Ca\(^{2+}\)/CALMODULIN AND Ca\(^{2+}\)/PHOSPHOLIPID-DEPENDENT PROTEIN KINASES IN THE NEURAL TISSUE OF THE HONEYBEE APIS MELLIFERA

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Abstract—The Ca\(^{2+}\)/calmodulin and Ca\(^{2+}\)/phospholipid-dependent protein kinases have been purified and characterized from neural tissue of the honeybee Apis mellifera. Ca\(^{2+}\)/calmodulin-dependent protein kinase appeared as a multisubunit complex composed of three subunits that co-migrate with kinase activity during all purification steps. The three subunits had molecular weights of 52,000, 57,000 and 60,000, termed α, β, and β, respectively. The α and β subunits are distinct peptides whereas β may have been generated from β by proteolysis. The Ca\(^{2+}\)/calmodulin-dependent protein kinase required 0.1 μM calmodulin and about 1 μMCa\(^{2+}\) for half-maximal activation. The Ca\(^{2+}\)/phospholipid-dependent protein kinase (protein kinase C) was purified from honeybee neural tissue by using DEAE-Sephacel and phosphatidylinerine-affinity chromatography. The molecular weight of the protein kinase C was about 80,000 as estimated by gel filtration. Subjection to SDS-PAGE gave a single band with M\(_r\) = 80,000, indicating that the enzyme exists as a monomer. The enzyme was fully activated by diacylglycerol in the presence of phospholipid and Ca\(^{2+}\).

Key Word Index: protein kinase C; Ca\(^{2+}\)/calmodulin-dependent protein kinase; brain; honeybee

INTRODUCTION

Phosphorylation/dephosphorylation reactions prove to play an important role in cellular regulation. Activation of protein kinases in the nervous system have been implicated in regulation of neurotransmitter release, ion channels, growth and differentiation, and neural plasticity (Nishizuka, 1986; Shacter et al., 1988). In the marine snail Hemitrida Ca\(^{2+}\)/calmodulin-dependent protein phosphorylation of K\(^{+}\)-channel proteins is involved in learning mechanisms (Alkon, 1984). Recent evidence indicates that the action of the Ca\(^{2+}\)-dependent protein kinase is implicated in processes for LTP induction (Malinow et al., 1988; Malenka et al., 1989).

Two groups of Ca\(^{2+}\)-dependent protein kinases are highly concentrated in neural tissue. Brain Ca\(^{2+}\)/calmodulin-dependent protein kinase II (CaM kinase II) has been isolated and characterized from subcellular fractions of several vertebrates (Schulman and Lou, 1989). The enzyme has been purified as an oligimer of M\(_r\) = 450,000–550,000 composed of α, β, and β polypeptides with M\(_r\) = 50,000, 58,000 and 60,000, respectively. These subunits display considerable homology, bind calmodulin, and autophosphorylate avidly (Bennett et al., 1983; Goldenzweig et al., 1983). The major polypeptide (M\(_r\) = 50,000) of the postsynaptic density has been identified as the α-subunit (Kennedy et al., 1983; Kelly et al., 1984). The α and β-subunits of the Ca\(^{2+}\)/calmodulin-dependent protein kinase II accounted to be the major kinase in neuronal nuclei (Sahyoun et al., 1984).

The Ca\(^{2+}\)/phospholipid-dependent protein kinase (protein kinase C) is a species of Ca\(^{2+}\)-dependent protein kinases which requires phospholipid as a cofactor and can be further activated by diacylglycerol. Protein kinase C is known to be a large family of proteins with multiple subspecies that exhibit distinct patterns of tissue expression, compartmentalization and differences in their activation mechanisms (Parker et al., 1984; Nishizuka, 1988; Sekiguchi et al., 1988).

The kinase is a monomeric polypeptide (M\(_r\) = 80,000) composed of a hydrophobic membrane-binding domain and a hydrophilic catalytic domain. Protein kinase C is a ubiquitous enzyme and has attracted particular attention because it plays a pivotal role in controlling many cellular functions and seems to be involved in modulation of signal transduction (Nishizuka, 1986, 1988).

