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Polyphosphoinositides as activators of PKC-dependent synapsin I phosphorylation

S.E. Severin and E.L. Moskvitina

Research Center of Molecular Diagnostics, USSR Ministry of Health, Moscow 113149, USSR and *All Union Endocrinology Research Center of AMN USSR, Moscow 117036, USSR*

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The effect of PIP_2 and diacylglycerol (products of polyphosphoinositide turnover) on the activation level of phosphorylation of the human brain neurospecific protein SI by PKC from the same source was studied. The apparent activation constant of the phosphorylation process was shown to decrease in the presence of PIP_2 from 1.1 μg/ml to 0.2 μg/ml for PI and from 0.8 μg/ml to 0.6 μg/ml for PS; the value of 0.4 μg/ml in the latter case was detected merely after the addition of DOG into the reaction mixture. Polyphosphoinositides are suggested to play a role in activating PKC-mediated phosphorylation of SI in nerve terminals.

Synapsin I; Protein kinase C; Phosphorylation; Polyphosphoinositide; Diacylglycerol; Activation

1. INTRODUCTION

In our previous studies [1,2], the neurospecific protein SI that acts either as a peculiar anchor of synaptic vesicles or as a means for their transportation in nerve terminals [3,4] was shown to be effectively and specifically phosphorylated not only by cAMP-dependent protein kinase and Ca^{2+}, calmodulin-dependent protein kinase II, which has been reported by other authors as well [5], but also by Ca^{2+}, phospholipid-dependent protein kinase (protein kinase C). The fact of PKC-dependent SI phosphorylation was earlier noted by Greengard et al. in some reviews [6], but the process was denied any physiological significance, unlike PK II-mediated phosphorylation which is believed to lead to the secretion of neurotransmitter [7].

We used homogeneous human brain SI for detailed kinetic studies of the protein phosphorylation by PKC as well as for establishing the relationship between this process and PK II-dependent SI phosphorylation. The maximal rates of the two above processes are virtually the same, and the K_m value with respect to SI phosphorylated by PKC amounts to 0.25 μM (80 kDa per M_r of SI), which is comparable and even lower than K_m for other protein kinases phosphorylating this neurospecific protein [8,9]. These results allow a suggestion that SI is a physiological substrate for PKC as well.

Correspondence address: S.E. Severin, Research Center of Molecular Diagnostics, USSR Ministry of Health, Moscow 113149, USSR

Abbreviations: SI, synapsin I; PKC, protein kinase C; PIP_2, phosphatidylinositol-4,5-bisphosphate; PS, phosphatidylserine; PI, phosphatidylinositol; DOG, dioleoylglycerol

We have also managed to demonstrate that the process of calmodulin and phospholipid-mediated SI phosphorylation are interrelated, i.e. PKC activators (fatty acids and acidic phospholipids) inhibit PK II-modulated phosphorylation, whereas calmodulin, a PK II stimulator, suppresses PKC-dependent SI phosphorylation [2].

The capacity of PKC for activating neurosecretion processes has been described by a number of authors [10–12]. The reported findings together with our data evidence possible occurrence of PKC-dependent SI phosphorylation in vivo, though the actual significance of the process remains yet unclear. In this connection we thought it of interest to undertake a detailed study of regulatory mechanisms of this phosphorylation.

2. MATERIALS AND METHODS

Purification procedure of human brain SI was that proposed by Ueda and Greengard [13] with some modifications detailed in [14].

PKC was isolated from human brain by the method similar to the previously described one [15].

Bovine brain PIP_2 was obtained as described elsewhere [16].

Phosphorylation of SI by PKC was carried out as described previously [15], except that SI was used as the substrate instead of histone H1.

Protein concentration was determined according to Bradford [17].

Reagents used in the study were [γ-^{32}P]ATP (1000 Ci/mmol) from Amersham; Tris, ATP, PS, PI and DOG, from Sigma; EGTA, $CaCl_2$ and other salts from Serva.

