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The common structure and activities of four subspecies of rat brain protein kinase C family

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Elucidation of the complete sequences of four cDNA clones (α, βI, βII, and γ) of the rat brain protein kinase C family has revealed their common structure composed of a single polypeptide chain with four constant (C_1-C_4) and five variable (V_1-V_5) regions. Although these sequences are highly homologous and closely related to one another V_3-, V_4-, and V_5-regions of γ-subspecies are slightly bigger than the corresponding regions of the other three subspecies. The first constant region, C_1, contains a tandem repeat of cysteine-rich sequence (6, total 12 cysteine residues). The third constant region, C_3, has an ATP-binding sequence which is found in many protein kinases. In adult rat whole brain, the relative activities of α-, βI-, βII-, and γ-subspecies are roughly 16, 8, 55, and 21%, respectively. γ-Subspecies is expressed after birth apparently only in the central nervous tissue, implying its role in the regulation of specific neuronal functions.

Protein kinase C; Complementary DNA

1. INTRODUCTION

Nucleotide sequence analysis of the cDNA for protein kinase C has predicted the existence of multiple subspecies of the enzyme in the mammalian brain [1–8]. Expression of rat brain cDNA clones in COS cells, and the comparison of these expressed forms with the biochemically fractionated subspecies of the enzyme have allowed identification of the primary structure of each subspecies present in the brain [9,10]. Comparison of the complete structures of the four subspecies encoded by α-, βI-, βII- and γ-cDNA clones that are all obtained from the rat brain library has elucidated the common structure of the four protein kinase C subspecies. The γ-subspecies is slightly different from the others, and biochemical and immunological analysis reveals that this subspecies appears to be expressed after birth apparently only in the central nervous tissue. The integrated nomenclature of α-, βI-, βII- and γ-cDNA clones and its correspondence to those of other workers [1,2,5,8] is as described in our preceding reports [9,10].

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2. MATERIALS AND METHODS

2.1. *Materials*

Sprague-Dawley rats were employed for the present studies. βI- and βII-cDNA clones of rat brain protein kinase C were obtained as described in [4]. α- and γ-cDNA clones of the enzyme were obtained by sequential plaque screening by the method of Maniatis et al. [11] using synthetic oligonucleotide probes, which corresponded to amino acid residues from 162 to 170 and 334 to 342 of bovine α-cDNA [1], and those from 165 to 173 and 338 to 346 of rat γ-cDNA [5], respectively, as described in [10]. Some of the properties of these cDNA clones have been described [9,10].

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Polyclonal antibodies that specifically recognize each subspecies of protein kinase C were prepared using synthetic polypeptides corresponding to the variable regions of the subspecies. Detailed procedures of preparation and properties of the antibodies will be described elsewhere. Other materials and chemicals were obtained from commercial sources.

2.2. *Nucleotide sequence analysis*

Nucleotide sequence analysis was made using the enzymatic chain termination method in conjugation with M13-derived vectors as described [3].

2.3. *Enzyme purification and assays*

Protein kinase C was assayed by measuring the incorporation of $^{32}P_i$ into calf thymus H1 histone from [γ-^{32}P]ATP in the presence of phosphatidylserine, diolein and Ca^{2+} as described in [12]. Phorbol ester-binding was assayed with [^{3}H]phorbol-12,13-dibutyrate using the filtration procedure described in [13].

Rat brain protein kinase C was purified from the soluble fraction by DE-52, threonine-Sepharose and TSK phenyl-5PW column chromatographies, and then resolved into three fractions, type I, II and III, by chromatography on a hydroxyapatite column, which was connected to a high-performance liquid chromatography (HPLC) (Pharmacia FPLC) system under the conditions described in [9,10]. The type I, II and III protein kinase C correspond to γ-, βI- plus βII- and α-subspecies, respectively [9,10].

2.4. *Other procedures*

Protein was determined by the method of Lowry et al. [14] with bovine serum albumin as the standard. Western blot and immunocytochemical analysis were performed as described in [15].

