockout mice (which still express synapsin III) to determine whether synapsin III changes in these mice. Our results demonstrate that synapsins form homo- and heterodimers in vivo and in vitro, thereby creating a multitude of combinations of synapsins on the synaptic vesicle surface.

**Experimental Procedures**

**Yeast Two-hybrid Screens**—Yeast two-hybrid screens of a rat brain cDNA library of postnatal day 8 in pVP16-3 were performed as described previously (18–20) with a bait vector containing full-length rat synapsin IIA fused to LexA cloned into the plasmid pLexN. 38 million transformants were screened, yielding 228 positive clones. Analysis of 100 clones showed that 99 clones exhibited activation of β-galactosidase. Of these, 65 clones were sequenced. 45 clones encoded synapsins, 9 clones encoded EST108711, 4 clones encoded 14-3-3 proteins, and the others encoded independent clones not identified in the data banks (Table I).

**Quantitative β-Galactosidase Assays of Yeast Two-hybrid Interactions**—Full-length or partial coding regions of synapsins Ia, IIA, and IIIa, synaptotagmin I, and lamin (as a negative control; a gift of Dr. S. Hollenberg, Vollum Institute) were cloned into the bait and prey yeast expression vectors pLexN and pVP16-3 (18–20). The following vectors were used containing the following inserts: 1) pLexNSynI-C, residues 110–420 of rat synapsin Ia; 2) pLexNSynIIa, residues 1–586 of rat synapsin IIA; 3) pLexNSynIIIC-C, residues 89–399 of rat synapsin IIIa; 4) pVP-SynI1–624, residues 1–624 of rat synapsin I; 5) pVP-SynII1–479, residues 91–479 of rat synapsin II; and 6) pVP-SynIII-C, residues 89–399 of rat synapsin IIIa. Yeast strain L405 was co-transformed with bait and prey vectors using lithium acetate. Transformants were plated on selection plates lacking uracil, tryptophane, and leucine. After 3 days of incubation at 30 °C, colonies were inoculated into supplemented minimal medium lacking uracil, tryptophane, and leucine and plated on a shaking incubator at 30 °C for 48 h. β-Galactosidase assays were performed on yeast extracts with protein concentrations of 20–40 mg/liter/assay (21).

**Antibodies**—Polyclonal pan-synapsin antibodies (E028) were raised against a peptide containing the N-terminal sequence found in all synapsins (NYLRRLSDSNFMALPQYMTDQITPPQ). Polyclonal synapsin III antibodies (U549 and U551) were generated against a peptide with a synapsin III-specific sequence (CATERRHPQLAAPSF). All peptides were coupled to keyhole limpet hemocyanin. The monoclonal antibody to synapsin I (C110.22) was a gift from Dr. R. Jahn, and the NMDA receptor antibody was a gift from Dr. N. Brose.

**Construction of Bacterial Expression Vectors and Expression and Purification of Recombinant Proteins**—All vectors encoding GST-synapsins were described previously (4, 17). For expression as a His6-fusion protein, a His6-tagged GST was fused to the C-domain of synapsins Ia and IIIa (3–5). More than 200 prey clones were isolated. Sequencing of 65 of these revealed that only three proteins were selected multiple times as independent overlapping clones (Table I). 45 of the 65 sequenced prey clones encoded synapsin I or synapsin III; thus, synapsins account for the majority of the clones isolated. Nine clones encoded an unidentified protein present as an EST sequence in the data banks (EST108711). RNA blots indicated that the mRNA for this protein is not enriched in brain but is ubiquitously distributed (data not shown), and tests with irrelevant bait vectors suggested that the EST108711 clones were mildly autoactivating. Thus, it seems probable that their interaction with synapsins is an artifact. Finally, four clones were from two distinct isoforms of 14-3-3 proteins that we have frequently isolated in yeast two-hybrid screens with other baits and may also represent an artifact. Indeed, synapsin immunoprecipitations failed to co-precipitate 14-3-3 proteins, indicating that they do not bind to synapsins physiologically (data not shown).

