Synapsins I and II Are ATP-binding Proteins with Differential Ca\(^{2+}\) Regulation*

(Received for publication, October 28, 1997, and in revised form, November 19, 1997)

Masahiro Hosaka and Thomas C. Südhof‡

From the Howard Hughes Medical Institute and Department of Molecular Genetics, University of Texas Southwestern Medical School, Dallas, Texas 75233

Synapsins I and II are abundant phosphoproteins that are localized to synaptic vesicles and have essential functions in regulating synaptic vesicle exocytosis. Synapsins contain a single evolutionarily conserved, large central domain, the C-domain, that accounts for the majority of their sequences. Unexpectedly, the crystal structure of the C-domain from synapsin I revealed that it is structurally closely related to several ATPases despite the absence of sequence similarities (Esser, L., Wang, C.-R., Hosaka, M., Smagula, C. S., Südhof, T. C., and Deisenhofer, J. (1998) *EMBO J.*, in press). We now show that the C-domains of both synapsin I and synapsin II constitute high affinity ATP-binding modules. The two C-domains exhibit similar ATP affinities but are differentially regulated; ATP binding to synapsin I is Ca\(^{2+}\)-dependent whereas ATP binding to synapsin II is Ca\(^{2+}\)-independent. In synapsin I, the Ca\(^{2+}\) requirement for ATP binding is mediated by a single, evolutionarily conserved glutamate residue (Glu\(_{373}\)) at a position where synapsin II contains a lysine residue. Exchange of Glu\(_{373}\) for lysine converts synapsin I from a Ca\(^{2+}\)-dependent protein into a Ca\(^{2+}\)-independent ATP-binding protein. Our studies suggest that synapsins I and II function on synaptic vesicles as ATP-binding proteins that are differentially regulated by Ca\(^{2+}\).

Ever since their discovery as major brain phosphoproteins and abundant components of synaptic vesicles, synapsins have been intensely studied (1, 2). In vertebrates, synapsins constitute a family of at least four homologous proteins (synapsins Ia, Ib, IIA, and IIB; collectively referred to as synapsins I and II) that are derived by alternative splicing from the primary transcripts of two genes (3, 4). The four synapsins are composed of common amino-terminal domains found in all synapsins (the short A- and B-domains and the long C-domain) and divergent carboxyl-terminal domains that are present in different combinations in each synapsin (D- to I-domains) (3). The C-domains account for more than half of the synapsin sequences and constitute their most conserved domain. In addition to the vertebrate synapsins, a synapsin gene was described in *Drosophila*; this gene also produces two protein products that differ at the carboxyl terminus (5). Only the C-domain is highly conserved between vertebrate and invertebrate synapsins, suggesting that it is the central functional domain.

The four vertebrate synapsins are co-expressed in most neurons (6). They are substrates for multiple protein kinases. All synapsins contain a phosphorylation site for Ca\(^{2+}\), calmodulin-dependent protein kinase I, and protein kinase A at the amino terminus (3). In addition, synapsin I but not synapsin II is phosphorylated by Ca\(^{2+}\), calmodulin-dependent protein kinase II, Cdk5, and mitogen-activated protein kinase at more carboxyl-terminal sites (7–9). The physiological roles of the synapsins have remained elusive because their sequences are not related to proteins of known functions and because they are sticky proteins that bind to a number of possible targets (actin filaments, microtubules, neurofilaments, spectrin, calmodulin, annexin, to name a few of the binding proteins described; reviewed in Refs. 10–12). In knockout mice, synapsins are essential for maintaining stable vesicles and for normal short term synaptic plasticity, suggesting that they constitute important regulatory molecules (13–15). These experiments established that synapsins function in mature synapses but did not reveal their actual activity in the nerve terminal.

