Minireview

Structure and Function of the Synapsins*

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The synapsins comprise a family of four highly related phosphoproteins, synapsins Ia and Ib and synapsins IIa and IIb. They were discovered as major brain substrates for cAMP-dependent and Ca^{2+}/calmodulin-dependent protein kinases and subsequently found to be neuron-specific [1–3] (see Ref. 4 for a detailed review). Their expression correlates with synaptogenesis [5–10]. In mature neurons, the synapsins are highly concentrated in nerve terminals irrespective of the neurotransmitter secreted. In nerve terminals, they selectively associate with the cytoplasmic surface of small synaptic vesicles [11–17]. In immature neurons, the synapsins are distributed throughout the distal part of the developing axon and in the growth cone, whereas after formation of synaptic contacts they concentrate specifically in the axon terminal [18].

Several lines of evidence implicate the synapsins in the regulation of neurotransmitter release from nerve terminals. Under conditions of physiological or pharmacological stimulation, Ca^{2+}-dependent neurotransmitter release and Ca^{2+}-dependent phosphorylation of the synapsins undergo parallel changes (for review, see Ref. 19). Synapsin I is able to undergo several cycles of phosphorylation/dephosphorylation within the nerve terminal correlated with cycles of depolarization/repolarization of the presynaptic membrane [20]. Microinjection of dephosphorylated synapsin I into the preterminal digit of the squid giant axon or into goldfish Mauthner neurons induces an inhibition of spontaneous and evoked neurotransmitter release. These effects are not observed when synapsin I which has been phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II)[1] is used [21, 22]. Fluctuation analysis reveals that the inhibition of neurotransmitter release is due to a decrease in the number of synaptic vesicles available for fusion with the presynaptic membrane and not to a change in the probability of fusion [23, 24]. In agreement with these data, microinjection of CaM kinase II induces a 3–7-fold increase in the number of neurotransmitter quanta released in response to presynaptic depolarization [21, 22]. These *in vivo* results were confirmed in experiments in which depolarization-induced glutamate or norepinephrine release from rat brain synaptosomes was evaluated after introduction of synapsin I or CaM kinase II by a freeze-thawing procedure [25, 26].

In addition to their short-term action of regulating neurotransmitter release, the synapsins appear to play a role in the cytoarchitectural and functional rearrangements which occur during maturation of developing synapses. Overexpression of synapsin IIb in neuroblastoma × glioma hybrid cell lines induces, during cell differentiation, a dramatic increase in the number of varicos-

ities along neurites and in the number of synapse-like contacts with other cells. Moreover, the number of synaptic vesicles per varicosity as well as the levels of synaptic vesicle proteins are greatly increased [27]. Microinjection of synapsin I into *Xenopus* embryos at the 2–8-cell stage accelerates the maturation of quantal secretion mechanisms in developing neuromuscular synapses in culture, as measured by both spontaneous and evoked neurotransmitter release [28].

These functional studies point to an involvement of the synapsins in both short- and long-term signal transduction mechanisms. The studies on the structure and biochemical properties of the synapsins described below have revealed some possible molecular mechanisms underlying their biological activities.

Primary Structure and Physicochemical Properties

Synapsins I and II are encoded by two distinct genes. The a and b isoforms of each protein derive from differential splicing of the primary transcripts. In the rat, the four mRNAs code for proteins of 704 (synapsin Ia), 668 (synapsin Ib), 586 (synapsin IIa), and 479 (synapsin IIb) amino acids. The four proteins share a common NH_2-terminal region which is highly conserved phylogenetically and comprises more than half of each molecule. The differences among the four proteins are restricted to the COOH-terminal region. Synapsins Ia and Ib differ only in a very limited region at the COOH-terminal end, the difference between the two isoforms being due to the differential use of two splice acceptor sites separated by 38 nucleotides which generates different reading frames. The COOH-terminal amino acid sequence of synapsin Ia is longer and unrelated to that of synapsin Ib. For synapsins IIa and IIb the alternative use of two different exons leads to a more extended region of dissimilarity [29].