**Analysis of Interactions between Synapsins Using Yeast Two-hybrid Assays**—The isolation of synapsin I and II prey clones in yeast two-hybrid screens with a synapsin IIA bait suggests that synapsin II forms dimers by self-association and forms heterodimers by association with synapsin I. Synapsin I may not have been identified as a prey because a large fraction of its prey is present at low levels in brain. In the synapsin I crystal structure, the C-domain forms a tight dimer with a large contact area (16). Thus, it seemed likely that the interaction of synapsins I and II with themselves and each other is mediated via their C-domain.

To test this hypothesis and to correlate the crystal structure...
with yeast two-hybrid methods, we measured the binding of synapsin Ila to various fragments of synapsin Ib in yeast two-hybrid assays (Fig. 1). Full-length synapsin Ib and fragments containing residues 117–451 interacted with synapsin Ila, whereas fragments composed of residues 123–415 or less were inactive. Thus, the full-length C-domain appears to be required for binding in the yeast two-hybrid assays, confirming the correlation between the yeast two-hybrid and structural approaches.

We next investigated whether synapsins I and II also interact with synapsin III in yeast two-hybrid assays and whether the various interactions exhibit similar strengths. For this purpose, we constructed prey and bait vectors containing synapsins I, II, and III. In the L40 yeast strain, interactions of bait and prey proteins that are expressed in the yeast cells activate a β-galactosidase gene (18). Therefore, as a quantitative measure of binding of the synapsins to each other and to control proteins (lamin and synaptotagmin 1), we determined the β-galactosidase activities in yeast cells that were transformed with the various synapsin and control bait and prey vectors. These experiments demonstrated that all three synapsins interact with themselves and also bind to each other (Fig. 2).

Synapsin II bound equally strongly to itself and to synapsins I and III. Similarly, the interactions of synapsin I with synapsin II and of synapsin II with synapsin III were similar in magnitude to the interactions of these synapsins with themselves. Only the binding of synapsin I to synapsin III was significantly weaker (Fig. 2). No binding of synapsins to control proteins or vectors was observed, indicating that the observed interactions are specific (Fig. 2).

Characterization of Synapsin Antibodies—The yeast two-hybrid studies indicated that synapsins I, II, and III form homo- and heterodimers via interactions between their C-domains, with the heterodimerization between synapsins I and III being much weaker than all other dimerization events. In preparation for an analysis of such interactions on the protein level, we raised two antibodies to a peptide from the N terminus of synapsin III and characterized the relative specificities of these and other antibodies to synapsins. In these experiments, we used COS cells transfected with the various synapsin expression vectors and utilized three types of antibodies: 1) a monoclonal antibody to synapsin I, 2) the two polyclonal antibodies against synapsin III, and 3) a polyclonal antibody generated against a peptide from the N terminus of synapsin I with a sequence that is similarly found in synapsins II and III.

The synapsin I antibody and one of the synapsin III antibodies were found to be specific for their respective isoforms, indicating that these antibodies can be used to probe only these synapsins (Fig. 3). The second synapsin III antibody also recognized synapsin I in addition to synapsin III (see below). The antibody against the conserved N-terminal peptide of synapsins reacted with all three synapsins, as expected, and therefore represents a pan-synapsin antibody. On the immunoblots, synapsin I is the largest protein, whereas synapsin III is the smallest protein, allowing an unequivocal identification of each synapsin on immunoblots, even with the pan-synapsin antibody (Fig. 3).

Analysis of Synapsin Heteromultimerization in Transfected COS Cells—To determine whether synapsin heteromultimerization occurs in vivo and is not an artifact of yeast two-hybrid assays, we co-transfected COS cells with synapsins I and II or with synapsin II alone (Fig. 4A, lanes 1 and 4). We then immunoprecipitated extracts from the transfected COS cells with the synapsin I monoclonal antibody and analyzed the immunoprecipitates by immunoblotting with the pan-synapsin antibody (Fig. 4 A, lanes 2 and 5). As a control, identical reactions were performed without the primary antibody (Fig. 4A, lane 3). The synapsin I antibody co-precipitated synapsin II. The ratio

| Prey clone | Encoded protein | Residue numbers | No. of isolates |
|------------|----------------|----------------|----------------|
| pPreySyn1–1" | Synapsin Ib | Full-length | 13 |
| pPreySyn1–2 | Synapsin Ib | Full-length | 1 |
| pPreySyn1–3 | Synapsin Ia/b | 1–624 | 5 |
| pPreySyn1–4 | Synapsin Ia/b | 117–617 | 2 |
| pPreySyn2–1 | Synapsin Ia | 1–540 | 23 |
| pPreySyn2–2 | Synapsin Ib | 91–479 | 1 |
| pPreyE108–1 | Unknown | (EST108711) | 5 |
| pPreyE108–2 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |
| pPreyE108–3 | Unknown | (EST108711) | 2 |