Recently, the crystal structure of the C-domain of bovine synapsin I was solved (16). The structure revealed that the C-domain constitutes a large, independently folding domain that forms a stable dimer. Surprisingly, data bank searches for three-dimensionally related proteins demonstrated that the C-domain of synapsin I is very similar to the structures of five ATP-utilizing enzymes: glutathione synthetase, d-alanine-d-alanine ligase, biotin carboxylase-a-chain, succinyl-CoA synthetase \(\beta\)-chain, and pyruvate-orthophosphate dikinase. No sequence similarity was detected between these proteins and the C-domain of synapsin I, but more than 80% of the Ca carbon atoms of the C-domain can be superimposed on those of glutathione synthetase or \(\beta\)-alanyne-\(\beta\)-alanyne ligase with a root mean square deviation of 0.32 nm. The five enzymes to which synapsin I is structurally related bind ATP and transfer phosphate from bound ATP to a substrate (17, 18), suggesting that synapsin I may also bind ATP and be a phosphotransfer enzyme. In support of this, the structure of a complex of the synapsin I C-domain with ATP\(_{\gamma}\)S\(_1\) and Ca\(^{2+}\) was solved (16). In this structure, ATP was bound by residues similar to the ATP-binding residues in the structurally related enzymes, and Ca\(^{2+}\) was coordinated by the pyrophosphate moiety of ATP\(_{\gamma}\)S and two glutamate residues (Glu\(_{373}\) and Glu\(_{386}\)).

The crystal structure of the synapsin I C-domain suggested the possibility that ATP binding may be regulated by Ca\(^{2+}\), an interesting hypothesis in view of the role of synapsins in Ca\(^{2+}\)-regulated exocytosis (13–15). Furthermore, because the C-domains of synapsins I and II are highly homologous, the results with the synapsin I C-domain raised the question if synapsin II also binds ATP. We have now addressed these issues using recombinant C-domains from synapsins I and II. Our results show that all synapsins bind ATP; surprisingly, ATP binding is

---

*This study was supported by a postdoctoral fellowship from the Human Frontiers Science Program (to M. H.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed. Tel.: 214-648-5022; Fax: 214-648-6426; E-mail: TSudho@mednet.swmed.edu.

‡ To whom correspondence should be addressed. Tel.: 214-648-5022; Fax: 214-648-6426; E-mail: TSudho@mednet.swmed.edu.

The abbreviations used are: ATP\(_{\gamma}\)S, adenosine 5'-O-(thiotriphosphate); GST, glutathione S-transferase.
directly regulated by Ca\textsuperscript{2+} only in synapsin I but not synapsin II. The distinct regulatory properties of synapsins depend on a single, evolutionarily conserved amino acid, indicating an evolutionary selection of their separate regulatory properties. These data suggest that synapsins may be differentially regulated phosphotransfer enzymes on the surface of synaptic vesicles.

**MATERIALS AND METHODS**

**Construction of Expression Vectors and Expression of Recombinant Proteins**—Synapsin expression vectors in pGEX-KG (29) with wild type or mutant C-domain sequences were obtained by polymerase chain reaction with oligonucleotide primers containing flanking restriction sites essentially as described using standard molecular biology techniques (20, 21). The following pGEX plasmids were used in the current study: pGEXrSynI-C encoding residues 113–421 of wild type rat synapsin I, pGEXrSynI-C/K269Q, pGEXrSynI-C/E373K, pGEXrSynI-C/E373S, and pGEXrSynI-C/E166D encoding the mutant C-domain of synapsin I with the indicated amino acid substitutions, and pGEXrSynI-NII-C encoding residues 113–421 of wild type rat synapsin II. Control plasmids used were described previously (21, 22). The baculovirus expression vector encoding full-length synapsin Ia fused at the carboxyl terminus with a hexahistidine sequence was constructed in the CspI/RsrII and KpnI sites of pFASTBAC1 (Life Technologies, Inc.) by polymerase chain reaction. All vectors were verified by DNA sequencing. Expression of GST fusion proteins was performed essentially as described (20–22). For baculovirus expression, the Bac-to-Bac expression system (Life Technologies, Inc.) was used according to the manufacturer’s specification. Recombinant protein was produced in High-five cells and purified on nickel-agarose. All recombinant proteins were analyzed by SDS-polyacrylamide gel electrophoresis and Coomasie Blue staining and quantified using known amounts of bovine serum albumin run on the gels.