Analysis of the primary structure of the four synapsins fails to reveal sequence homology with any other protein sequence determined so far and suggests the domain model for the protein family shown in Fig. 1 [29]. The common region comprises domains A to C. Domain A contains one phosphorylation site (site 1, Ser^9 for synapsin I and Ser^{10} for synapsin II) for cAMP-dependent protein kinase and Ca^{2+}/calmodulin-dependent protein kinase I (CaM kinase I) [30]. Domain C, rich in hydrophobic residues, exhibits the greatest similarity between synapsins I and II (78% identity) and the greatest degree of interspecies conservation. Domain D, present only in synapsin I, contains two phosphorylation sites (sites 2 and 3, Ser^{566} and Ser^{603}, respectively) for CaM kinase II [30] and one phosphorylation site (Ser^{551}) for a nerve growth factor-sensitive, proline-directed protein kinase [31]. Domain D is rich in basic residues, has an unusually low number of acidic residues, and is very sensitive to collagenase due to the abundance of glycine and proline. Collagenase digestion of synapsin I produces a 46-kDa fragment [32] which roughly corresponds to domains A–C and is referred to as the "head region" of the molecule. The collagenase-sensitive portion of synapsin I is referred to as the "tail region". In synapsin II, domain C is flanked by a short proline-rich region (domain G). Domains E, F, H, and I represent the portions of the molecules generated by alternative splicing. The strong sequence homology observed in the C-terminal 50 amino acids of synapsins Ia and IIa suggests that a gene duplication event involving the a isoforms might have generated synapsins I and II [29]. An analysis of the structure of the coding region of the synapsin I gene reveals that the distribution of the exons correlates with the domains predicted by comparing the primary structures of the four synapsin isoforms [33].

The neuron-specific expression of the synapsin I gene has prompted the search for a neuron-specific promoter in the 5'-flanking region of the gene. This analysis reveals the presence of a constitutive promoter as well as of positive and negative *cis*-

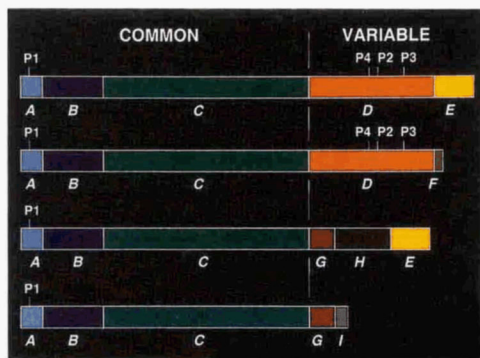*Minireview: Structure and Function of the Synapsins*

FIG. 1. **Structure of the synapsins.** The individual and shared domains of the four synapsins are represented by letters *A–I*. The NH₂-terminal region shared by the four isoforms is composed of domains A–C. In the COOH-terminal variable region, the proline- and glycine-rich D domain is shared by synapsins Ia and Ib, the proline-rich G domain is shared by synapsins IIa and IIb, and the COOH-terminal domain E is present in both synapsin Ia and synapsin IIa. Phosphorylation sites are indicated by *P1–P4* (*P1*, site for cAMP-dependent protein kinase and CaM kinase I; *P2* and *P3*, sites for CaM kinase II; *P4*, site for proline-directed protein kinase). The synapsin isoforms shown in the figure are (from *top* to *bottom*): synapsin Ia, synapsin Ib, synapsin IIa, and synapsin IIb (modified from Ref. 29; copyright 1989 by the AAAS).

TABLE I

Physicochemical properties of the synapsins

The dephospho form of synapsins purified from bovine brain was used.