- Two different full-length clones for these proteins were isolated in the screens.

**TABLE I**

**Yeast two-hybrid identification of Synapsin interacting molecules**

Legend: 38 million transformants of a rat brain (postnatal day 8) cDNA library in the prey vector pVP16–3 were screened with a bait construct containing the full-length sequence of synapsin Ila fused to LexA in pLexN. 100 positive clones from a total of 228 positives were analyzed; 98 were β-galactosidase-positive, and the plasmid DNA of 65 clones could be rescued and was sequenced. In addition to those clones listed, we isolated seven independent single isolates with no homology in the data banks and multiple transcription factors that probably represent artifacts.

**Fig. 1.** Yeast two-hybrid analysis of the sequences of synapsin Ib that are essential for heterodimerization with synapsin Ila. The domain structure of synapsin Ib is shown schematically at the top, and the locations of the sequences encoded by the various prey clones used for analysis are depicted below the domain structure. Yeast cells were co-transformed with a synapsin Ila full-length bait construct and the indicated prey constructs. Cells were selected on supplemented minimal plates lacking uracil, tryptophan, and leucine. The β-galactosidase activities and resistance to -THULL selection of the yeast strains harboring both vectors were then estimated on selection plates that also lacked histidine (-THULL) or on nitrocellulose filters soaked with X-GAL as described previously (18–20).
of synapsin II to I was lower in the immunoprecipitates than in the COS cells, which is consistent with the notion that synapsin I is complexed both into homodimers and into heterodimers. In the absence of synapsin I, no synapsin II was precipitated by synapsin I antibodies, demonstrating specificity (Fig. 4A, lane 5). Similarly, no synapsins were brought down in control immunoprecipitations (Fig. 4A, lane 3). These data show that synapsins I and II heterodimerize in transfected COS cells.

We next performed similar experiments for synapsins II and III, using the synapsin III-specific antibody for immunoprecipitation and the pan-synapsin antibody for immunoblotting (Fig. 4B). However, in this case, the antibodies used for immunoprecipitation and immunoblotting are both polyclonal, leading to an increased background labeling of IgG. For this reason, we performed the immunoprecipitations with the two independent synapsin III antibodies. Despite the background reactivity for IgG, the two bands corresponding to co-precipitated synapsins II and III could be clearly identified in the immunoprecipitates with synapsin III antibodies from co-transfected COS cells but not in those from COS cells transfected with synapsin II alone (Fig. 4B, arrows in lanes 3 and 6). However, when we attempted similar experiments for synapsins I and III, we were unsuccessful in demonstrating an interaction (data not shown). Therefore, synapsins II and III dimerize not only in yeast two-hybrid assays but also in transfected COS cells, whereas the binding of synapsin I to synapsin III is less certain.

Lack of Stable Heterodimerization between Synapsins I and III—The relatively weak interactions of synapsins I and III in yeast two-hybrid assays compared with other synapsin pairs and our failure to demonstrate synapsin I/III heterodimers in immunoprecipitations raise the possibility that the binding of synapsins I and III may be too weak to allow the formation of a stable dimer. To test this directly, we expressed GST-synapsins I, II, and III and His6-synapsin I singly or in combination in bacteria and purified the recombinant proteins on glutathione-agarose and nickel columns. The presence of GST- and His6-synapsin I dimerization with GST-synapsin I and GST-synapsin II but not with GST-synapsin III was then examined using Coomassie Blue-stained SDS gels and immunoblotting (Fig. 5; data not shown). The results show that the GST-fusion proteins exhibited much higher expression levels, resulting in a very low abundance of heterodimers in glutathione-agarose and nickel columns. The presence of GST- and His6-synapsin I dimerization with GST-synapsin I and GST-synapsin II but not with GST-synapsin III could be detected (Fig. 5, top panel). When we turned the experiment around and purified His6-
synapsin I on nickel columns, a nearly stoichiometric binding of GST-synapsins I and II was found, whereas synapsin III was absent (Fig. 5, bottom panel). Because GST-synapsin III was abundantly expressed in the bacteria, these results indicate that synapsin III indeed does not stably dimerize with synapsin I.