\[^{35}S\text{ATP}\text{S} \text{and }[^{32}P]\text{ATP Binding Measurements and ATPase Assays}—\]Purified GST fusion proteins immobilized on glutathione beads were washed 3 times with buffer A (50 mM HEPES-NaOH, pH 7.4, 25 mM NaCl) containing 2 mM EGTA \( \pm 2.1 \text{ mM Ca}^{2+} \). Aliquots of the beads with a control of recombinant protein were used in 0.1-ml binding assays containing buffer A with 10 nM [\(^{35}\text{S}\text{ATP}\text{S}\) and 2 mM EGTA \( \pm 2.1 \text{ mM Ca}^{2+} \) and the indicated additions of nucleotides or Mg\textsuperscript{2+}. For the determination of the Ca\textsuperscript{2+} concentration dependence of [\(^{35}\text{S}\text{ATP}\text{S}\) binding, Ca\textsuperscript{2+}/EGTA buffers were used in buffer A with 10 nM [\(^{35}\text{S}\text{ATP}\text{S}\) and the concentration of free Ca\textsuperscript{2+} was calculated using the Chelator program (23). After a 1-h incubation at room temperature, beads were washed three times in the incubation buffer without [\(^{35}\text{S}\text{ATP}\text{S}\] but the radioactivity bound to the beads was determined. Binding measurements to full-length baculovirus synapsin I were performed similarly using synapsin I immobilized to nickel-agarose beads, with beads lacking synapsin I used as a control. Beads were washed with buffer A, and binding measurements were performed as described before for the GST fusion proteins except that all buffers lacked EGTA. [\(^{32}P\text{ATP}\) binding experiments were performed analogously. Binding data were analyzed using GraphPad PRIZM software by nonlinear regression assuming a single binding site. ATPase assays were performed with purified recombinant proteins as described (24).

**RESULTS**

**High Affinity ATP Binding by Synapsins I and II**—To study the ATP binding properties of synapsins, we expressed their C-domains as GST fusion proteins and used [\(^{35}\text{S}\text{ATP}\text{S}\] as a non-hydratable ATP ligand. We immobilized the GST fusion proteins on glutathione-agarose beads, incubated them with [\(^{35}\text{S}\text{ATP}\text{S}\] under a variety of conditions, and washed them with the respective incubation buffers. Bound ATP\textsuperscript{γS} was then measured by scintillation counting, and background binding was determined using GST alone or GST fusion protein of other synaptic proteins. With this method, we found that the C-domains from both synapsin I and synapsin II avidly and specifically bound ATP\textsuperscript{γS}, with background binding accounting for less than 0.1\% of the specific signal (Fig. 1).

Next we investigated the nucleotide specificity and effect of Mg\textsuperscript{2+} on ATP binding to synapsins. Mg\textsuperscript{2+} inhibited but did not abolish ATP\textsuperscript{γS} binding to both synapsin C-domains, similar to its effect on GTP binding to Rab proteins (Fig. 1) (25). Only ATP but not GTP competed for ATP\textsuperscript{γS} binding. To evaluate the relative ATP affinities of synapsins, we measured ATP displacement curves. We observed an almost identical EC\textsubscript{50} for ATP of approximately 0.1 \( \mu \text{M} \) for both synapsins, indicating that both proteins bind ATP with a similar high affinity (Fig. 2).

To test if full-length synapsin I also binds ATP\textsuperscript{γS}, we purified recombinant synapsin Ia produced in High-five cells as a fusion protein with a hexahistidine sequence. Binding experiments were performed with synapsin I immobilized on nickel-agarose beads as described for the GST fusion proteins above, except that EGTA could not be added to the incubations because it would have eluted the nickel from the column matrix. We observed robust ATP binding with synapsin I beads but not with control beads (Fig. 3A). The ATP affinity of full-length synapsin was similar to that of the C-domain (EC\textsubscript{50} \( \approx 0.12 \mu \text{M} \)). Much higher concentrations of ADP than ATP were required to displace ATP\textsuperscript{γS} (EC\textsubscript{50} \( \approx 6 \mu \text{M} \)), and GTP was again inactive (Fig. 3B). Together these studies demonstrate that the C-domains from both synapsins constitute ATP-binding modules with a high nucleotide specificity and with similar ATP affinity and that ATP binding is also a property of full-length synapsin.