	Ia	Ib	IIa	IIb
Molecular weight (SDS-PAGE)[a]	86,000	80,000	74,000	55,000
Molecular weight (actual)	74,474	70,395	62,334	53,331
Isoelectric point	>10.5	>10.5	6.9	6.7
Stokes radius (Å)[b]	59	59	ND[c]	34
Sedimentation coefficient (S)	2.9	2.9	ND	2.9
Frictional ratio	2.2	2.2	ND	1.5
Axial ratio[d]	>20	>20	ND	9

[a] SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
[b] Deduced from gel filtration.
[c] ND, not determined.
[d] Calculated from the frictional ratio, for a prolate ellipsoid.

TABLE II

Comparison of surface area and collapse pressure of some surface-active proteins

These data are modified from Ref. 54.

Protein	Limiting area	Collapse pressure
	Å²/residue	dynes/cm
Apolipoprotein A-I	24	22
Apolipoprotein A-II	nd	24
Apolipoprotein B	17	38
Apolipoprotein E	24	14
Synapsin I[a]	22	60

[a] Values are for dephosphorylated synapsin I. Phosphorylation had no significant effect on either the limiting area or the collapse pressure.

acting DNA sequences. The 5'-flanking region lacks TATA and CAAT consensus elements and is enriched in G + C, in analogy with two other neuronally expressed genes, those for brain-specific aldolase C and nerve growth factor receptor. It also contains a consensus sequence for the cAMP-responsive element (34, 35).

Synapsin I and synapsin II are fairly abundant proteins, representing 0.4 and 0.2% of total brain protein, respectively, and are present, in most brain areas of various species, in molar ratios of 2:4:1:2 (Ia:Ib:IIa:IIb) (32, 36, 37). All four synapsins are excellent substrates for cAMP-dependent protein kinase and CaM kinase I, exhibiting a K_m of 2 μM for both kinases (38–41). In addition, synapsin I (but not synapsin II) is the best known substrate for CaM kinase II, exhibiting a K_m of 0.4 μM (38, 42). These phosphorylation reactions have been demonstrated to occur *in vivo* in response to increased levels of second messengers (43, 44). Synapsin I is also phosphorylated *in vivo* (PC12 cells) and *in vitro* by a nerve growth factor-sensitive, proline-directed protein kinase (31, 45). Since this phosphorylation reaction has not been shown to be physiologically relevant, it will not be

discussed further. Purified synapsin I can also be phosphorylated to a limited extent by protein kinase C (46, 47), but this phosphorylation reaction has not yet been shown to occur *in vivo* (44). In the synapsins, potential *O*-glycosylation sites exist. However, the stoichiometry of glycosylation appears to be rather low, so that only a subset of the synapsins contain *O*-linked *N*-acetylglucosamine (48).

Synapsin I has a very basic isoelectric point which is contributed by the tail region, whereas synapsin II as well as the head region of synapsin I exhibit isoelectric points close to neutrality (32, 37) (Table I). Hydrodynamic studies and fluorescence anisotropy studies suggest that synapsin I has an asymmetric elongated structure (32, 49). It has been reported to have a lollipop-like appearance when observed at the electron microscope after rotary shadowing (50, 51). However, rotary shadowing of quick frozen, deep-etched samples of freshly prepared synapsin I failed to detect an elongated structure.[2] The tail region of synapsin I makes a major contribution to the elongated shape of the molecule, due to its enrichment in proline and basic residues and its solubility in aqueous buffers. On the contrary, synapsins IIa and IIb, having a much shorter proline-rich region (IIa and IIb) and a very short variable region (IIb), have a less elongated structure (39) (Table I).

Several types of data demonstrate that the head region of synapsin I, which is also shared by synapsin II, has a hydrophobic character. The hydrophobicity plot calculated from the primary structure of synapsin I reveals a fairly high degree of hydrophobicity involving primarily domain C, in the absence of apparent transmembrane regions (29). The spectral distribution of the intrinsic fluorescence of synapsin I, contributed by four tryptophan residues which are all located in domain C, is remarkably blue-shifted, as expected for tryptophan residues buried inside the protein. A very low accessibility of the tryptophans can also be demonstrated by fluorescence quenching experiments using polar quenchers. These data suggest that in solution the head region of synapsin I is arranged in a tertiary structure which keeps the hydrophobic residues largely segregated from the external aqueous medium (49). In agreement with these hydrophobic properties, synapsin I and synapsin II have a tendency to self-associate at high protein concentrations (32, 52, 53).

