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Synapsin I phosphorylated by Ca^{2+}, calmodulin-dependent protein kinase II is able to self-degrade

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Phosphorylation of the neurospecific protein synapsin I (SI) by various cellular protein kinases was studied. The analysis of functional properties of phosphosynapsins showed that in the case of PK II-mediated modification of the protein, it becomes capable of self-degradation. The latter process was found to be specific and did not appear to be characteristic of the phosphoforms emerging after the protein modification by PK C or PK A. A possible involvement of the process in the regulation of neurotransmitter secretion is discussed.

Synapsin I; Calmodulin-dependent protein kinase II; Phosphorylation; Self-degradation

1. INTRODUCTION

Synapsin I, a neurospecific protein described for the first time by Greengard [1], is localized in synaptic terminals of nerve cells. It was found that SI is associated with the cytoplasmic surface of synaptic vesicles and, in all probability, acts as an anchor for vesicles, because it has potency of binding to cytoskeletal proteins as well [2]. The protein can be phosphorylated by various cellular protein kinases both in vivo and in vitro [3,4]. Phosphorylation of SI is considered as one of the mechanisms of the regulation of neurotransmitter secretion and synaptic vesicle functions.

The main goal of this study was to investigate possible changes in the structure and function of an SI molecule following its modification by various protein kinases.

2. MATERIALS AND METHODS

The following reagents were used (γ-^{32})ATP (1000 Ci/mmol) from Amersham; Tris, ATP, EGTA, $CaCl_2$ from Serva, and phosphatidylserine from Sigma.

PK C was isolated as described in [5]. The isolation procedure of human brain calmodulin was as in [6] with modifications. Human brain SI was obtained according to [7]. PK II was isolated from rat brain following the technique given in [8]. The peptide mapping procedure was the same as in [4]. Limited proteolysis of proteins in a gel was performed by the modified method of Cleveland [9]. SI was phosphorylated by PK C according to [10], except that SI was used instead of histone HI, by PK A and PK II as in [3] with slight modifications. Protein electrophoresis was run under denaturing conditions on 20×15×0.75 cm plates by the method of Laemmli [11].

The catalytic subunit of pig brain cAMP-dependent protein kinase was kindly provided by M.V. Nesterova of the Research Center of Molecular Diagnostics, USSR Ministry of Health.

Abbreviations: SI, synapsin I; PK C, protein kinase C; PK A, cAMP-dependent protein kinase; PK II, calmodulin-dependent protein kinase.

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3. RESULTS AND DISCUSSION

In our experiments both homogeneous and partially purified human brain SI was used. Homogeneous SI is represented in the electrophoregram as a double band corresponding to polypeptides of 86 kDa (synapsin Ia) and 80 kDa (synapsin Ib) [7].

In our previous studies we demonstrated that human brain SI can be phosphorylated by PK A and PK II and also by PK C isolated from the same source [10]. It was found that these 3 kinases phosphorylate SI at different sites (data not shown).

Our investigation of SI phosphorylation by the above protein kinases using the method of limited proteolysis of proteins according to Cleveland, revealed that PK II-dependent SI phosphorylation leads to degradation of the SI molecule.

SI phosphorylation by the kinases was carried out in the presence of (γ-^{32}P)ATP and corresponding activators for 15 min at 37°C. The samples were fractionated by polyacrylamide gel electrophoresis using the system of Laemmli [11]. The gel slab containing SIa and SIb were cut out, incubated in the equilibration buffer and placed into wells of the second gel with extended stacking gel (5 cm). The electrophoresis conditions maintained allowed proteins to be kept in the concentrating gel for about 1.5 to 2 h, whereupon they were separated under standard conditions in the PAAG gradient. In modification of the procedure proposed by Cleveland,

