Site-specific Phosphorylation of Synapsin I by Mitogen-activated Protein Kinase and Cdk5 and Its Effects on Physiological Functions*

(Received for publication, January 29, 1996, and in revised form, June 10, 1996)

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Site-specific modifications of synapsin I, a major phosphoprotein in synaptic terminals, were studied by mass spectrometry. In addition to a well known phosphorylation site by calmodulin-dependent protein kinase II (CaM kinase II), a hitherto unrecognized site (Ser553) was found phosphorylated in vivo. The phosphorylation site is immediately followed by a proline, suggesting that the protein is an in vivo substrate of so-called proline-directed protein kinase(s). To identify the kinase involved, three proline-directed protein kinases expressed highly in the brain, i.e. mitogen-activated protein (MAP) kinase, Cdk5-p23, and glycogen synthase kinase 3β, were tested for the in vitro phosphorylation of synapsin I. Only MAP kinase and Cdk5-p23 phosphorylated synapsin I stoichiometrically. The phosphorylation sites were determined to be Ser553 and Ser555 with Cdk5-p23, and Ser562, Ser567, and Ser569 with MAP kinase. Upon phosphorylation with MAP kinase, synapsin I showed reduced F-actin bundling activity, while no significant effect on the interaction was observed with the protein phosphorylated with Cdk5-p23. These results raise the possibility that the so-called proline-directed protein kinases together with CaM kinase II and CAMP-dependent protein kinase play an important role in the regulation of synapsin I function.

Synapsin I has been characterized as one of the major phosphoproteins in nerve terminals and is thought to be involved in the regulation of neurotransmitter release (for reviews see Refs. 1 and 2). Synapsin I cross-links synaptic vesicles and cytoskeleton, and the interactions of the protein with actin filaments and synaptic vesicles are regulated by phosphorylation by calmodulin-dependent protein kinase II (CaM kinase II). 1

1 This work was supported in part by Grants-in-Aid from the Fujita Health University, Grants-in-Aid for Scientific Research (C) (06680773), and Grants-in-Aid for Scientific Research on Priority Areas (06253218, 06276218, 07268221, and 07279242) from the Ministry of Education, Science and Culture, Japan. 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.

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1 The abbreviations used are: CaM kinase, calmodulin-dependent protein kinase; LC/MS, liquid chromatography/mass spectrometry; MARCKS, myristoylated alanine-rich protein kinase C substrate, a major in vivo protein kinase C substrate protein; MAP kinase, mitogen-activated protein kinase; Cdk5-p23, cyclin-dependent protein kinase and CAMP-dependent protein kinase (3–6). To understand the regulatory mechanisms of synapsin I function, it is necessary to know the posttranslational modifications of the protein in detail.

Recently we have applied electrospray mass spectrometry to studies on in vivo posttranslational modifications of various phosphoproteins. The high precision (within a few Da) and the high resolution (on the order of 10 Da) achieved by the method has made it possible to analyze protein phosphorylation and myristoylation of isolated proteins directly (7–9). Liquid chromatography/electrospray mass spectrometry (LC/MS), in which a capillary high performance liquid chromatography is connected online to an electrospray mass spectrometer, was found very useful in analyzing the in vivo posttranslational modifications including protein phosphorylation. Application of the methodology to brain-specific phosphoproteins revealed that prominent in vivo substrate proteins such as myristoylated alanine-rich protein kinase C substrate (MARCKS) or GAP-43 are phosphorylated by proline-directed protein kinases such as mitogen-activated protein (MAP) kinase and Cdk5 (10, 11). This was surprising, since these two proteins have been believed to be major and specific substrates of protein kinase C. This prompted us to reexamine in vivo phosphorylation sites of various major phosphoproteins systematically.

In the present study, the posttranslational modifications of synapsin I isolated from bovine brain were studied, and the LC/MS analysis revealed a novel phosphorylation site. To identify the protein kinase(s) involved in the phosphorylation at the novel site, we have examined in vitro phosphorylation of synapsin I by three proline-directed protein kinases expressed highly in the brain, namely MAP kinase, Cdk5-p23 (tau protein kinase II), and glycogen synthase kinase 3β (GSK 3β) (tau protein kinase I) (12–17). Effects of the phosphorylation on physiological functions of synapsin I were further assessed by examining the interaction of the protein with cytoskeletal proteins.