Classical conditioning of the nictitating membrane/eyelid response in the rabbit induces long-term translocation of the protein kinase C in hippocampal CA1 cells (Bank et al., 1988; Olds et al., 1989). Hippocampal long-term potentiation is also associated with translocation of protein kinase C from cytosol to membrane fraction (Akers et al., 1986; Malenka et al., 1989; Malinow, 1988).

Related to observations in Drosophila and Hemitrida, pharmacological and biochemical evidence also indicate an important role of Ca\(^{2+}\)-dependent protein kinases in processes of learning and memory in honeybees (Menzel et al., 1989; Sugawa et al., 1989).
In the present work we report the purification and characterization of the Ca\textsuperscript{2+}/calmodulin and the Ca\textsuperscript{2+}/phospholipid-dependent protein kinases in neural tissue of the honeybee *Apis mellifera*.

**MATERIALS AND METHODS**

**Materials**

We used stock and worker bees of *Apis mellifera*. (γ-\textsuperscript{32}P)ATP was obtained from Amersham. For the purification of DEAE-Sephasel (Pharmacia), CM-Sephasel C-50 (Sigma), Sephadex G-200 (Sigma) and calmodulin-agarose (phosphorylase kinase gel, 3′,5′-cyclic nucleotide activated, bovine brain) were used. Calmodulin, histone II A and II S, diolein, and phosphatidylserine were obtained from Sigma. Molecular weight kits were purchased from Sigma. Tissue-Tek II was obtained from Lab-Tek Products. All chemicals were of analytical grade.

**Preparation of brain crude homogenate**

Stock and worker bees were randomly caught from the hive. The preparation of their brains was carried out under permanent liquid nitrogen cooling. The heads were cut off with a scalpel and fixed with Tissue-Tek II to a brass block and subsequently cooled down to liquid nitrogen temperatures. The head capsules were broken and removed. The gland tissue—either the food glands facing the front or the head glands facing the back of the head—were scraped off and the brain could be levered out of the fixed head. The brains were collected and stored at −70°C. Freeze-drying of the heads at 10\textsuperscript{−3} bar at −20°C for 2 h facilitated the separation of the brains from non-neural tissue.

50–100 frozen brains (80–160 mg) were transferred into an ice-cooled glass–glass potter and homogenized in 15 ml extraction-buffer (50 mM Tris–HCl, pH 7.5, 100 mM NaCl, 2 mM EDTA, 10 mM β-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, and 1 mM benzamidine) at 4°C. All following steps were carried out at 4°C. The homogenate was centrifuged at 50,000 \texttimes g for 20 min. The pellet was resuspended in 8 ml of extraction-buffer and centrifuged again. Both supernatants were combined and dialyzed against DEAE-buffer (50 mM Tris–HCl, pH 8.1, 1 mM EDTA, 10 mM β-mercaptoethanol) and subsequently used for further purification.

**Preparation of endogenous Ca\textsuperscript{2+}/calmodulin-dependent protein kinase substrate from honeybees**

CaM kinase substrate was enriched from honeybee brain using a procedure described for purification of synapsin (Ueda and Greenberg, 1977). The pellet obtained by centrifugation of the brain homogenate was resuspended in distilled water and adjusted to pH 3. After 15 min of repeated shaking, the suspension was centrifuged at 50,000 \texttimes g for 15 min at 4°C. The supernatant was adjusted to pH 6 followed by a further centrifugation. The resulting supernatant was stored at −70°C and subsequently used within 4 weeks.