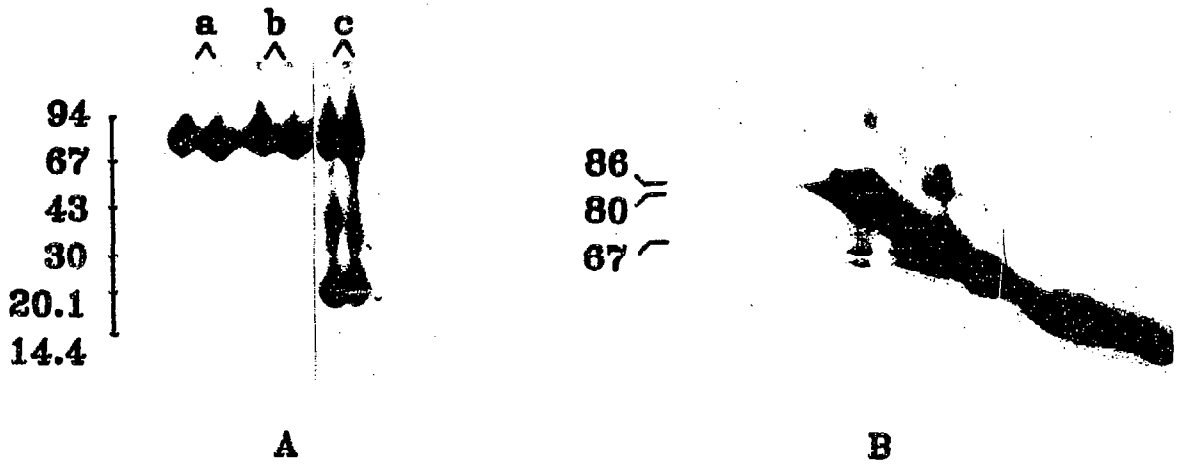

Fig. 1. (A) Degradation of various phosphoforms of SI phosphorylated by: (a) PK C; (b) PK A; and (c) PK II. (B) Degradation of the partially purified preparation of SI phosphorylated by PK II. (see details in section 3).

we introduced no proteases into our samples. Fig. 1A,c demonstrates that the SI preparation phosphorylated by PK II produces SI fragments of about 35 kDa and 18 kDa. No degradation under similar experimental conditions was detected in the case of nonphosphorylated SI or SI phosphorylated by PK A and PK C (Fig. 1A,a,b); SI did not degrade in the presence of PK II but in the absence of ATP (data not shown). Hence self-degradation of phospho-SI appears to be a specific Ca^{2+}, calmodulin-dependent process.

To confirm the above mentioned results, we repeated the experiment in a slightly modified form. Using a partially purified SI preparation, we cut out the whole track of gel after running the first electrophoresis, then treated it as described above and placed it into the second gel with an extended stacking gel. The autoradiogram of this gel revealed degradation of proteins of 86–80 kDa and 67 kDa (Fig. 1B), the latter of which being an SI fragment as evidenced by immunoblotting (data not shown).

Our assumption that the observed effect is not an artefact and has no relation to the source of SI (human brain) is well supported by the findings reported by Fukunaga et al. [12]. In the experiments on rat brain SI phosphorylated by purified PK II, the authors revealed an enhancement of phosphorylation of proteins in the region of 44–64 kDa and suggested that these proteins may be products of proteolytic degradation of SI, as they were always observed when SI was phosphorylated by purified PK II. Degradation of SI in the nerve terminal was reported by Bahler et al. [13].

In as much as the observed effect was found to be specific merely for SI phosphorylated by PK II, one can suppose a certain physiological significance for this phenomenon. Our findings allow us to propose the following hypothetical scheme of neurotransmitter secretion.

The depolarization-induced increase in the concentration of calcium in a synaptic terminal activates protein kinase C which phosphorylates B50 protein whose dephosphoform is capable of binding to calmodulin [14]. After B50 phosphorylation, the released calmodulin activates PK II which, in turn, phosphorylates SI. SI phosphorylated by PK II undergoes proteolytic degradation, which results in the release of the synaptic vesicle from the cytoskeleton and subsequent secretion of neurotransmitters.

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