EXPERIMENTAL PROCEDURES

Materials—Synapsin I, purified from bovine brain using acid extraction or detergent extraction under nondenaturing conditions as described (18, 19), was stored in 25 mM Tris-HCl buffer (pH 8.0), containing 175 mM NaCl and 0.1 mM EGTA, at −80 °C. Both preparations gave essentially the same results in terms of in vitro phosphorylation. Actin prepared from acetonitrile powder of rabbit skeletal muscle as described (20) was further purified by gel filtration on a Superose 12 column (Pharmacia Biotech Inc.). Tubulin was prepared from bovine brain homogenates by three cycles of temperature-dependent assembly and disassembly in 0.1 M Mes-NaOH buffer (pH 6.8), containing 1 mM EGTA, 0.5 mM MgCl2, and 1 mM GTP, followed by phosphocellulose column chromatography (21). MAP kinase was purified from bovine brain 5-p23 complex; GSK 3β, glycogen synthase kinase 3β; PTH, phenylthiohydantoin; DTT-Ser, dithiothreitol adduct of dehydroalanine; Mes, 4-morpholinethanesulfonic acid.

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**RESULTS**

LC/MS Analysis of Synapsin I Protease Digests—Synapsin I isolated from bovine brain was digested with trypsin, and the resulting peptide mixture was subjected to LC/MS analysis as described under "Experimental Procedures." Peaks are numbered according to the elution order, and the peptides identified are shown in Table I.

| Peak | Peptide | Residues | Mass of MH* Da |
|------|---------|----------|----------------|
| 1    | T31     | 325-328  | 540.2          |
| 2    | T54(lb) | 663-670  | 769.3          |
| 24   | T24     | 270-279  | 1012.5         |
| 3    | T9      | 129-131  | 457.1          |
| 28   | T28     | 312-315  | 552.2          |
| 50   | T50     | 577-587  | 1053.4         |
| 48   | T48     | 557-565  | 925.4          |
| 5    | T30     | 319-324  | 722.3          |
| 5    | T49     | 566-576  | 1099.4         |
| 5    | T52 + P1| 603-612  | 1322.2         |
| 6    | T12     | 135-142  | 925.1          |
| 7    | T52     | 603-612  | 1042.4         |
| 5    | T51     | 588-602  | 1485.6         |
| 9    | T32     | 329-336  | 878.3          |
| 10   | T37     | 412-419  | 786.3          |
| 11   | T47 + P1| 532-556  | 2517.4         |
| 12   | T47     | 532-556  | 2437.9         |
| 14   | T47     | 532-556  | 2437.9         |
| 14   | T46     | 508-531  | 2466.0         |
| 14   | T44     | 447-507  | 3951.3         |
| 15   | T38     | 404-413  | 1154.7         |
| 17   | T53(la) | 613-663  | 5081.8         |
| 17   | T53(la) | 623-663  | 4016.5         |
| 18   | T53(lb) | 623-663  | 3902.8         |
| 19   | T27     | 300-311  | 1356.8         |
| 20   | T6      | 86-108   | 2052.5         |
| 21   | T23     | 257-269  | 1454.2         |
| 22   | T45     | 477-507  | 3226.7         |
| 22   | T53-54(lb)| 613-670 | 5718.5       |
| 24   | T16     | 177-186  | 1188.2         |
| 25   | T33     | 337-352  | 1725.5         |
| 26   | T54(la) | 664-689  | 2823.9         |
| 27   | T37     | 382-403  | 2435.5         |
| 28   | T21-22  | 238-256  | 2777.1         |
| 29   | T25-26  | 280-299  | 2227.8         |
| 30   | T5      | 664-689  | 2823.1         |
| 31   | T26     | 282-299  | 2001.0         |
| 32   | T22     | 259-269  | 2149.5         |
| 33   | T4      | 8-53     | 4706.2         |
| 34   | T13     | 143-169  | 2950.0         |