**SDS-PAGE and quantitative determination of the \textsuperscript{32}P-incorporation into the substrate proteins**

The standard SDS-polyacrylamide gel electrophoresis was performed according to the procedure of Laemmli (1970). After electrophoresis, gels were fixed and stained conventionally with Coomasie Blue R250. Labeled protein bands were visualized by exposing the dried gel to Kodak X-Omat AR films. Autoradiograms were scanned with a Kontron Uvikon 810 spectrophotometer. For quantitative evaluation of the \textsuperscript{32}P-incorporation into the substrate proteins, conditions were chosen so that the exposure of the films was kept in the linear range. The linear range of the individual substrate proteins was determined by cutting out the labeled bands of the SDS-gel and count the slices in a scintillation counter. This procedure allows the simultaneous determination of \textsuperscript{32}P-incorporation into specific substrates of different protein kinases. Native gels were run in a modified procedure under omission of SDS.

**Ca\textsuperscript{2+}/calmodulin-dependent protein kinase assay**

Kinase activity was assayed using an endogenous CaM kinase substrate. The reaction mixture with a total volume of 30 µl contained 50 mM Tris–HCl, pH 7.5, 20 mM NaCl, 10 mM MgCl\textsubscript{2}, 10 mM β-mercaptoethanol, 10 µM ATP, 0.1 µCi (γ-\textsuperscript{32}P) ATP, 0.1 µM calmodulin, CaM kinase substrate with or without 2 mM CaCl\textsubscript{2}. The reaction was started by addition of 10 µl of the CaM kinase substrate. The reaction was stopped after 15 min (25°C) with 6 µl of SDS sample buffer (0.5 M Tris–HCl, pH 6.8, 5% 2-mercaptoethanol, 5% SDS, 20% glycerol). The samples were subjected to SDS-PAGE and the incorporation of labeled phosphate into the substrate was quantified by densitometric scanning of the autoradiograms. For autophosphorylation of the CaM kinase the reaction was carried out under the same conditions omitting CaM kinase substrate.

**Protein kinase C assay**

Protein kinase C was assayed by phosphorylation of a mixture of histones I A and II S with or without addition of phosphatidylserine. The mixture with a final volume of 20 µl contained 50 mM Tris–HCl, pH 7.5, 10 mM MgCl\textsubscript{2}, 10 mM β-mercaptoethanol, 10 µM ATP, 0.1 µCi (γ-\textsuperscript{32}P) ATP, 0.5 mM CaCl\textsubscript{2}, 1 µg histone I A, 1 µg histone II S, 0.02 µg diolein with or without 0.8 µg phosphatidylserine. The mixture was prepared according to Uchida and Filburn (1984) with simplifications as following. Phosphatidylserine (5 mg/ml) and diolein (0.05 mg/ml) were dissolved in 95% chloroform/5% methanol. Appropriate amounts were filled into a reaction tube, and the solvent evaporated at room temperature. After evaporation, the other compounds of the mixture were added and vortexed. The phosphorylation was started by adding 10 µl of the sample to 10 µl of the reaction mixture. After incubation at 25°C the reaction was terminated by addition of 4 µl of SDS sample buffer. The samples were subjected to SDS-PAGE and the incorporation of \textsuperscript{32}P into the substrate was quantified by densitometric scanning of the autoradiograms. For autophosphorylation of the protein kinase C the reaction was carried out as described omitting the histones.

**Preparation of phosphatidylserine affinity gel**

For preparing the affinity column, we followed the instruction of Uchida and Filburn (1984). Phosphatidylserine (1 mg) and cholesterol (5 mg) dissolved in chloroform were combined and evaporated at room temperature. 1 ml of a solution of 15% acrylamide and 5% bisacrylamide was added and mixed vigorously. The polymerization was started by addition of 15 µl ammonium persulfate solution (140 mg/ml) and 2 µl TEMED. The mixture polymerized within 30 min to a white rigid gel. The resulting gel was rinsed with water, minced with a razor blade and homogenized in a loose-fitting glass–glass potter. The homogenized gel was allowed to settle down for about 5 min, the supernatant was decanted, and this procedure was repeated for five times after resuspension in water. The settled gel particles were washed and equilibrated in buffer A (50 mM Tris–HCl, pH 7.5, 10 mM β-mercaptoethanol, 100 mM NaCl) containing 2.5 mM CaCl\textsubscript{2}.