**Synapsin IIIa Levels in Synapsin I/II Double Knockout Mice Are Decreased**—Knockout mice lacking synapsins I and II are viable and fertile but exhibit severe changes in the regulation of neurotransmitter release (13, 14). In addition, synaptic vesicles are destabilized in these mice, and the levels of vesicle proteins are decreased. At the time of the analysis of these knockout mice, however, the existence of a third synapsin isoform (synapsin III) was unknown. Synapsin III is expressed only at low levels; it seems unlikely that it could functionally substitute for synapsins I and II at physiological expression levels. However, it is possible that synapsin III expression may have been induced in the knockouts and obscured a more severe synapsin knockout phenotype by compensating for the loss of synapsins I and II. Conversely, some proteins that form heteromultimeric complexes with other proteins become unstable when their binding partners are not available (e.g., see Ref. 24). Thus, it is also possible that synapsin III levels could actually be decreased in the double knockout mice. To address these questions, we investigated the levels of synapsin IIIa compared with those of a synaptic vesicle protein, synaptophysin, and to a general synaptic marker, NMDA receptor, in wild type and synapsin I and II double knockout mice.

Brain homogenates from wild type and double knockout mice were analyzed by immunoblotting with four antibodies: the two independent antibodies to synapsin III, an antibody to the NMDA receptor as a control protein that does not change in the knockouts, and an antibody to synaptophysin as a synaptic vesicle protein that was previously shown to decrease by approximately 30% in the double knockouts (14). Because the endogenous synapsin III levels in the brain are very low, synapsin III immunoreactivity is weak, and peptide blocks were used to ensure that the various bands were specific. One of the synapsin III antibodies we raised cross-reacts with synapsin I; the corresponding band is absent in the knockouts, whereas the lower synapsin III band is still recognizable (Fig. 6, top panel). Both synapsin III antibodies react with additional brain proteins in a manner insensitive to peptide competition, indicating that this band is not immunologically related to synapsin III (asterisks). Blots were visualized by ECL.

On the immunoblots, synapsin III can be clearly recognized in the double knockout mice, but it seems to be decreased, not increased. This suggests that synapsin III is not induced in the knockout but may actually be destabilized (Fig. 6). To obtain a quantitative measure of this, we performed immunoblots with 125I-labeled secondary antibodies and detected the signal with a PhosphorImager. In agreement with previous results, an analysis of multiple mice showed that synaptophysin levels were decreased by approximately 30% in the double knockouts compared with the NMDA receptor levels. Synapsin IIIa levels, however, were depressed even further (by 50%; Fig. 7). Statistical analysis showed that the decrease in synaptophysin and synapsin IIIa levels from wild type to double knockout mice was highly significant. More importantly, the relative decrease...
in synapsin IIIa levels compared with synaptophysin levels in the double knockouts was also statistically validated \((p < 10^{-8})\). This result demonstrates that the decrease in synapsin IIIa is not only due to the loss of synaptic vesicles but represents a further decrease that cannot be accounted for by the general destabilization of synaptic vesicles (Fig. 7).

**DISCUSSION**

Synapsins are peripheral membrane proteins of synaptic vesicles that are stoichiometrically phosphorylated upon stimulation (1–5). There are at least three synapsin genes (I, II, and III) directing the synthesis of at least five synapsins (Ia, Ib, IIa, IIb, and IIIa). The various synapsins are composed of similar N-terminal and central domains followed by variable C-terminal domains (3). Their most striking feature is the presence of a large central domain, the C-domain, which binds ATP with high affinity and is evolutionarily the most conserved domain of synapsins (3, 10, 16, 17). Although the precise functions of synapsins are unknown, analysis of knockout mice revealed an essential role for synapsins I and II in regulating synaptic vesicle exocytosis and in stabilizing synaptic vesicles (13–15). Together with the crystallographic and biochemical analysis of ATP binding to synapsins, the current data suggest that synapsins are enzymes that act on the synaptic vesicle in an unknown manner.