3. RESULTS

3.1. *The common structure of four protein kinase C subspecies*

The complete amino acid sequences encoded by βI-, βII- and γ-cDNAs of rat brain have previously been reported from this and other laboratories [3,5]. The amino acid sequences predicted by rat γ-cDNA clones obtained in the two different groups were identical. The amino acid sequence of α-subspecies from rat brain is shown in fig.1. This sequence differs slightly from the subspecies encoded by bovine [1], human [2] and rabbit [8] brain α-cDNA clones. However, in general, the animal species difference in each enzyme subspecies is much smaller than the enzyme subspecies difference found in a single animal species. Nevertheless, the structures of the four enzyme subspecies present in rat brain are highly homologous and closely related to one another (fig.1).

Comparison of the structures of the four subspecies reveals their common structure composed of a single polypeptide chain having four constant (C_1–C_4) and five variable (V_1–V_5) regions as schematically represented in fig.2. The first constant region, C_1, contains a tandem repeat of cysteine-rich sequence (6, total 12 residues) that is analogous to 'Zn^{2+}-finger' found in some metalloproteins and DNA-binding proteins [16]. The third constant region, C_3, has an ATP-binding sequence, GXGXXG-----K, which is commonly found in many protein kinases. This region contains only one cysteine residue. The fourth constant region, C_4, has four to six cysteine residues. This constant region contains an additional glycine-rich sequence similar to the ATP-binding site, GXGXXG-----(K). The significance of this repeat is not known at present.

Treatment with Ca^{2+}-dependent protease (calpain) yielded a fragment with about 51 kDa, that is fully active catalytically in the absence of phospholipid, diacylglycerol (or phorbol ester), and Ca^{2+} [17]. Despite the presence of many cysteine residues, the phorbol ester-binding domain, that is presumably located in the amino-terminal half, was relatively resistant to *N*-ethylmaleimide, whereas the protein kinase domain was sensitive to this reagent as shown in fig.3. *N*-Ethylmaleimide did not interfere with the substrate, histone. No obvious structure for the binding of calcium and phospholipid, such as EF hand that is found in proteins such as calmodulin [18], is apparent in the enzyme molecules.

3.2. *Relative activities of protein kinase C subspecies*

Biochemical fractionation on a hydroxyapatite column has resolved the brain protein kinase C into three fractions, type I, II and III as described

