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Minireview

Protein kinase C in transmembrane signalling

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Protein kinase C functions as the transducer of a second messenger, diacylglycerol, and is the major receptor for tumour-promoting phorbol esters. The enzyme is a family of proteins with closely but distinct structures and individual enzymological properties. Members of the family are differently distributed in particular cell types and limited intracellular locations from lower organisms to mammalian tissues. The enzyme appears to interact with many signalling pathways, and display functions in the processing and modulation of cellular responses to external stimuli. Presumably, each member of the family plays discrete roles in the control of a variety of membrane functions and activation of gene transcription.

Signal transduction; Protein kinase C, molecular heterogeneity; Protein kinase C, signal routes for activation; Protein kinase C down regulation; Protein kinase C, physiological roles

1. INTRODUCTION

Protein kinase C (PKC), a multifunctional serine/threonine protein kinase, plays crucial roles in signal transduction [1]. The enzyme requires Ca^{2+} and phospholipid, particularly phosphatidylserine for its activation. Under physiological conditions diacylglycerol greatly increases the affinity of PKC for Ca^{2+} and phospholipid, thereby activating the enzyme. Although the receptor-mediated hydrolysis of inositol phospholipids was once thought to be the sole mechanism leading to the activation of PKC, recent studies suggest the existence of several other routes to provide the diacylglycerol that is needed for enzyme activation. In addition, it is now clear that there are multiple subspecies of PKC with closely related structures but subtly different properties and perhaps with different physiological functions. While the extent of the diversity of the PKC family is not fully clarified at present, some aspects of current studies and the prospective of this enzyme family will be briefly discussed.

2. MOLECULAR HETEROGENEITY AND STRUCTURES

Molecular cloning and biochemical analysis have revealed the enzyme to exist as a family of multiple subspecies having closely related structures (see [2] for a review). Initially, four cDNA clones, α, βI, βII and γ were isolated [3–8]. The enzymes with βI- and βII-sequence are derived from a single RNA transcript by alternative splicing [8]. Each of the four PKC subspecies consists of a single polypeptide with four conserved (C1–C4) and five variable (V1–V5) regions. The amino-terminal half, containing region C1 and C2, is the regulatory domain that interacts with Ca^{2+}, phospholipid and diacylglycerol or phorbol ester. The region C1 contains a tandem repeat of a cysteine-rich zinc-finger-like sequence. This sequence seems to be essential for the binding of phorbol ester, and possibly by analogy diacylglycerol, implying its involvement in the membrane-PKC interaction [9]. The region C2 may be necessary for the Ca^{2+} sensitivity of the enzyme [10]. The carboxyl-terminal half, containing region C3 and C4, is the protein kinase domain that shows sequence homology with many other protein kinases. The region C3 involves the catalytic site of the enzyme.

Another group of cDNA clones, encoding at least three further subspecies that have the δ-, ϵ- and ζ-sequence, have also been isolated from the rat brain library [11–13]. The members of this group lack region C2 of the regulatory domain. The translational products of δ-, ϵ- and ζ-cDNA expressed in COS cells do not require Ca^{2+} for their enzymatic activity [12–14]. The molecular mass of ζ-subspecies is smaller (64 kDa) than that of the others (approximately 80 kDa), since this subspecies contains only one zinc-finger-like sequence in region C1.

Although the biochemical mechanism of PKC activation has not yet been fully clarified, the activity of this enzyme appears to be normally covered by interaction

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between the catalytic and regulatory domains. It has been proposed that a small part of the amino-terminal region of the regulatory domain encodes a unique sequence, termed 'pseudosubstrate' [15]. This sequence has a strong affinity to the active site of the enzyme. A synthetic peptide containing the pseudosubstrate sequence has been shown to be a potent inhibitor of PKC, while this peptide becomes an excellent substrate for the enzyme when the alanine in this sequence is replaced by serine. The proteins which are capable of serving as substrates for PKC normally contain basic amino acids such as arginine or lysine at the carboxyl-terminal side adjacent to the seryl or threonyl residue to be phosphorylated [16–19]. The antibody directed against this pseudosubstrate peptide has been shown to activate PKC in the absence of Ca^{2+}, phospholipid and diacylglycerol [20].