**RESULTS**

**Purification of the CaM Kinase**

The dialyzed supernatant of the brain homogenate was applied to a DEAE-Sephasel column equilibrated with DEAE-buffer and eluted with a linear

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1989).
Ca²⁺-dependent protein kinases in honeybee brain

NaCl-gradient (0–0.3 M NaCl). Ca²⁺/calmodulin-dependent protein kinase activity was eluted as a single peak at 0.09–0.12 M NaCl (Fig. 1). Beside this peak, no other Ca²⁺/calmodulin-dependent kinase activity was detectable in the supernatant of the brain homogenate. Active fractions were pooled and CaCl₂ was added to a final concentration of 2.5 mM. The solution was immediately applied to a calmodulin-Sepharose column (1 ml) equilibrated with buffer B (50 mM Tris-HCl, pH 7.5, 10 mM 2-mercaptoethanol, 0.5 mM EDTA and 100 mM NaCl) containing 2.5 mM CaCl₂. The calmodulin-affinity column was washed with buffer B containing 1 mM CaCl₂ and the CaM kinase activity was eluted with Buffer B containing 10 mM EGTA [Fig. 2(A)]. Bovine serum albumin was added to a final concentration of 1 mg/ml to the eluted samples to stabilize protein kinase activity.

Structure of the CaM kinase

Incubation of the purified kinase fraction with [γ-³²P]ATP in the presence of Ca²⁺ and calmodulin resulted in the autophosphorylation of the CaM kinase (Fig. 3). Since the small amounts of the purified honeybee CaM kinase did not allow gel-filtration, the molecular weight of the kinase holoenzyme was estimated by using non-denaturing polyacrylamid gel electrophoresis. In the gel system (7% separation gel) the autophosphorylated CaM kinase exhibited electrophoretic mobility corresponding to a Mr of 550,000–650,000, which is in the range of the Mr of CaM kinase II from vertebrates (Bennett et al., 1983; McGuinness et al., 1985). Separation of the autophosphorylated CaM kinase on SDS-PAGE showed that the enzyme-fraction contained ³²P-labelled Mr = 52,000, 57,000 and 60,000 polypeptides (Fig. 3). This is in accordance with the polypeptide composition of CaM kinase II holoenzyme reported in other sources (Bennett et al., 1983; McGuinness et al., 1985). The autophosphorylated polypeptides termed α, β, and β respectively in order of their apparent molecular weights, peaked in the same fractions of kinase activity during DEAE-chromatography and bound specifically to a calmodulin affinity column. This supports evidence that these polypeptides are subunits of the CaM kinase II holoenzyme complex. Since the phosphopeptide composition after limited digestion of the phosphorylated forms of Mr = 57,000 and 60,000 subunits (β and β respectively) are very similar. Phosphopeptide maps of the α and β (β') subunits show characteristic differences (Fig. 4).

Properties of Ca²⁺/calmodulin-dependent protein kinase

The purified CaM kinase revealed its activity only in the presence of both Ca²⁺ and calmodulin. Since casein and several types of histones were phosphorylated poorly or not at all, we used an endogenous substrate from honeybee brain. This substrate was enriched according to the procedure described for purification of synapsin (Ueda and Greengard, 1977). After separation of SDS-PAGE the endogenous substrate phosphorylated by CaM kinase showed a double of ³²P-labeled protein bands with electrophoretic mobility corresponding to Mr = 66,000 and Mr = 65,000, respectively. Using these proteins as substrates the concentrations of Ca²⁺ and calmodulin required for half-maximal activation of CaM kinase were 1 and 0.1 μM, respectively.

Fig. 1. DEAE-Sepharose chromatography of honeybee brain Ca²⁺/calmodulin and Ca²⁺/phosphatidyserine dependent protein kinases. Ca²⁺/calmodulin kinase activity (○) was assayed using an endogenous substrate in absence and presence of Ca²⁺ and calmodulin. Protein kinase C activity (□) was measured in a mixture containing Ca²⁺ and diolein with or without phosphatidyserine. The activities were normalized with respect to their maximal values.