Assignment of synapsin I tryptic peptides

| Peak | Peptide | Residues | Mass of MH* Da |
|------|---------|----------|----------------|
| 1    | T31     | 325-328  | 540.2          |
| 2    | T54(lb) | 663-670  | 769.3          |
| 24   | T24     | 270-279  | 1012.5         |
| 3    | T9      | 129-131  | 457.1          |
| 28   | T28     | 312-315  | 552.2          |
| 50   | T50     | 577-587  | 1053.4         |
| 48   | T48     | 557-565  | 925.4          |
| 5    | T30     | 319-324  | 722.3          |
| 5    | T49     | 566-576  | 1099.4         |
| 5    | T52 + P1| 603-612  | 1322.2         |
| 6    | T12     | 135-142  | 925.1          |
| 7    | T52     | 603-612  | 1042.4         |
| 5    | T51     | 588-602  | 1485.6         |
| 9    | T32     | 329-336  | 878.3          |
| 10   | T37     | 412-419  | 786.3          |
| 11   | T47 + P1| 532-556  | 2517.4         |

**TABLE I**

| Peak | Peptide | Residues | Mass of MH* Da |
|------|---------|----------|----------------|
| 1    | T31     | 325-328  | 540.2          |
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| 5    | T51     | 588-602  | 1485.6         |
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| 10   | T37     | 412-419  | 786.3          |
| 11   | T47 + P1| 532-556  | 2517.4         |

**Peak**

**Peptide**

**Residues**

**Mass of MH* Da**

**Average mass. Other values are monoisotopic mass.**

**N-terminal acetylation is assumed.**

**Produced by partial deavage of Arg**

**Produced by pyroglutamte formation of N-terminal Gin.**

pared with the theoretical one calculated from the deduced amino acid sequence (24). Most of the peptides that account for more than 98% of the whole sequences of both synapsin Ia and Ib were easily identified solely from the mass as shown in Table I. One interesting point to note is that the N-terminal peptide is clearly N"-acetylated.

Two tryptic peptides were found partially phosphorylated as shown in Fig. 2. One peptide corresponds to T52 (from Gln<sup>525</sup> to Arg<sup>533</sup>) and the other corresponds to T47 (from Gln<sup>154</sup> to Arg<sup>162</sup>). The former contains one of the two phosphorylation sites by CaM kinase II, i.e. Ser<sup>529</sup>. Since the peptide contains only one phosphorylatable amino acid, we conclude that the Ser<sup>529</sup> is the major in vivo phosphorylation site. The latter peptide, T47, contains more than one possible phosphorylatable residue and was subjected to further analysis. A similar
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**Fig. 2.** Deconvoluted mass spectra of phosphopeptides. The electrospray mass spectra between peaks 5 and 7 shown in Fig. 1 were combined and deconvoluted to get a mass spectrum (a). Peptide T52 (1041.4 Da) was accompanied by a monophosphopeptide (1121.3 Da). A similar spectrum was obtained from spectra between peaks 11 and 12 shown in Fig. 1 (b). Peptide T47 (2436.8 Da) was observed with its corresponding phosphopeptide (2516.4 Da). About 50% of peptide T52 and about 20% of peptide T47 were in phosphorylated form.

**Fig. 3.** In vitro phosphorylation of synapsin I by proline-directed protein kinases. Synapsin I purified from bovine brain using detergent extraction (19) was incubated with MAP kinase (C), Cdk5-p23 (p), GSK3β (×), or a mixture of Cdk5-p23 and GSK3β (■) in the presence of [32P]ATP as described under "Experimental Procedures." The incorporation of the radioactivity was measured by counting gel slices.

### Table II

| Cycle | PTH-Ser | DTT-Ser | PTH-Ser/DTT-Ser |
|-------|---------|---------|-----------------|
| 2     | 4.6     | 17.2    | 0.27            |
| 20    | 0.6     | 2.9     | 0.22            |
| 22    | NDa     | 2.6     | 0.0             |

* ND: Not detected.