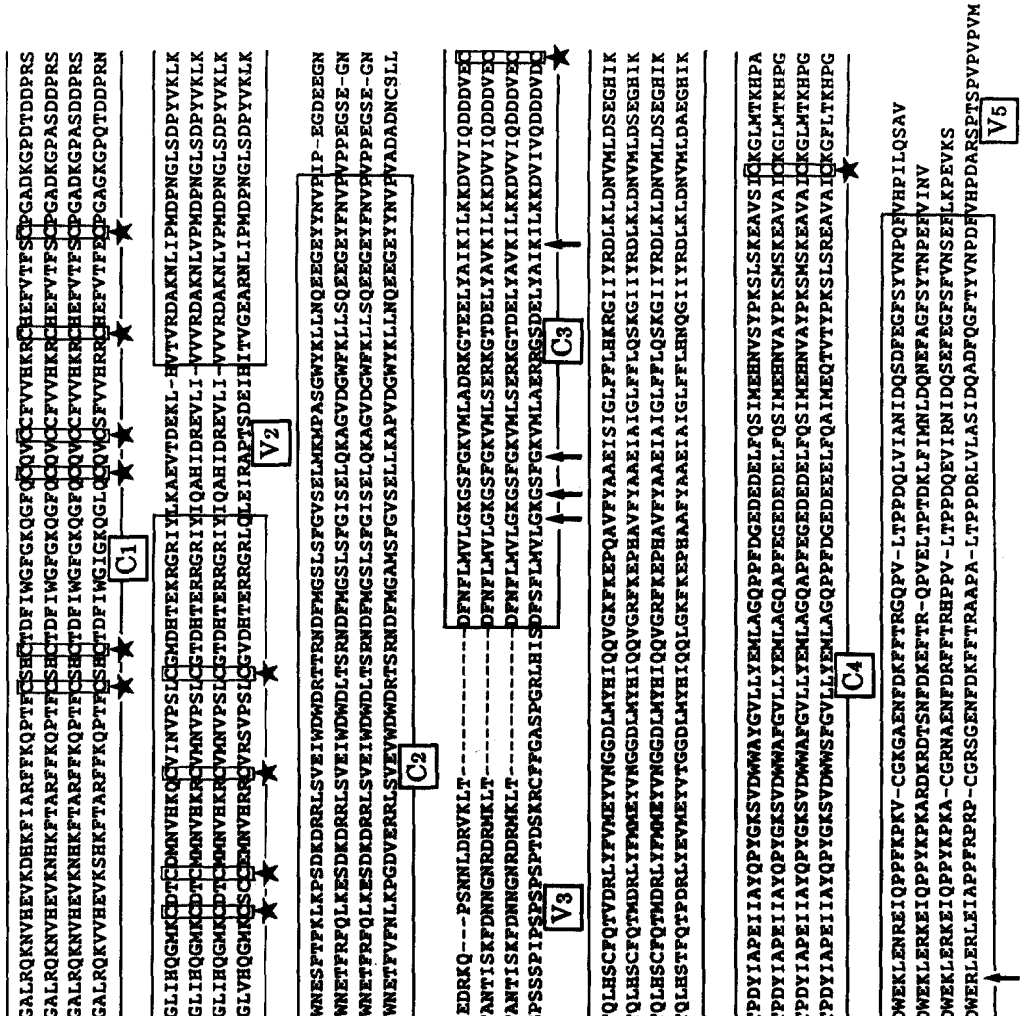

Fig.1. Amino acid sequences of rat protein kinase C subspecies predicted by analysis of four cDNA clones obtained from the rat brain cDNA library. Amino acids were shown by one-letter abbreviations. The α-sequence of the rat enzyme was clarified in this study. The βI- and βII-sequences were taken from [4]. The present study confirmed that the γ-sequence coincided well with the amino acid sequence reported by Knopf et al. [5]. The sequences in the boxes indicate the constant regions (C_1–C_4). V_1–V_5 indicate the variable regions. Stars indicate cysteine residues commonly present in the four subspecies. Arrows in the C_3 and C_4 regions show ATP-binding and its analogous sequences.

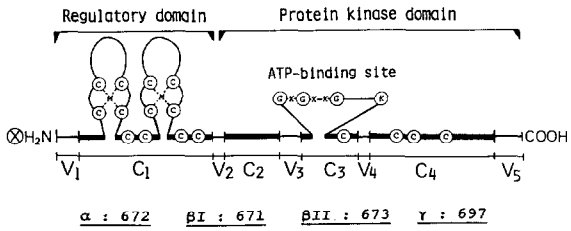

Fig.2. Common structure of four rat protein kinase C subspecies. The amino-terminals appeared to be blocked. The numbers in this figure indicate the amino acid residues composing each subspecies. C, cysteine; G, glycine; K, lysine; M, metal; and X, any amino acid.