Fig. 2. Affinity chromatography of Ca²⁺/calmodulin-dependent protein kinase and protein kinase C. (A) DEAE-Sepharose fractions with Ca²⁺/calmodulin-dependent kinase activities were pooled and CaCl₂ was added to a final concentration of 2.5 mM. The solution was applied to a calmodulin affinity column. The column was washed with buffer B containing 1 mM CaCl₂ and the CaM kinase activity was eluted with buffer B containing 10 mM EGTA. (B) Affinity chromatography of pooled. DEAE-fractions with protein kinase C activity on polycrylamide immobi-

ized phosphatidyserine. Elution procedure as described in (A). Kinase activities were assayed as described for Fig. 1. The activities were normalized with respect to their maximal values.
Compartmentalization of the Ca\textsuperscript{2+}/calmodulin-dependent protein kinase

The autophosphorylated subunits (α, β, and β′) in soluble and particulate fractions of honeybee brain extract showed the same peptide pattern after partial digestion with protease from Staphylococcus aureus V8 as the corresponding subunits of the purified CaM kinase. The major amount of Ca\textsuperscript{2+}/calmodulin-dependent protein kinase activity could be detected in the particulate fraction.

Calmodulin in the honeybee brain

Calmodulin was eluted from the DEAE-Sephadex column as a single peak at 0.27–0.33 M NaCl. These fractions were concentrated and chromatographed on Sephadex G-200. As a single peak at a position corresponding to M<sub>r</sub> = 20,000. The fractions containing calmodulin were combined, lyophilized, redisolved in a small amount of water, and dialyzed against 50 mM Tris–HCl, pH 7.5, and 10 mM 2-mercaptoethanol. For quantitative determination of the amount of honeybee brain calmodulin, calmodulin purified from bovine brain was used as a standard. Honeybee calmodulin and known amounts of bovine brain calmodulin were applied to SDS-gels and stained with Coomassie. The bands were scanned at λ = 590 nm and the areas of the peaks were measured. The content of calmodulin in honeybee brain tissue was calculated to be about 1 μM. Calmodulin mixed with either 2 mM EGTA or 2 mM Ca\textsuperscript{2+} prior to SDS-electrophoresis showed electrophoretic mobilities corresponding to M<sub>r</sub> = 20,000 and M<sub>r</sub> = 17,000, respectively. Calmodulin purified from honeybee brain activated the CaM kinase from honeybee at the same concentrations as calmodulin from bovine brain. All these properties are in accordance with those of calmodulin reported from other tissues (Klee et al., 1980).

Protein Kinase C

Purification of protein kinase C

The brain homogenate dialyzed against DEAE-buffer was applied to a DEAE-Sephacel column equilibrated with its buffer. The column was eluted with a linear NaCl gradient (0–0.3 M NaCl). Ca\textsuperscript{2+}/phosphatidylserine-dependent protein kinase activity was eluted as a single peak at 0.08–0.1 M NaCl (Fig. 1). Beside this peak no other Ca\textsuperscript{2+}/phosphatidylserine-dependent kinase activity was detected. Immediately after DEAE-chromatography active fractions were pooled, CaCl\textsubscript{2} was added to a final concentration of 2.5 mM and the solution was subsequently applied to a phosphatidylserine-affinity column (1 ml). The column was washed with buffer A containing 1 mM CaCl\textsubscript{2} and the protein kinase activity was eluted with buffer A containing 10 mM EGTA (Fig. 2B). Immediately after elution bovine serum albumin was added to a final concentration of 1 mg/ml to the eluted sample to stabilize protein kinase activity.