**Analysis of the Phosphorylation Site by Edman Degradation**—To determine the phosphorylation sites, the phosphopeptide T47 was isolated by reversed-phase column chromatography and subjected to Edman degradation. Since serine forms both phenylthiohydantoin-derivative of serine (PTH-Ser) and dithiothreitol adduct of dehydroalanine (DTT-Ser), phosphoserine can be detected unambiguously by comparing the yields of the two products (10, 11, 26). As shown in Table II, the first two of three serine residues in the peptide showed peaks of PTH-Ser and DTT-Ser. The ratios of the two peaks were 0.27 and 0.21, at the second cycle (Ser533) and at the 20th cycle (Ser551), respectively. These values correspond well to those observed with normal serine under the conditions employed. On the contrary, the yield of PTH-Ser peak at the 22nd cycle was under the detection limit, although that of DTT-Ser (2.6 pmol) was comparable with that observed at the 20th cycle (2.9 pmol). Since the phosphopeptide fraction subjected to the analysis contained only singly phosphorylated species as was confirmed by mass spectrometry, these results indicate that Ser553 is the sole phosphorylation site in the peptide. The phosphorylation site is immediately followed by a proline residue, suggesting that synapsin I is an in vivo substrate of so-called proline-directed protein kinase.

**In Vitro Phosphorylation of Synapsin I by Proline-Directed Protein Kinases**—To identify the protein kinase involved in the in vivo phosphorylation of synapsin I at the novel sites identified, three protein kinases with serine/threonine-proline specificity that are highly expressed in the brain were tested for their ability to phosphorylate purified synapsin I in vitro. As shown in Fig. 3, significant incorporation of radioactivity was observed with MAP kinase and Cdk5-p23. On the other hand, very little, if any, phosphorylation was observed with GSK3β. Since phosphorylation of tau protein by Cdk5-p23 is a prerequisite for the subsequent phosphorylation by GSK3β (22), a mixture of the two kinases was also tested. The extents of the phosphorylation did not differ appreciably between the sample incubated with Cdk5-p23 alone and that with the mixture. The final level of the phosphorylation reached around 3 mol/mol of synapsin I with MAP kinase, while that obtained with Cdk5-p23 was around 1 mol/mol.

Analysis of the Phosphorylation Site by LC/MS—To determine the in vitro phosphorylation sites, synapsin I phosphorylated by MAP kinase or by Cdk5-p23 was digested with trypsin, and the resulting peptide mixtures were directly analyzed by LC/MS as described above. Since most of the peptides have already been assigned, phosphorylated peptides were easily detected by the increase of their mass by 80 Da. Peptide T47 that contained the newly found in vivo phosphorylation site, Ser553, was almost stoichiometrically phosphorylated when MAP kinase was used (Fig. 4, a and b). Interestingly, only singly phosphorylated peptide with a mass of 2516 Da was observed, and no peak corresponding to doubly phosphorylated species was detected. Another peptide found phosphorylated was peptide T5 (from Ala54 to Lys85) (Fig. 4, c and d). In this case quantitative incorporation of two phosphoryl groups was observed. While the doubly phosphorylated species was the major product, very little, if any, singly phosphorylated or triply phosphorylated species were observed. The peak areas of the deconvoluted mass spectra correlate well with the amounts of the peptides under the conditions employed (10, 11). Peptide T4 (positions 8–53) near the N terminus was also found phosphorylated to varying degrees depending on the incubation conditions (less than 30% data not shown). Since no other peptides were found phosphorylated to a significant degree, we concluded that the MAP kinase phosphorylates synapsin I at three major sites and probably at one minor site. This corresponds well to the stoichiometry determined from the phosphorylation experiments using [32P]ATP as described above.