3. DIFFERENTIAL DISTRIBUTION AND PROPERTIES

The diversity of the sequence in the variable regions allows separation of PKC into several subfractions by chromatography on a hydroxyapatite column [21–23]. Three subfractions, type I, II and III, have been obtained which correspond to γ-, β (βI + βII)- and α-subspecies, respectively [22,24]. With the aid of biochemical, immunohistochemical and in situ hybridization procedures the relative activity and individual pattern of expression of multiple PKC subspecies in several tissues and cell types has been examined extensively (see [2] for a review).

PKC with the γ-sequence (type I) is expressed exclusively in the central nervous system, and the highest enzyme activity has been found in the hippocampus, cerebral cortex, amygdaloid complex, cerebellar cortex and spinal cord [25,26]. This PKC subspecies is associated with most of the membranous structure, particularly in the hippocampal pyramidal cell and Purkinje cell. The enzyme is poorly expressed in the nerve endings which terminate on these cells, implying that this PKC subspecies plays roles in the post-synaptic processes in these cell types.

Perhaps the most striking feature of the γ-subspecies is that this enzyme can be activated in vitro by micromolar concentrations of free arachidonic acid and some of its metabolites such as lipoxin A (see [2] for a review). This activation does not require Ca^{2+} and phospholipid. The second messenger role of arachidonic acid and its metabolites, however, has not yet been established.

PKCs with the βI- and βII-sequences (type II) are present in variable ratios in many tissues and cell types including the brain. Normally, the amount of the βII-subspecies far exceeds that of the βI-subspecies. These two subspecies differ from each other only in their carboxyl-terminal end-region corresponding to V5. In certain tissues such as cerebellar cortex, a distinct cellular expression of these two enzymes is apparent [2]. In neuronal cells, the βI-subspecies is sometimes associated with plasma membranes, whereas the βII-subspecies is often localized in the Golgi complex (see [2,27] for reviews). PKC with the β-sequence has been shown to exhibit a significant activity in the absence of added Ca^{2+}, although the Ca^{2+} dependence depends largely on the phosphate acceptor protein employed [28].

PKC with the α-sequence (type III) is widely distributed in many tissues and cell types. It is the most sensitive to diacylglycerol for activation. Some cell types such as NIH 3T3 fibroblasts [29] and COS cells [8] express only the α-subspecies, but most cells so far examined including T-lymphocytes [30–32] and HL-60 cells [33] co-express several PKC subspecies in different ratios. Several PKC subspecies, by light-microscopy resolution, apparently show distinct intracellular locations, depending upon the state of differentiation or proliferation.

At present, the distribution and biochemical properties of the enzymes encoded by the δ-, ϵ- and ζ-sequence have not been determined. These subspecies have been expressed in COS cells, but their correspondence to subfractions obtained by chromatography from tissues has not been identified. Histone is usually a poor substrate for this subgroup of enzymes.

In addition to the PKC family described above, several structurally undefined subspecies have been isolated from various tissues such as adrenal cortex [34], platelets [35] and HL-60 cells [33], which respond to Ca^{2+}, phospholipid and diacylglycerol in different ways. A fraction of enzyme, which is phospholipid-dependent but is inhibited by Ca^{2+}, has been obtained from rat brain tissues [36]. Several PKC subspecies so far isolated from many tissues and cell types exhibit subtly different kinetic properties and substrate specificities [28,34,37]. In short, mammalian tissues and organs appear to contain a variety of PKC subspecies, but the extent of this diversity is not fully clarified at present.

The enzyme has been found also in lower organisms including *Drosophila* [38,39], *Xenopus laevis* [40], sea urchin egg [41], *Dictyostelium discoideum* [42,43] and *Saccharomyces cerevisiae* [44] as determined by molecular cloning analysis. It is worth noting that the recently isolated yeast enzyme shows a substrate specificity entirely different from that of mammalian PKC [45]. The enzyme shows characteristics similar to mammalian PKC, and is activated by diacylglycerol but not by phorbol esters [45].