Finally we investigated if the C-domains of synapsin I or II or full-length synapsin I exhibit ATPase activity. No ATP hydrolysis by synapsins was detected even at high protein concentrations under a variety of conditions, such as the presence of different lipids or various divalent cations (data not shown). Thus synapsins are not constitutively active ATPases. This result is not unexpected if synapsins are functionally similar to the enzymes that they resemble structurally (glutathione synthetase, d-alanine:d-alanine ligase, biotin carboxylase α-chain,
succinyl-CoA synthetase β-chain, and pyruvate, orthophosphate dikinase) (16). These enzymes transfer phosphates to a substrate during ATP hydrolysis and are unlikely to be active in the absence of substrate.

**Regulation of ATP Binding by Ca²⁺**—ATPγS binding to immobilized GST-C-domain fusion proteins from synapsin I (Fig. 2A) or synapsin II (Fig. 2B) was measured as described for Fig. 1 with or without Ca²⁺. Binding reactions were carried out in the presence of increasing concentrations of unlabeled ATP or GTP as shown. Data were fit to a single binding site, resulting in the half-maximal inhibition constants (EC₅₀) shown on the right. Note that GTP does not compete for binding and that ATP exhibits almost identical competition curves for both synapsins but only synapsin I requires Ca²⁺ for binding.

**FIG. 2.** ATP binding affinities of the C-domains from synapsin (Syn) I and II. [³⁵S]ATPγS binding to immobilized GST-C-domain fusion proteins from synapsin I (A) or synapsin II (B) was measured as described for Fig. 1 with or without Ca²⁺. Binding reactions were carried out in the presence of increasing concentrations of unlabeled ATP or GTP as shown. Data were fit to a single binding site, resulting in the half-maximal inhibition constants (EC₅₀) shown on the right. Note that GTP does not compete for binding and that ATP exhibits almost identical competition curves for both synapsins but only synapsin I requires Ca²⁺ for binding.

**FIG. 3.** ATPγS binding to full-length synapsin I. A, recombinant synapsin Iα was produced with a baculovirus expression system in High-five cells as a fusion protein with a carboxyl-terminal hexahistidine sequence. Nickel-agarose beads containing immobilized synapsin Iα (Synapsin Iα) or control protein from uninfected High-five cells (Control) were incubated with 10 nM [³⁵S]ATPγS in the presence of 2 mM Mg²⁺ and/or Ca²⁺ as indicated and washed, and bound ATPγS was measured. B, ATPγS binding reactions with synapsin Iα and control beads were carried out in the presence of increasing concentrations of unlabeled ATP, ADP, or GTP. Data were fit to a binding curve predicted for a single binding site; half-maximal inhibition concentrations are shown next to the curves.

What about the C-domain of synapsin II, which is homologous to the C-domain of synapsin I (78% sequence identity) and binds ATP with a similar affinity (Fig. 2)? Surprisingly, Ca²⁺ neither inhibited nor enhanced ATPγS binding to synapsin II (Figs. 1 and 2). To exclude the possibility that these results were artifacts caused by sequence variations, we confirmed them with several independent cDNA clones. Thus ATP binding to synapsins I and II is differentially regulated by Ca²⁺ despite their high degree of homology and similar ATP binding affinities.