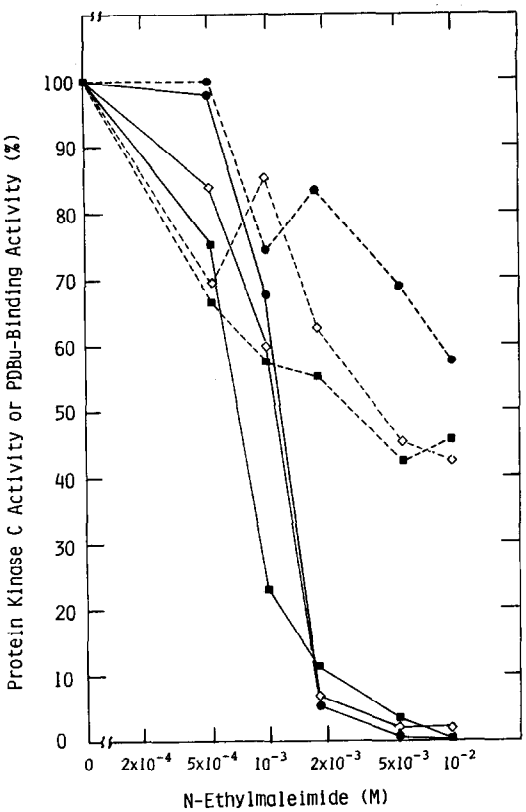

Fig.3. Susceptibility of protein kinase and phorbol ester-binding activities to *N*-ethylmaleimide. Each of the purified type I, II and III protein kinase C was assayed in the presence of the reagent as indicated. (◇) Type I, (●) type II, and (■) type III enzyme. (Broken lines) Phorbol ester-binding activity, and (solid lines) protein kinase C activity.

[9,10,19,20]. Comparison of each type with the enzyme subspecies separately expressed in COS cells by transfection of the cDNA-containing plasmids indicates that type I corresponds to γ-subspecies, type II is a mixture of βI- and βII-subspecies which are derived from alternative splicing, and that type III corresponds to α-subspecies [9,10]. In adult rat brain, these four subspecies are all expressed, and the relative enzyme activities in the brain cytosol are roughly: α, 16%; βI, 8%; βII, 55%; and γ, 21%. The ratio of βI- and βII-subspecies eluted in the type II enzyme fraction was estimated by immunoblotting using antibodies which specifically recognized each subspecies.

Biochemical fractionation analysis indicated that γ-subspecies (type I) was not detected in the newborn rat brain. This subspecies was expressed slowly after birth, and did not reach its maximum level until after 3–4 weeks. It was also noted that γ-subspecies was apparently only found in the central nervous tissue and not in other tissues and organs so far tested, including adrenal gland and peripheral nerve. Table 1 shows the distribution of this subspecies in the central nervous tissue of rat. The highest specific activity was found in the cerebellar cortex and hippocampus.

4. DISCUSSION

Protein kinase C was previously thought to be a single entity, but recently was realized to be a mixture of multiple subspecies. The primary structures of the four subspecies are highly homologous and

Table 1

Distribution of γ-subspecies protein kinase C in rat central nervous tissues

	Specific activity (pmol $^{32}P_i$/min per mg wet tissue)
Whole brain	3.9
Cerebrum	3.0
Cerebellum	18.8
Hippocampus	12.8
Hypothalamus	1.3
Spinal cord	0.2

Protein kinase C in the soluble fraction of each region was fractionated by DE-52 column, followed by chromatography on a hydroxyapatite column connected to HPLC as described in section 2. The specific activity given in this table was estimated from the relative ratio of the activity of type I enzyme (γ-subspecies) over the total enzyme activity

closely related to one another. However, V_3-, V_4- and V_5-regions of γ-subspecies are slightly bigger than the corresponding regions of the other three subspecies. Since this subspecies of protein kinase C is expressed only after birth in specific regions of the central nervous tissue, it may play a role in regulating some specialized neuronal functions, such as long-term potentiation and depression.

The three cDNA clones, α, β and γ, from bovine brain [1], that correspond to α, βII and γ from rat brain, respectively [10], are shown to be encoded by distinct chromosomes [2]. The βI- and βII-cDNA clones, on the other hand, are derived from a single mRNA transcript, and the two subspecies differ from each other only in the short ranges of about 50 amino acid residues in their carboxyl-terminal regions [4,8,9]. These two subspecies show almost identical physical and kinetic properties, and are distinguished thus far only in their immunochemical properties. However, it is possible that the three fractions of protein kinase C that are separated on a hydroxyapatite column still contain additional subspecies of the enzyme. A partial sequence of a cDNA clone recently isolated from the rat brain library [6] is apparently similar to but clearly different from any of the four subspecies described in this paper.

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