3. RESULTS AND DISCUSSION

Earlier, in our investigations of PKC-mediated histone H1 phosphorylation [15] we carefully analysed the kinetics of rat brain PKC activation by acidic

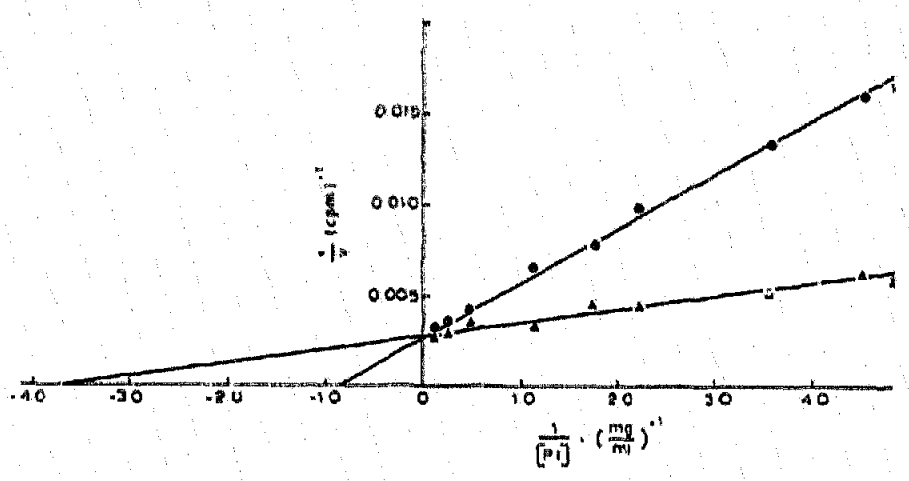

Fig. 1.

phospholipids. A highly effective stimulating action of PI, comparable to that exhibited by the recognized activator PS was detected. Some arguments were produced against the originally supposed decisive role of PS alone in the PKC activation in vivo. Any facts in support of possible involvement of polyphosphoinositides as physiological modulators in this phosphotransferase system are extremely important for better, more comprehensive understanding of the extracellular signal transduction, including the lipid metabolism system as an integral part of this process [18].

Analysis of Ca^{2+}, phospholipid-dependent phosphorylation of the neurospecific protein synapsin I from this point of view appears of a particular interest, because it could provide new experimental data relating to the neurotransmission pathways, provided that the process in question is assumed to be one of the ways of the neurosecretion regulation.

Since acidic phospholipids proved to be effective activators of PKC-mediated SI phosphorylation [2], we studied the effect produced by the products of the phosphoinositide turnover phosphatidylinositol-4,5-bisphosphate and diacylglycerol on the activation level of this process by PS and PI. It should be noted that the actual ratio of polyphosphoinositides and diacylglycerol in the membranes of nerve cells was taken into account [19].

As can be seen in Fig. 1, the addition into the incubation medium of PIP_2 (at a concentration of 0.5 μg/ml) was followed by a 5-fold decrease in the K'_a value for PI: viz. from 1.1 to 0.2 μg/ml, the analogous experiments for DOG show K'_a about 0.5 μg/ml (Table I). It is noteworthy that in case of PKC-mediated phosphorylation of histone H1, similar experimental conditions did not affect so essentially the affinity of the phospholipid for the enzyme (data not shown) as was the case with SI.