**A Single Residue Controls Ca²⁺ Regulation of ATP Binding to Synapsins**—The result that the C-domains from synapsin I and II exhibit differential regulation of ATP binding by Ca²⁺ is surprising in view of their high degree of sequence identity. The crystal structure of synapsin I showed that ATP is bound by several residues that are conserved in the enzymes to which synapsin I is structurally homologous. These residues include Lys²²⁶, Lys²⁶⁹, and Gly²⁷⁶. Ca²⁺ is coordinated by the β- and γ-phosphates of ATP and by two glutamate residues (Glu³⁷³ and Glu³⁸⁶). These residues are evolutionarily conserved in
human, rat, mouse, and bovine synapsin I, and the binding properties of bovine and rat synapsin I are similar. Analysis of the sequence of synapsin II shows that all of the residues involved in ATP binding are also conserved in agreement with their similar ATP binding properties. Of the Ca$^{2+}$-coordinating residues, however, the residue corresponding to Glu$^{373}$ is a lysine (Lys$^{374}$) in synapsin II, whereas Glu$^{386}$ is also a glutamate in synapsin II. Similar to Glu$^{373}$ in synapsin I, Lys$^{374}$ is evolutionarily conserved in synapsin II. This suggests that synapsins were diversified in evolution in Ca$^{2+}$-regulated and Ca$^{2+}$-independent forms by a single point mutation similar to the diversification of synaptotagmins in Ca$^{2+}$-regulated and Ca$^{2+}$-independent forms (26).

To test this hypothesis, we analyzed the structural determinants of ATP binding to the C-domains of synapsin I by site-directed mutagenesis. We expressed mutant synapsin I C-domains with single amino acid substitutions and analyzed their ATP$^S$ binding properties. The different mutants did not exhibit increased instability, suggesting that there was no major impairment of the folding of the C-domain in the different mutants (data not shown).

First we studied a mutation in Lys$^{369}$ of synapsin I because the crystal structure indicated that this lysine should be essential for ATP binding. Substitution of Lys$^{369}$ for glutamine completely abolished ATP$^S$ binding as predicted (Fig. 5). This result confirms the crystallographic model of ATP binding and validates the specificity of the ATP$^S$ binding assays. As a positive control, we analyzed a substitution of Glu$^{166}$ to asparagine in synapsin II, which had no effect on binding. We then studied single amino acid substitutions in the presumptive Ca$^{2+}$ binding site of synapsin I. Exchange of Glu$^{373}$ in synapsin I for lysine had no effect on overall ATP binding but eliminated the requirement for Ca$^{2+}$ (Fig. 5). Thus the E373K substitution transformed the Ca$^{2+}$-dependent ATP binding activity of the C-domain of synapsin I into a Ca$^{2+}$-independent ATP binding activity. However, when Glu$^{373}$ was substituted for serine, ATP$^S$ binding was abolished, suggesting that a serine in this position in synapsin I is not compatible with ATP binding. These data demonstrate that substitutions of single amino acid residues either transform its Ca$^{2+}$ regulation of ATP binding or abolish ATP binding altogether.

DISCUSSION

Synapsins have been at the center of attention for many years because of their abundance, stoichiometric phosphorylation by multiple protein kinases, and strategic localization to synaptic vesicles. Few proteins are associated with so many detailed mechanistic models and postulated functions as synapsins. They have been implicated in neurogenesis, gene expression, axonal extension, synaptogenesis, and neurotransmitter release (for a review of these proposed extended functions, see Ref. 10). Analyses of knockout mice that lack synapsins confirmed an essential role in regulating synaptic vesicle exocytosis and suggested a primary, possibly exclusive, function for synapsins in the mature nerve terminal (13–15).

However, these and many of the other studies on synapsins, although technically elegant, failed to reveal the molecular nature of synapsin action because they suffered from limitations that made precise functional definitions difficult. These limitations include the use of synapsin fragments that contain parts of domains instead of complete domains, the high surface activity of synapsins (27), which may explain why synapsins bind with a high apparent affinity to so many different proteins (especially cytoskeletal elements (28)), and the use of complex biological systems in which direct and indirect effects are difficult to distinguish (13–15). We have now attempted to overcome these limitations by correlating structural and functional approaches. Our data show that synapsins are ATP-binding proteins that are differentially regulated by Ca$^{2+}$ and may serve as phosphotransfer enzymes.