Analysis of the primary structure of the synapsins reveals the presence of multiple sequence stretches with the potential to form amphipathic α-helices and β-sheets. These stretches are particularly concentrated in domain C, which is both hydrophobic (39% hydrophobic residues) and highly charged (27% charged residues) (29). Direct measurements show that synapsin I has an extraordinarily high surface activity; it forms a highly stable monolayer at the air-water interface which can be compressed with a lateral force as high as 60–65 dynes/cm before collapsing (Table II). Analysis of fragments of synapsin I reveals that the surface activity is contributed primarily by the head region. Analysis of the secondary structure of synapsin I by circular dichroic spectra reveals significant amounts of α-helix and β-strand structures which can be hydrophobic or amphiphilic. The amount of secondary structure is significantly increased when synapsin I is exposed to amphiphilic environments which simulate the membrane surface. Under these conditions, the limiting surface area of one molecule of synapsin I is 150–180 nm², an exceptionally high value when compared with the molecular weight of the protein (54).

The possibility that site-specific phosphorylation of synapsin I might induce conformational transitions which could account for the changes in its biological activity has been investigated by studying the decays of the intrinsic fluorescence and of the fluorescence anisotropy in the nanosecond time scale (49). Changes in the relative amplitudes and shifts in the spectral distribution of the three components of tryptophan fluorescence decay (with lifetimes of 0.2, 3, and 7 ns) have been detected after phosphorylation of site 1 or sites 2 and 3; these findings demonstrate that the effects of synapsin I phosphorylation are detectable in sequences far beyond those surrounding the phosphorylation sites. Analysis of the fluorescence anisotropy decay demon-

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[2] J. Heuser, F. Benfenati, and F. Valtorta, unpublished results.

strates that, after phosphorylation of sites 2 and 3, but not of site 1, the rotational correlation time and the related Stokes radius of synapsin I are markedly decreased (Table III), indicating that synapsin I undergoes a major conformational change upon phosphorylation of sites 2 and 3.

Interaction of Synapsins with Synaptic Vesicle Protein(s) and Phospholipids

The synapsins are specifically associated with the membrane of small synaptic vesicles although they are not integral membrane proteins (13). It is possible to remove the endogenous synapsins from purified synaptic vesicles and to study the reassociation of purified exogenous synapsins with these vesicles. The association of synapsin I with synaptic vesicles has been characterized in detail. Synaptic vesicles exhibit a high affinity binding (K_D, 10 nM in 40 mM NaCl) for the dephosphorylated form of both synapsins Ia and Ib. The binding is specific for synaptic vesicles, reversible and saturable, and the maximal amount of synapsin I which can be bound (6–7% of total vesicle protein) corresponds to the amount of endogenous synapsin I copurifying with synaptic vesicles. Phosphorylation of synapsin I on sites 2 and 3 by CaM kinase II causes a 5-fold decrease in the affinity of synaptic vesicle binding. In contrast, phosphorylation of site 1 causes only a slight decrease in affinity. In both cases, the amount of synapsin I bound at saturation is unchanged (55). The high limiting surface area determined by biophysical studies (150–180 nm^2) (54) and the number of synapsin I molecules estimated from binding studies (55) and immunocytochemical experiments (12, 16) (10–20 per vesicle) suggest that a large portion of the surface of synaptic vesicles is covered by synapsin I.