4. SIGNAL ROUTES FOR ACTIVATION AND DOWN-REGULATION

Although the receptor-mediated hydrolysis of in-

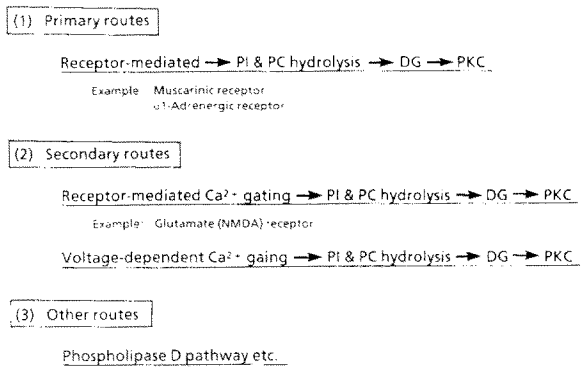

Fig. 1. Signal routes for PKC activation. The detailed explanation is given in the text. PI, phosphatidylinositol and its mono- and bis-phosphate; PC, phosphatidylcholine; DG, diacylglycerol; and NMDA, *N*-methyl-D-aspartate.

ositol phospholipids was once thought to be the sole mechanism leading to the activation of PKC [1], recent studies suggest that there are several additional routes to provide the diacylglycerol that is needed for enzyme activation, as schematically given in Fig. 1. For instance, phosphatidylcholine may also be hydrolysed to produce diacylglycerol at a relatively later phase of cellular responses, particularly those to long-acting signals such as some growth factors (see [46,47] for reviews). For instance, early studies with T-lymphocytes have suggested that interleukin-1, an accessory signal to cell activation, stimulates the production of diacylglycerol from phosphatidylcholine [48]. However, several isoforms of the Ca^{2+}-dependent phospholipase C so far identified do not react with phosphatidylcholine, and it has been postulated that phospholipase D followed by phosphatidic acid phosphatase may be involved in such receptor-mediated phospholipid degradation. In fact, agonist-stimulated activation of phospholipase D has been shown in several tissues and cell types such as hepatocytes [49], HL-60 cells [50,51], platelets [52] and fibroblasts [53], although the precise mechanism of this activation remains to be explored. It is possible that PKC itself may take part in this phospholipid degradation cascade.

In addition, both receptor-mediated and voltage-dependent Ca^{2+}-gate opening may cause phospholipid degradation, that is probably initiated by the activation of phospholipase C and also phospholipases D and A_2 due to the Ca^{2+}-influx. As briefly noted above, arachidonic acid and its metabolites are able to activate some subspecies of PKC under certain conditions. Thus, the signal routes leading to the activation of PKC may greatly vary with cell types, extracellular signals, and perhaps with the time after cell stimulation.

The enzyme was originally detected as an undefined protein kinase which was activated by limited proteolysis with calpain, a Ca^{2+}-dependent neutral protease [54]. Indeed, PKC is very susceptible to limited proteolysis. This reaction generates a catalytically fully active fragment, previously called protein kinase M [55]. A protein kinase known for a long time as histone kinase II has been proved to be the proteolytically activated fragment of PKC [18]. Calpain I, that is active in the micromolar range of Ca^{2+} concentrations, cleaves preferentially the active form of PKC [56]. In in vitro systems, PKC subspecies with the α-, β- and γ-sequence are cleaved at different rates at one or two specific sites in region V3, that connects the regulatory and protein kinase domains.

Although the physiological significance of this limited proteolysis has not been established, it is possible that this reaction is a process for activating PKC persistently as postulated in platelets and neutrophils [57,58]. Alternatively, the proteolysis may also be a process for initiating the degradation, termed down-regulation, of the PKC molecule itself [59–62]. In most cells, the catalytically active fragment is not always recovered from the cell, and is probably degraded further by the action of other proteases [56]. In fact, on treatment with phorbol esters, several PKC subspecies co-expressed in a single cell disappear at different rates, which may reflect their susceptibility to the calpain action [63,64].