The crystal structure of the C-domain from synapsin I demonstrated that it constitutes an autonomous, independently folding domain (16). The C-domain is a dimer in which the amino and carboxyl termini of each subunit are in close proximity. Comparison of the C-domain structure with data bases uncovered a high degree of structural similarity to a group of enzymes: glutathione synthetase, d-alanine:d-alanine ligase, ...
ATP Binding to Synapsins I and II

1429

biotin carboxylase α-chain, succinyl-CoA synthetase β-chain, and pyruvate,orthophosphatase dikinase. All of these enzymes bind ATP and transfer phosphate from bound ATP to a substrate (17, 18). The crystallographic data raise a number of questions. Do synapsins bind ATP? What are the relative ATP binding properties and affinities of different synapsins? Is ATP binding to synapsins regulated by Ca\(^{2+}\)? We have now addressed these issues to explore the functional implications of the structural observations. Our data showed that the C-domains of both synapsins bind ATP, but not GTP, with similar high affinity, thereby characterizing synapsins as ATP-utilizing proteins on the vesicle surface.

Although we were unable to measure ATPase activity in synapsins, the following data support the notion that ATP binding to synapsins is part of an enzyme reaction. 1) All five proteins to which the three-dimensional structure of C-domains is closely related are ATPases. 2) In glutathione synthase and d-alanine–d-alanine ligase, a flexible catalytic loop with a central arginine/lysine residue is essential for activity. The synapsin I C-domain has a flexible loop at the same position that is 100% conserved in synapsin II and also contains a central lysine residue. 3) Synapsins bind ADP with a much lower affinity than ATP, indicating that after hydrolysis, ADP would be exchanged for ATP. The fact that we could not detect ATPase activity in synapsins is not surprising because the enzymes to which synapsins are structurally homologous transfer the phosphate to the substrate and would not be expected to be active in the absence of substrate. Together our data suggested that synapsins are probably dimeric enzymes of unknown substrate specificity on the vesicle surface.

This description paints a unitary view of synapsins in agreement with their high degree of homology. Unexpectedly, however, we found that ATP binding was differentially regulated in the two synapsins despite their similar ATP affinity. Ca\(^{2+}\) was required for ATP binding to synapsin I but not synapsin II. The difference in regulation could be traced to a single amino acid difference between the two synapsins: Glu\(^{373}\) in synapsin I, which corresponds to Lys\(^{374}\) in synapsin II. Substitution of Glu\(^{373}\) to a lysine converted synapsin I into a Ca\(^{2+}\)-independent ATP-binding protein. This finding has functional and evolutionary implications. Evolutionarily, it implies that synapsins I and II co-evolved with the C-domain as the main functional domain and that at some time a divergence in regulation occurred that is based on the substitution of a single amino acid. A similar evolutionary switch between Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent isoforms in a protein family by a change in a single amino acid was recently observed in synaptotagmins (26). Functionally, this observation suggests that synapsin II may be constitutively active whereas synapsin I may only be activated upon increases in Ca\(^{2+}\). This hypothesis agrees well with the more severe phenotype of synapsin II knockouts than synapsin I knockouts, despite the greater abundance of synapsin I (14). A view of synapsins emerges from these studies in which the major central domain of synapsins, the C-domain, represents a differentially regulated ATP-binding domain that is flanked by amino- and carboxyl-terminal extensions and may serve as a phosphotransfer enzyme of unknown specificity.

Acknowledgments—We thank Drs. J. Deisenhofer and L. Esser for advice, Drs. X.-S. Xie and D. K. Stone for help with the ATPase assays, and Dr. K. Ichtenko for producing the synapsin baculovirus construct.