PIP_2 is known to be capable of stimulating under certain conditions histone H1 phosphorylation [20]. Besides that, selectivity of phospholipids in PKC ac-

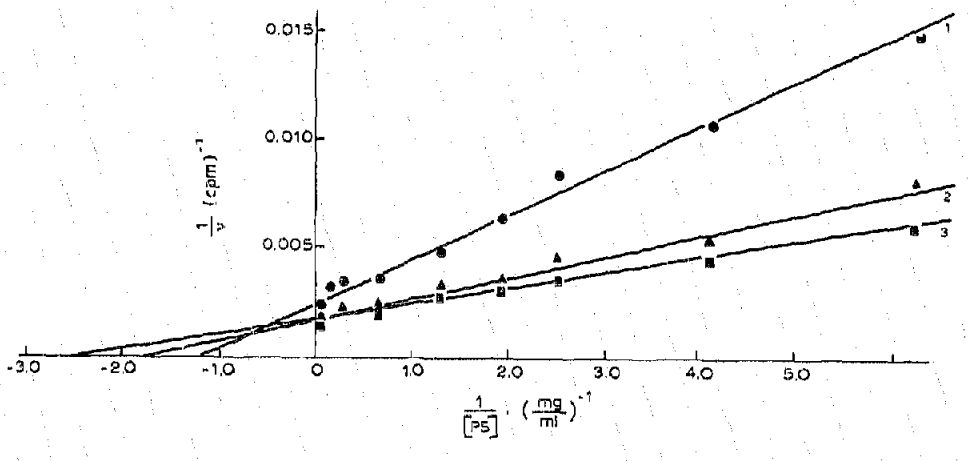

Fig. 2.

tivation depends to a great extent on the character of the substrate-phospholipid interaction [21]. Apparently, in our case, too, the introduction into the lipid bilayer of an additional negative charge appears more preferable for this substrate and thus provides another evidence in favor of physiological significance of Ca^{2+}, phospholipid-dependent SI phosphorylation.

A similar comparison was carried out for a conventional PKC activator PS, in which case the K_a' value of PKC-mediated SI phosphorylation of 0.8 μg/ml (close to that for PI) remained almost the same (0.6 μg/ml) in the presence of PIP_2 and decreased 2-fold (0.4 μg/ml) after the addition of DOG, a recognized physiological activator of PKC (Fig. 2). So the affinity of the lipid for the enzyme in the latter case was even lower than that for the PI + PIP_2 mixture (K_a' = 0.2 μg/ml) (Table I).

It is a well-known fact that PKC activation by membrane lipids is preceded by elevation of intracellular calcium and the following enzyme translocation to the membrane [22].

Since the PS content in the membranes of nerve cells is 2–3 times higher compared to the PI concentration [19] and the affinity of PKC for PI in the presence of PIP_2 is greater than for PS in the presence of both PIP_2 and DOG, it is reasonable to suggest that PIP_2, like diacylglycerol, can be a physiological activator of PKC-dependent SI phosphorylation in nerve cells. However, while DAG modulates PKC activity in case of receptor-dependent degradation of phosphoinositides (particularly PIP_2, as this leads to an increased concentration of intracellular Ca^{2+}), PIP_2 will be potent of activating PKC in the absence of a hormonal signal.

One should mind though that in nerve terminals depolarization and subsequent elevation of intracellular calcium will be followed by PKC translocation both to the cytoplasmic membrane and the membranes of intracellular organelles, in particular those of synaptic vesicles. In the meantime, no data are available yet in literature concerning the presence in the synaptic vesicle membranes of the enzyme complex: receptor/G-protein/phospholipase C, and consequently, the entire system of intracellular realization of an external signal, including products of the receptor-dependent degradation of PI (DOG). So, the primary role in activating PKC-mediated SI phosphorylation belongs in this case in all likelihood to polyphosphoinositides, rather than diacylglycerols. The advanced supposition correlates well with the kinetic characteristics of the process under consideration that are summarized in Table I.

Table I

Activation of PKC-dependent SI phosphorylation by acidic phospholipids

Activation constant (μg/ml)	Lipid	Lipid + PIP_2	Lipid + DOG
K_a' (PI)	1.1 ± 0.1	0.20 ± 0.03	0.50 ± 0.05
K_a' (PS)	0.8 ± 0.1	0.60 ± 0.08	0.40 ± 0.05

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