When similar experiments were conducted with Cdk5-p23, only one peptide, namely T47, was found phosphorylated to a significant extent (Fig. 5). Judging from the peak intensities of the deconvoluted mass spectra, about 80% of the peptide was found in the singly phosphorylated state. No peak corresponding to doubly phosphorylated species was detected. Peptides T5 and T4 and other peptides identified by LC/MS were not phosphorylated to a significant degree, suggesting that Cdk5-p23
The results obtained indicate that the one phosphoserine is distributed between the two positions. When we assume the PTH-Ser/DTT-Ser ratios of 0.03 and 0.20 for serine and phosphoserine, respectively, we can roughly estimate the contents of phosphoserine. At cycle 20, roughly 55% is phosphoserine, and at cycle 22 about 35% is phosphoserine. Since the sum (90%) corresponds fairly well to the theoretical value of 100%, such a calculation is feasible. When synapsin I purified by detergent extraction, which lacks the in vivo phosphorylated species, was used, about 50% of Ser$^{551}$ and 40% of Ser$^{553}$ were found phosphorylated. These results suggest that Cdk5-p23 phosphorylates synapsin I both at Ser$^{551}$ and Ser$^{553}$ and that the former is slightly favored over the latter. Furthermore, the phosphorylation at the two positions is mutually exclusive, since doubly phosphorylated species was not observed to significant extent as described above (Fig. 5). This was also the case with the MAP kinase-dependent phosphorylation. Although the synapsin I preparation used contained in vivo phosphorylated species, no doubly phosphorylated T47 peptide was observed with the MAP kinase phosphorylated synapsin I as shown in Fig. 4b. Thus, MAP kinase did not phosphorylate Ser$^{551}$ when Ser$^{553}$ had been already phosphorylated.

Effect of Phosphorylation of Synapsin I on its F-actin Bundling Activity—To understand physiological function of phosphorylation by MAP kinase or Cdk5-p23, we studied the effect of the phosphorylation on the F-actin bundling activity of synapsin I (23). The bundling of actin filaments was measured by light scattering, suggesting that the phosphorylation of synapsin I abolishes the F-actin bundling activity of the protein. These results were further confirmed by sedimentation experiments. Under the conditions employed, only large aggregates of bundled F-actin can be precipitated by low speed centrifugation, while individual actin filaments and synapsin I remain in the supernatant. Consistent with the light-scattering measurements, the addition of dephosphosynapsin I to F-actin led to sedimentation of bundled actin filaments, and most of the synapsin I

![Figure 4](image1.png)

**FIG. 4.** Deconvoluted mass spectra of phosphopeptides found in MAP kinase-phosphorylated synapsin I. Synapsin I phosphorylated by MAP kinase at 37°C for 5 h to get maximal phosphorylation was digested with trypsin and subjected to LC/MS analysis as described under “Experimental Procedures.” Peptide T47 (a) was almost quantitatively converted to corresponding monophosphopeptide (b). Peptide T5 (c) was converted to doubly phosphorylated species (d).

![Figure 5](image2.png)

**FIG. 5.** Deconvoluted mass spectra of phosphopeptide T47 found in Cdk5-p23-phosphorylated synapsin I. Synapsin I phosphorylated by Cdk5-p23 at 30°C for 5 h was digested with trypsin and subjected to LC/MS analysis. Only peptide T47 was significantly phosphorylated. About 80% of the peptide was found in singly phosphorylated form. Note that no doubly phosphorylated form was detected.

The results obtained indicate that the one phosphoserine is distributed between the two positions. When we assume the PTH-Ser/DTT-Ser ratios of 0.03 and 0.20 for serine and phosphoserine, respectively, we can roughly estimate the contents of phosphoserine. At cycle 20, roughly 55% is phosphoserine, and at cycle 22 about 35% is phosphoserine. Since the sum (90%) corresponds fairly well to the theoretical value of 100%, such a calculation is feasible. When synapsin I purified by detergent extraction, which lacks the in vivo phosphorylated species, was used, about 50% of Ser$^{551}$ and 40% of Ser$^{553}$ were found phosphorylated. These results suggest that Cdk5-p23 phosphorylates synapsin I both at Ser$^{551}$ and Ser$^{553}$ and that the former is slightly favored over the latter. Furthermore, the phosphorylation at the two positions is mutually exclusive, since doubly phosphorylated species was not observed to significant extent as described above (Fig. 5). This was also the case with the MAP kinase-dependent phosphorylation. Although the synapsin I preparation used contained in vivo phosphorylated species, no doubly phosphorylated T47 peptide was observed with the MAP kinase phosphorylated synapsin I as shown in Fig. 4b. Thus, MAP kinase did not phosphorylate Ser$^{551}$ when Ser$^{553}$ had been already phosphorylated.