The phosphatidylserine-affinity column is a critical step in the purification procedure. Since the binding ability of the protein kinase C to the phosphatidylserine-affinity column decreases with time ("age of the protein kinase C-fraction"), the affinity step had to be performed as immediately as possible after DEAE-chromatography. Addition of cephalostatin, a calpain inhibitor, has no effect on the observed decrease in the binding ability of protein kinase C to the phosphatidylserine-affinity column. Therefore, the loss of binding ability to the affinity column is not caused by proteolytic degradation of the protein kinase C by calpain (Huang et al., 1989; Uchida and Filburn, 1984).

Characterization of protein kinase C

Fractions of DEAE chromatography containing protein kinase C activity were pooled, concentrated, and applied to a Sephadex G-200 column equilibrated with 50 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA and 10 mM 2-mercaptoethanol. Protein kinase activity eluted in a single peak at apparent M<sub>r</sub> = 80,000 (Fig. 5). The protein kinase C was fully activated and underwent autophosphorylation in the presence of diacylglycerol, phospholipid and Ca\textsuperscript{2+}. Subjection of autophosphorylated protein kinase to SDS-PAGE resulted in a single band with M<sub>r</sub> = 80,000, indicating that the enzyme exists as a monomer (Fig. 3).

Compartmentalization of the protein kinase C

Ca\textsuperscript{2+}/phosphatidylserine-dependent protein kinase activity was found in both, soluble and particulate fractions of honeybee brain extracts. There was no detectable variation in the total levels of protein kinase C activities in mushroom-bodies or central brain and optic lobes. Since only small amounts of brain tissue were available, an exact determination of the intracellular distribution and localization of kinase activity in distinct regions of honeybee brain was not possible at that time.

Fig. 5. Gel-chromatography of protein kinase C activity from DEAE-Sephacel. The Sephadex G-200 column was calibrated with (1) aldolase from rabbit muscle (M<sub>r</sub> = 158,000), (2) bovine serum albumin (M<sub>r</sub> = 67,000) and (3) hen egg albumin (M<sub>r</sub> = 42,000). DEAE-fractions with protein kinase C activity were pooled, concentrated and applied to the Sephadex G-200 column. The PKC was assayed as described for Fig. 1 and the activities were normalized with respect to their maximal values. The arrow indicates M<sub>r</sub> = 80,000.
Fig. 3. SDS-polyacrylamide gel showing autophosphorylated subunits of Ca²⁺/calmodulin-dependent protein kinase and autophosphorylated protein kinase C. Affinity purified Ca²⁺/calmodulin-dependent kinase were autophosphorylated in presence of Ca²⁺ and calmodulin. Protein kinase C eluted from the phosphatidylerine affinity column was autophosphorylated with Ca²⁺, diolein and phosphatidyserine. The proteins were separated on SDS-PAGE, dried and exposed on Kodak X-Omat AR films. Arrows point to the P-labeled α, β and β′ polypeptides of CaM kinase and to the protein kinase C.

Fig. 4. Comparison of the phosphopeptide composition of autophosphorylated Ca²⁺/calmodulin-independent protein kinase subunits. The bands from autophosphorylated α, β and β′ polypeptides (see Fig. 3) were cut out, placed in the sample wells of a SDS-gel and overlaid with Staphylococcus aureus V8 protease. Digestion proceeded in the stacking gel. P-labeled bands were visualized by exposing the gel to Kodak X-Omat films.
**DISCUSSION**

The Ca\(^{2+}\)/calmodulin and Ca\(^{2+}\)/phosphatidylerine dependent protein kinases isolated from honeybee brain demonstrated purification characteristics, subunit compositions, and activation properties similar to those of the corresponding protein kinases from vertebrate brain tissue (Bennett et al., 1983; Kuret and Schulman, 1984; McGuiness et al., 1985).