The binding of the synapsins to synaptic vesicles consists of multiple interactions of distinct sites of the synapsins with phospholipid as well as protein components (56). The binding constants for synapsin I interactions with vesicle phospholipids are similar to those observed for the binding to native synaptic vesicles, but they are not altered by phosphorylation. The binding is specific for acidic phospholipids (phosphatidylserine and phosphatidylinositol) and involves the head region of synapsin I. In contrast, the tail region, though very basic, is not able to bind phospholipids, suggesting that electrostatic forces are not sufficient to account for the interaction with acidic phospholipids. Although synapsin I is not an integral membrane protein, the use of photoactivatable hydrophobic probes has shown that the binding to phospholipids is accompanied by the penetration of a portion of the head region into the hydrophobic core of the synaptic vesicle membrane (56, 57). The amphipathic regions with high potential for surface and hydrophobic interactions identified in domain C may account for these observations.

Studies performed with synapsin I fragments indicate that the COOH-terminal region interacts with a protein component of synaptic vesicles and that this interaction is markedly reduced upon phosphorylation of sites 2 and 3 (56). Photoaffinity labeling experiments have allowed the identification of the major vesicle-binding protein for synapsin I as the α-subunit of CaM kinase II (58). It is possible that, by virtue of a complex between dephosphorylated synapsin I and CaM kinase II on the vesicle membrane, increases in the cytoplasmic Ca^{2+} level lead to an extremely rapid phosphorylation of synapsin I.

Based upon its substantial homology with the head region of synapsin I, it seems likely that synapsin II is also able to interact with phospholipid bilayers. The fact that synapsin II exhibits a tighter hydrophobic interaction than does synapsin I with synaptic vesicles (see Ref. 4) may be accounted for by its lack of a highly charged tail region. Analysis of the binding activity of several fusion proteins containing different portions of the NH$_2$-terminal region of synapsin II suggests the presence in domain B of an additional site involved in binding to a vesicle protein (59).

Effects of Synapsin I on Actin Dynamics

Synapsin I interacts *in vitro* with various components of the cytomatrix (namely F-actin, microtubules, neurofilaments, and spectrin) (60–64). This ability has prompted the idea that synapsin I might mediate the attachment of synaptic vesicles to the cytoskeleton and regulate their traffic within the axon and the nerve terminal. Since actin is one of the major cytoskeletal elements of the nerve terminal (50, 51), we have paid particular attention to the interactions between synapsin I and actin.

The interaction of synapsin I with F-actin *in vitro* induces the formation of thick bundles of actin filaments, an effect which is reduced by phosphorylation of site 1 and abolished by phosphorylation of sites 2 and 3. Phosphorylation of sites 2 and 3 also reduces the maximal binding of synapsin I to actin filaments by approximately 50%. The affinity of the binding of synapsin I to actin (K_D, 1.5–2.0 μM) is in the same range as for other actin-binding proteins (62, 63). However, no sequence homology is present between synapsin I and other actin-binding proteins (29). (Previous reports of such homology were based on an erroneous sequence of synapsin I (65, 66).) The synapsins may therefore be envisaged as members of a novel family of actin-binding proteins.

Analysis of fragments of synapsin I reveals the presence of a major actin-binding site located in domain C of the molecule (52). The presence of a second binding site in domain D, which might explain the bundling effect, has been postulated based on fragment and cross-linking studies (52, 67).

Actin filaments are highly dynamic structures which undergo cycles of polymerization and depolymerization within the cell (68, 69). Synapsin I has recently been shown to increase the initial rate of actin polymerization and to induce the rapid growth of a higher number of shorter actin filaments, even under conditions in which the formation of filaments is highly unfavorable. These effects can be attributed to a tight binding of synapsin I to actin monomers, which gives rise to the formation of actively elongating pseudonuclei (70–72). The effects are markedly reduced upon phosphorylation of synapsin I. The observation that the concentration of monomeric actin in the cytoplasm is generally high and increases during stimulation of neurotransmitter release (73) suggests a possible functional role for the phosphorylation-dependent nucleating activity of synapsin I.
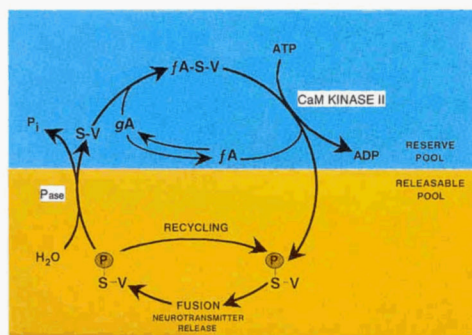