5. PHYSIOLOGICAL ROLES FOR CELLULAR REGULATION

It is well recognized that the synergistic interaction between PKC and Ca^{2+} pathways underlies a variety of cellular responses to external stimuli (see [1,65] for reviews). Although studies on the detailed biochemical action of individual PKC subspecies are only at their outset, one can anticipate that members of the family may each have different roles in the processing and modulation of a wide variety of cellular functions. Up to now, many physiological functions have been assigned to PKC, including involvement in secretion and release of various cellular constituents from endocrine, exocrine and neuronal tissues, smooth muscle contraction and metabolic processes such as steroidogenesis [34].

Major roles of PKC appear to lie in positive and negative interactions with various ion channels ([66], also see [67] for a review) and other cell-signalling pathways involving Ca^{2+} and cyclic AMP (see [2,65] for reviews). A large body of evidence also indicates that PKC exerts negative-feedback control or down-regulation of various receptors, that are coupled to inositol phospholipid hydrolysis, and those of some growth factors (see [2,65] for reviews). Epidermal growth factor receptor (see [68] for a review) and T-cell surface CD3 complex [69] are typical examples. Presumably, some coupling factor in membranes is a possible target of PKC action [70].

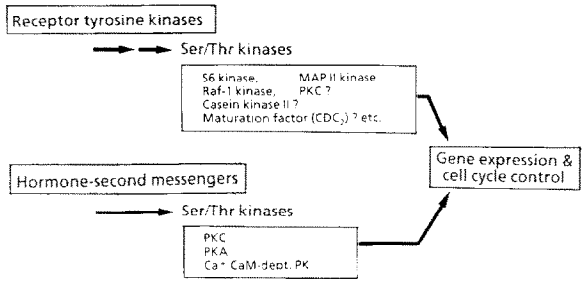

Fig. 2. Hypothetical signal cascades involving various protein kinases, eventually leading to gene activation and cell cycle control. Search has been made in recent years to elucidate a possible link between various growth factor receptors having direct or indirect tyrosine kinase and serine/threonine kinase activities. MAP II kinase, microtubulus-associated protein II kinase; PKA, cyclic AMP-dependent protein kinase; Ca^+ CaM-dept. PK, Ca^{2+} and calmodulin-dependent protein kinase.

On the other hand, it is becoming clearer that PKC may play roles of crucial importance for the regulation of gene expression and cell proliferation. Apparently, sustained activation of PKC is needed to cause gene activation, eventually leading to cell proliferation. It has recently been demonstrated that, with purified T-cells, multiple and repeated additions of membrane-permeable diacylglycerol result in both interleukin-2 receptor expression and cell proliferation [71,72]. Unlike phorbol esters which activate PKC persistently [73], a single dose of permeable diacylglycerol, which causes only transient activation of PKC, cannot elicit T-cell activation [31]. The cell activation and proliferation process is obviously the result of an interaction of a number of signalling pathways. One of these is PKC activation, which is required for a prolonged length of time, brought about, physiologically, by a combination of various signals involving the phospholipid degradation cascade discussed above. This may be needed for maintenance of phosphorylation of a protein whose activity is critical in the regulation of gene activation, such as transcription factors. Presumably, the signalling pathways, including tyrosine kinases and Ca^{2+}-dependent kinases, may play critical roles in this process. Recently, considerable interest has centered on cross-talk between a variety of different protein kinases in a direct or indirect fashion as schematically given in Fig. 2 (see also [74] for a review). It has been proposed that integration of two signalling pathways involving tyrosine and serine/threonine kinases is needed for activation of a protein kinase, MAP kinase [75]. It is likely then, that gene activation and cell proliferation may result from dynamic interactions between PKC and many other signalling pathways.

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