The honeybee neural Ca\(^{2+}\)/calmodulin kinase II is a multisubunit complex that consists of three subunits a (M\(_{r}\) = 52,000), \(\beta\) (M\(_{r}\) = 60,000), and \(\beta'\) (M\(_{r}\) = 57,000). In agreement with brain CaM kinase II from other sources the a and \(\beta\) subunits are distinct peptides, whereas, \(\beta'\) may have been generated from \(\beta\) by proteolysis. All three of these polypeptides co-migrate with kinase activity during the purification steps and can bind to a calmodulin-affinity column in the presence of Ca\(^{2+}\). Due to the small amounts of honeybee CaM kinase we have not been able to determine the stoichiometry of the subunit composition. Evidence from rat show the existence of isozyme forms of CaM kinase II in various brain regions which differ in their relative subunit ratios (McGuiness et al., 1985). The differences in subunit ratios may be the basis for the differential localization of the kinase in intracellular compartments, because the rat forebrain isoyme is more enriched in post synaptic densities, whereas the cerebellar isoyme has a greater association with other particulate structures (Miller and Kennedy, 1986).

All three of the purified honeybee CaM kinase subunits show autophosphorylation in the presence of Ca\(^{2+}\) and calmodulin. Studies have shown that the rat brain CaM kinase can become autonomous when appropriately autophosphorylated (Lai et al., 1986; Lou et al., 1986; Miller and Kennedy, 1986). However, autonomous kinase continues to autophosphorylate without Ca\(^{2+}\)/calmodulin, the autonomy is turned off. An additional effect of autophosphorylation may be the translocation of the kinase. Serotonin treatment of *Aplysia* ganglia leads to dissociation of the cytoskeleton-associated CaM kinase (Saith and Schwartz, 1983). A particulate Ca\(^{2+}\)/calmodulin kinase from *Drosophila* heads also translocates after autophosphorylation in the presence of Ca\(^{2+}\)/calmodulin (Willmund et al., 1986). These regulatory properties of the autophosphorylation of the CaM kinase fit the theoretical description of a memory model (Lisman, 1985; Lisman and Golding, 1988).

Protein kinase C is a large family of enzymes ubiquitously present in animal kingdom. The protein kinase C in invertebrates (Devay et al., 1989; Qu et al., 1990a,b; Rosenhal et al., 1987; Schaeffer et al., 1989) show considerable homology with the protein kinase C in vertebrates (Huang, 1989; Nishizuka, 1988). The results in this report provide first evidence that the protein kinase C in honeybee brain shows remarkable similarities to the protein kinase in vertebrates. The similarities are: (i) monomeric polypeptide of M\(_{r}\) = 80,000, (ii) elution position from DEAE-Sephacel chromatography (0.08–0.1 M NaCl), (iii) Ca\(^{2+}\)-dependent binding to phosphatidyserine affinity column, (iv) kinase activity depends on Ca\(^{2+}\) and phosphatidyserine, and (v) autophosphorylation of the enzyme in the presence of Ca\(^{2+}\), phosphatidyserine and diolein.

Molecular cloning analysis and biochemical evidence (Nishizuka, 1988) indicates that protein kinase C exists as a family of multiple subtypes. Protein kinase C isozymes show subtly different enzymatic properties, different tissue distribution and intracellular compartmentalization (Huang et al., 1986; Sekiguchi et al., 1988). Since only milligram amounts of honeybee brain tissue was available, we have not been able to characterize subtypes of the kinase.

Several research groups have shown that protein kinase C is clef by a Ca\(^{2+}\)-dependent neutral proteinase, calpain, to produce a catalytically active fragment (Huang et al., 1989). The physiological significance of this proteolysis has not been unequivocally established. Nevertheless, it may be related to down-regulation of the protein kinase C. Recent studies have provided evidence that the protein kinase C—and possibly the calpain action on protein kinase C—play a role in controlling the release of neurotransmitter and registration of long-term memory (Hu et al., 1987; Mamenka et al., 1989; Malinow et al., 1988). Since the Ca\(^{2+}\)-dependent neutral proteinases calpain I and II have been detected and characterized in honeybee brain (Mller and Altfelder, 1991), the differential down-regulation of protein kinase C may also play a role in the modulation of protein kinase C activity involved in processes of learning and memory in the honeybee *Apis mellifera*.

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**REFERENCES**