The crystal structure of the C-domain of synapsin I uncovered two striking properties of this large, autonomously folded domain: 1) its structural homology to ATP-dependent synthases, and 2) its association into tightly bound dimers (16). Biochemical analysis of the C-domains from all synapsins showed that each C-domain constitutes an ATP-binding module with strikingly different regulatory properties (4, 17). The homology between the synapsin C-domains and their similar ATP binding properties now raise the questions of whether other synapsins besides synapsin I form homodimers and whether synapsins associate into heterodimers in addition to homodimers. This is a potentially important question because homo- and heterodimerization of synapsins would imply that synaptic vesicles are coated by a multitude of different synap-

sin dimers with distinct regulatory properties. Because different synapsins are differentially expressed in neurons in a regulated manner (3, 25–28), the exact composition of the coat of synapsin dimers on the synaptic vesicle surface could serve as a mechanism by which the properties of the vesicles are regulated. Four findings reported in the current study support the conclusion that all synapsins associate into such homo- and heterodimers:

1) In yeast two-hybrid screens with full-length synapsin IIa as a bait, synapsins I and II were isolated in 45 of 65 preys analyzed. Synapsin III presumably was not found because of its low abundance. Because yeast two-hybrid screens represent an unbiased approach to evaluating protein-protein interactions, the dominance of synapsins in the prey selected suggests that the major binding partner of a synapsin molecule is another synapsin molecule.

2) Quantitative measurements using yeast two-hybrid assays confirmed that all three synapsin C-domains homodimerize and heterodimerize in strong, pairwise interactions. We observed an almost equal degree of binding between synapsins I and II and between synapsins II and III. In addition, a strong but less intense interaction was observed between synapsins I and III. These findings agree well with the fact that ATP binding is Ca\(^{2+}\)-dependent for synapsin I, Ca\(^{2+}\)-independent for synapsin II, and Ca\(^{2+}\)-inhibited for synapsin III. Thus, by the strength of the interactions, dimers between synapsins that are either induced by Ca\(^{2+}\) or inhibited by Ca\(^{2+}\) would be least likely to form.

3) In transfected COS cells, immunoprecipitation of synapsin I co-precipitated co-transfected synapsin II. Similarly, immunoprecipitation of synapsin III co-precipitated co-transfected synapsin II. These data show that the synapsin I/II and II/III heterodimers occur at the protein level. No synapsin I/III dimers were observed. The heterodimerization of synapsins I and II and the lack of stable synapsin III heterodimers were confirmed in bacterial expression experiments that revealed that even in the presence of high levels of synapsins I and III, no stable binding occurred.

4) Finally, in knockout mice lacking synapsins I and II, we found that the levels of synapsin III were reduced beyond the general decrease observed for synaptic vesicle proteins in these knockout mice. Previous studies had shown that synaptic vesicles are destabilized in synapsin I/II double knockout mice, leading to an overall reduction in vesicle proteins of approximately 30% (14). This was confirmed in the present study in which the level of the synaptic vesicle protein synaptophysin was reduced by 30%. The level of synapsin III, however, was decreased significantly more to approximately 50%. This result indicates that synapsin III is disproportionately destabilized, which is consistent with the notion that it is normally present in a dimer with other synapsins and is destabilized in the absence of these synapsins.

In summary, our results demonstrate that synapsins homo- and heteromultimerize with variable efficiency. All synapsins strongly homodimerize; in addition, synapsin II forms strong heterodimers with synapsins I and III, whereas synapsins I and III interact only weakly, if at all. These data suggest that synapsins exist on the vesicle surface as obligatory dimers. As a result, up to 13 distinct combinations of synapsin dimers may be formed by the five different synapsins on the synaptic vesicle surface. The data portray synapsins as a multigene family of synaptic vesicle proteins that are more variable than previously imagined.

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