REFERENCES

1. Johnson, E. M., Ueda, T., Maeno, H., and Greengard, P. (1972) J. Biol. Chem. 247, 5650–5652
2. Huttner, W. B., Schiebler, W., Greengard, P., and DeCamilli, P. (1983) J. Cell Biol. 96, 1374–1388
3. Sudhof, T. C., Czernik, A. J., Kao, H., Takei, K., Johnston, P. A., Horichi, A., Wagner, M., Kanazir, S. D., Perin, M. S., DeCamilli, P., and Greengard, P. (1989) Science 245, 1474–1480
4. Sudhof, T. C. (1990) J. Biol. Chem. 265, 7849–7852
5. Klagges, B. R., Heinbeck, G., Godenschwege, T., Hofbauer, A., Pflugfelder, G. O., Reinfigerste, R., Reisch, D., Schaupp, M., Buchner, S., and Buchner, E. (1990) J. Neurosci. 16, 3154–3165
6. Ulrich, B., and Sudhof, T. C. (1995) Neurpharmacology 34, 1371–1377
7. Czernik, A. J., Pang, D. T., and Greengard, P. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 7518–7522
8. Matsubara, M., Kasubata, M., Ishiguro, K., Uchida, T., Tisani, K., and Taniguchi, H. (1996) J. Biol. Chem. 271, 21108–21113
9. Jovanovic, J. C., Benfenati, F., Siow, Y. L., Sihra, T. S., Sanghera, J. S., Pelech, S. L., Greengard, P., and Czernik, A. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3679–3683
10. Greengard, P., Valtorta, F., Czernik, A. J., and Benfenati, F. (1993) Science 259, 780–785
11. Sudhof, T. C. (1995) Nature 375, 645–653
12. Sudhof, T. C. (1996) Uchbara Memorial Foundation Symposium on Integrative and Molecular Approaches to Brain Function, pp. 3–11, Elsevier Science B. V., Amsterdam
13. Rosahl, T. W., Geppert, M., Spillane, D., Herz, J., Hammer, R. E., Malekna, R. C., and Sudhof, T. C. (1993) Cell 75, 661–670
14. Rosahl, T. W., Spillane, D., Missler, M., Herz, J., Selig, D. K., Wolff, J. R., Hammer, R. E., Malekna, R. C., and Sudhof, T. C. (1995) Nature 375, 488–493
15. Ryan, T. A., Li, L., Chin, L. S., Greengard, P., and Smith, S. J. (1996) J. Cell Biol. 134, 1219–1227
16. Esser, L., Wang, C.-R., Hosaka, M., Smagula, C. S., Sudhof, T. C., and Deisenhofer, J. (1998) EMBO J., in press
17. Matsuda, K., Mirzuguchi, K., Nishioka, T., Kato, H., Go, N., and Oda, J. (1996) Protein Eng. 9, 1083–1092
18. Arutyunin, P. J., Poirrette, A. R., Rice, D. W., and Willett, P. (1996) Nat. Struct. Biol. 3, 128–132
19. Guan, K., and Dixon, J. E. (1991) Anal. Biochem. 192, 262–267
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Davletov, B. A., and Sudhof, T. C. (1993) J. Biol. Chem. 268, 26386–26390
22. McMahon, H. T., Missler, M., Li, C., and Sudhof, T. C. (1995) Cell 83, 111–119
23. Schoenmakers, T. J. M., Visser, G. J., Flik, G., and Theuvenet, A. P. R. (1992) J. Cell Biol. 1374–1388
24. Stone, D. K., Xie, X.-S., and Racker, F. (1984) J. Biol. Chem. 259, 2701–2703
25. Shoja, I., Rikurhi, A., Kuroda, S., and Takai, Y. (1989) Biochem. Biophys. Res. Commun. 162, 273–281
26. von Poser, C., Ichtenko, K., Shao, X., Rizo, J., and Sudhof, T. C. (1993) J. Biol. Chem. 278, 14314–14319
27. Ho, M., Buhr, M., Czernik, A. J., Schiebler, W., Kezdy, F. J., Kaiser, E. T., and Greengard, P. (1991) J. Biol. Chem. 266, 5600–5607
28. Tang, J. X., and Janmey, P. A. (1996) J. Biol. Chem. 271, 8556–8563