**TABLE III**

| Cycle | PTH-Ser | DTT-Ser | PTH-Ser/DTT-Ser |
|-------|---------|---------|----------------|
| 2     | 2.6     | 13      | 0.20           |
| 3     | 2.5     | 13      | 0.19           |
| 9     | 0.24    | 9.9     | 0.024          |
| 14    | 0.17    | 7.4     | 0.023          |
| 17    | 0.87    | 3.6     | 0.24           |
| 18    | 0.87    | 4.0     | 0.22           |

**TABLE IV**

| Cycle | PTH-Ser | DTT-Ser | PTH-Ser/DTT-Ser |
|-------|---------|---------|----------------|
| MAP kinase | | | |
| 2     | 5.8     | 26.0    | 0.22           |
| 20    | 0.21    | 5.8     | 0.036          |
| 22    | 0.72    | 3.6     | 0.20           |
| Cdk5-p23 | | | |
| 2     | 3.1     | 17.0    | 0.18           |
| 20    | 0.45    | 4.0     | 0.11           |
| 22    | 0.51    | 3.6     | 0.14           |

A similar analysis of the phosphopeptide T47 obtained from synapsin I phosphorylated with Cdk5-p23 gave more complex results (Table IV). The ratio of the yields of PTH-Ser and those of DTT-Ser showed intermediate values between 0.2 (serine) and 0.02 (phosphoserine) both at cycle 20 (Ser$^{551}$) and cycle 22 (Ser$^{553}$). Since the isolated phosphopeptide was confirmed by mass spectrometry to contain only singly phosphorylated species free from nonphosphorylated and doubly phosphorylated species (data not shown), the total content of phosphoserine in the peptide should be exactly one mol/mol of peptide.
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**DISCUSSION**

Synapsin I, one of the prominent endogenous phosphoproteins in the nerve terminals, has been characterized as a substrate protein of various protein kinases such as CaM kinases I and II and cAMP-dependent protein kinase (2). Physiological functions of synapsin I, i.e. the cross-linking between synaptic vesicles and cytoskeletons seems to be regulated by phosphorylation by these kinases (1, 2). The detailed analysis on the in vivo phosphorylation site described in the present study, however, revealed a novel phosphorylation site. The phosphorylated serine is immediately followed by a proline, suggesting that synapsin I is an in vivo substrate of so-called proline-directed protein kinase. We have previously shown that the two prominent in vivo substrate proteins of protein kinase C, MARCKS and GAP-43, are also phosphorylated by these kinases in vivo (10, 11). These results suggest that the physiological functions of various proteins are regulated by multiple protein kinases in a very complex manner and that cross-talks between various signaling pathways occur not only upstream of the pathways but also at the substrate protein level.

Of the three proline-directed protein kinases tested, only MAP kinase and Cdk5-p23 phosphorylated synapsin I in vitro, and GSK3β did not phosphorylate the protein to a significant extent. The phosphorylation by the two kinases was site-specific; only one of two serine residues (Ser551 and Ser553) was phosphorylated by Cdk5-p23, while three serine residues were phosphorylated by MAP kinase. Since bovine synapsin I contains 11 Ser (Thr)-Pro motifs, there should be structural determinants other than the adjacent proline in the substrate recognition by the kinases. As for MAP kinase, Ser551 is within a well-known recognition sequence of the kinase, Pro-Xaa-Ser, where Xaa is usually a small neutral amino acid (27, 28). Since the other two sites have a proline either at −3-position or at −1-position, the presence of a proline preceding the phosphorylation site serine/threonine may be important for the recognition. It should be noted that peptide T4 from Leu to Arg, which was phosphorylated to some extent, contains a single Ser-Pro motif (Ser46), which is preceded by a proline. On the other hand, only one phosphopeptide was observed with synapsin I phosphorylated by Cdk5-p23. This kinase seems to phosphorylate both Ser551 and Ser553, but the phosphorylation at these two sites is mutually exclusive, suggesting that the incorporation of negative charges in the neighborhood changes the substrate specificity. Ser551 is preferentially phosphorylated, although the difference may not be significant. The recognition sequence of the kinase has yet to be defined, but an arginine at +3-position may be an important determinant.