FIG. 2. **Simplified scheme of the possible role of synapsin I in regulating the distribution of synaptic vesicles between reserve and releasable pools.** Elevation of free Ca^{2+} in the nerve terminal, by activating CaM kinase II and phosphorylating synapsin I, disrupts the ternary complex of F-actin-synapsin I-synaptic vesicle. The liberated synaptic vesicles are now free to move from the reserve pool to the releasable pool. Following fusion with the plasma membrane and endocytotic retrieval, the vesicles undergo one of two fates, being either recycled within the releasable pool or resequestered within the reserve pool. In the latter case, resequestering occurs because dephosphorylation of synapsin I promotes actin nucleation on the vesicle membrane, thus embedding the retrieved vesicle in the cytoskeletal network. *V*, synaptic vesicle; *fA*, filamentous actin; *gA*, monomeric actin; *S*, synapsin I; *Pase*, protein phosphatase(s) responsible for dephosphorylation of synapsin I.

TABLE III

Phosphorylation-dependent conformational changes in synapsin I measured by fluorescence anisotropy decay

These data are modified from Ref. 49.

	Dephospho-synapsin I	Phospho-synapsin I (site 1)	Phospho-synapsin I (sites 2, 3)	Phospho-synapsin I (sites 1–3)
ϕ at 25 °C (ns)^a	246	220	72	74
Stokes radius (Å)^b	63.1	60.7	41.9	42.3

^{a} ϕ, rotational correlation time of pyrene-labeled synapsin I.
^{b} Calculated from ϕ using the Stokes-Einstein equation.

Synapsin I: A Dynamic Link between Synaptic Vesicles and the Actin-based Cytoskeleton?

The involvement of synapsin I in the regulation of neurotransmitter release and its ability to bind to synaptic vesicles and to actin suggest a model in which synapsin I reversibly cross-links synaptic vesicles to the cytoskeletal network of the nerve terminal, thereby regulating the availability of synaptic vesicles for exocytosis (74). The results of the structure-function analysis (see above), which has mapped the major actin- and synaptic vesicle-binding sites to distinct parts of the synapsin I molecule, are compatible with such a model (52, 56). This hypothesis has been supported by electron microscopy and by the ability of synaptic vesicle-bound synapsin I to affect actin assembly (72).

The effects of site-specific phosphorylation on the binding of synapsin I to synaptic vesicles, to actin filaments,and to both simultaneously have been tested by computer simulations based on the experimentally determined binding constants. This analysis suggests that the fraction of synaptic vesicles anchored to the cytoskeleton is strongly decreased upon phosphorylation of synapsin I by CaM kinase II. The dissociation of synapsin I seems to occur preferentially from the actin sites (75). Results compatible with those obtained by computer modeling have been obtained by analyzing the distribution of synapsin I in resting and stimulated neuromuscular junctions (76) and synaptosomes (77).

Two functional compartments of synaptic vesicles have been identified in nerve terminals: a pool of vesicles immediately available for exocytosis and a reserve pool of vesicles which can be recruited upon activity (78, 79). We hypothesize that synapsin I, by undergoing cycles of phosphorylation and dephosphorylation, is involved in the movement of synaptic vesicles between these two pools (Fig. 2) and thus in the regulation of the efficiency of neurotransmitter release from adult nerve terminals. This model is consistent with a variety of experimental observations implicating actin in nerve terminal function (50, 51, 72, 73, 79). Whether the ability of the synapsins to induce rearrangements in the actin-based cytoskeleton of the nerve terminal also contributes to the ability of these phosphoproteins to induce the formation and maturation of functional synapses in the nervous system is a fascinating topic for further investigation.

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