Only one kinase, so-called proline-directed protein kinase has been so far reported to phosphorylate synapsin I in these regions (30). The kinase has later been identified as Cdc2-cyclin A complex (31). Since Cdc2 kinase is not expressed in the brain to a significant extent, this phosphorylation reaction lacks physiological relevance. However, it is of interest to note that the kinase also phosphorylates Ser551 preferentially (30). Whether the kinase phosphorylates Ser553 is not clear, because of the limitation of the technique used in determining the phosphorylation site. The radiosequencing employed in the study suffers from a massive carryover, which obscures the determination of successive phosphorylation sites. In any case, it is interesting that the three proline-directed protein kinases so far tested phosphorylate Ser551 exclusively or preferentially. Only Cdk5-p23 phosphorylates Ser553, but the kinase phosphorylates Ser551 as well, although only the former was found phosphorylated in vivo as has been shown in the present study. One possibility is that a protein kinase or kinases other than the
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Phosphorylation sites of synapsin I. The in vivo phosphorylation sites found in the present study and the in vitro phosphorylation sites by various protein kinases are summarized. Of the two successive Ser-Pro sequences at the P4 site (1), only the second one (Ser553) was found phosphorylated in vivo. MAP kinase and Cdk2-cyclin A phosphorylate the first serine (Ser551), while Cdk5-p23 phosphorylates both residues.

ones tested are responsible for the phosphorylation of Ser553. The other explanation is that Ser553 is preferentially dephosphorylated by protein phosphatase(s). Since the present study on the in vivo phosphorylation state of synapsin I represents only a “snapshot” of the total brain, similar studies conducted with cells and tissues under various physiological stimulations may give an answer to the question. The in vivo and in vitro phosphorylation sites so far identified are summarized in Fig. 8.

Phosphorylation of synapsin I at Ser568 and at Ser605 by CaM kinase II, Cdk5-p23, and cAMP-dependent protein kinase. The phosphorylation by Cdk5-p23 or that by Cdc2-cyclin A does not cause any detectable mobility shift (30) suggests that the protein kinase involved in nerve growth factor-dependent phosphorylation of synapsin I in PC12 is MAP kinase. In conclusion, proline-directed protein kinases that include MAP kinase and by cAMP-dependent protein kinase. The phosphorylation of synapsin I at Ser568 and at Ser605 by CaM kinase and by cAMP-dependent protein kinase. The phosphorylation has previously been attributed to that by Cdk2-cyclin A (1, 30), but the present study demonstrated that the mobility shift is induced only by MAP kinase-dependent phosphorylation but not by Cdk5-p23-dependent phosphorylation. The fact that phosphorylation by Cdk5-p23 or that by Cdk2-cyclin A does not cause any detectable mobility shift (30) suggests that the protein kinase involved in nerve growth factor-dependent phosphorylation of synapsin I in PC12 is MAP kinase. In conclusion, proline-directed protein kinases that include MAP kinase, Cdk5-p23, and (probably) other unknown protein kinases seem to play important roles in the physiological regulation of cytoskeletal components during neurotransmitter release, and there seem to be complex interactions between various protein kinases not only upstream of the signal transduction pathways but also at the substrate protein level (10, 11).

Acknowledgments—We are grateful to Masami Suzuki for excellent technical assistance.

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J. Biol. Chem. 1996, 271:21108-21113.
doi: 10.1074/jbc.271.35.21108

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