Structural Distinction Between Soluble and Particulate Protein Kinase C Species

David S. Lester,1,2 Nadav Orr,1 and Vlad Brumfeld1

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A number of peripheral membrane proteins functioning as regulatory enzymes are distributed between soluble and particulate fractions upon homogenization and subcellular fractionation. One such enzyme, the Ca2+/phospholipid-dependent protein kinase, protein kinase C, was analyzed in order to examine this characteristic of differential localization. The soluble and particulate forms of this enzyme were purified to relative homogeneity, and their biochemical and biophysical properties were analyzed and compared. Based on biochemical activities, the particulate form required lower phospholipid concentrations for maximal activation than for the soluble species. The particulate species had a more hydrophobic structure as demonstrated by a hydrophobic fluorescence probe, and had almost 50% more α-helical structures according to secondary structure estimation, determined from far ultra-violet-circular dichroism spectra (200-250 nm). Using Fourier transform infrared spectroscopy, specific lipid spectra were detected associated with the soluble protein kinase C species. Further analyses with a fluorescent neutral membrane probe suggested that there was more lipid associated with the purified particulate form, which was of a less mobile nature than those associated with the soluble species. These structural differences provide an explanation for the preferential localization of the enzyme and may prove to be the basis for distribution of other membrane-active peripheral membrane regulatory enzymes.

KEY WORDS: Protein kinase C; hydrophobic; membrane associated; cytosol; lipids.

1. INTRODUCTION

Upon cellular or tissue disruption and subsequent extraction, numerous important regulatory enzymes are distributed between the soluble (or cytosolic) and particulate (or membrane) fractions (Burn, 1988). These proteins are part of a larger class of membrane proteins known as the amphitrophic proteins. Some of these enzymes, such as the phospholipases and protein kinase C (PKC), are supposedly active in the form of peripheral membrane proteins (Nishizuka, 1986; Waite, 1987). Thus, their membrane localization has been considered to be representative of the enzyme in its active state. In order to extract the particulate forms, detergents are generally required, indicating their tight association with and possible insertion into the membrane (Tanford and Reynolds, 1976). To date, the nature of this differential localization has not been addressed. Due to the relatively high quantity of membrane-associated protein kinase C in the rat brain (Kikkawa et al., 1982; Lester, 1989) and our capability of purifying sufficiently large quan-
2. MATERIALS AND METHODS

2.1. Materials

1,6-diphenylhexatriene (DPH), phorbol myristate acetate (PMA), histone III-S, leupeptin, soybean trypsin inhibitor, phenyl methylsulfonyl fluoride, delipidated and nondelipidated bovine serum albumin (fraction V, BSA), poly-L-lysine (M_w = 100,000), and Nonidet P-40 were purchased from Sigma Chemical Co. Bovine pancreatic phospholipase A_2 (PLA_2) was obtained from Boehringer-Mannheim. 2-p-toluidinynaphthalene-6-sulfonate (TNS) was from Molecular Probes, Inc. Synthetic 1,2-dioleoylglycerol (DAG) was obtained from Serdary Research Products. Bovine spinal cord phosphatidylserine (PS) and egg lecithin (PC) were from Avanti Lipid Products. (γ-32P)ATP (3500Ci/nmol) and (3H)phorbol dibutyrate (21.5 Ci/mmole) were purchased from New England Nuclear.

All buffers were prepared from double distilled, deionized water that was further washed against Chelex 100 (Bio-Rad) and Amberlite MB-3 mixed bed monovalent resin to reduce contaminating divalent and monovalent ions. All buffers were corrected for pH (7.5) after divalent cation addition.

2.2. Purification of PKC Species

Protein kinase C was purified as previously described (Huang et al., 1986; Brumfeld and Lester, 1990). Briefly, brains (25) of 6-week-old male Wistar rats were homogenized by a Polytron (30 s at setting "6") in 20 mM Tris buffer containing 10 mM ethylene glycol tetraacetic acid (EGTA), 2 mM ethylene diaminotetraacetic acid (EDTA), 10 mM β-mercaptoethanol, and a mixture of protease inhibitors, including 0.5 mM phenylmethlysulfonyl fluoride, 5 μg/ml leupeptin, and 10 μg/ml soybean trypsin inhibitor. The homogenate was centrifuged at 100,000 g at 4°C in a Beckman Ti60 rotor for 1 hr. The supernatant (soluble or cytosolic fraction) was removed and the pellet (particulate fraction) suspended in the same homogenization buffer containing 0.5% Nonidet P-40, stirred for 45 min at 4°C, and then centrifuged as described above. The detergent extract (particulate fraction) was removed and further fractionated. The same purification procedure was suitable for purification of both forms and found to result in a purified protein. The procedure included the following fractionation steps: DEAE-Sepharose Fast Flow anion exchanger (Pharmacia), Phenyl sepharose CL-4B hydrophobic chromatography (Pharmacia), Amicon ultrafiltration (10,000 Da cut-off filter), Ultrogel Aca44 gel filtration (IBF), and polylysine agarose affinity chromatography (Sigma). Generally, 0.5 mg of purified protein from the cytosolic fraction and between 0.05-0.1 mg of membrane species could be obtained from 25 rat brains. Samples were concentrated in a Filtron 10 ml ultrafiltration unit (10,000 Da cut-off filter) and buffer (20 mM Tris, pH 7.5, 0.5 mM EGTA, 0.5 mM EDTA, 10% glycerol, 10 mM β-mercaptoethanol) added and concentrated again. Samples could be stored at −80°C for at least 1 month without significant loss of activity. The purified samples had similar specific activities of 600-1000 U/mg protein.

Protein was measured according to the procedure of Bradford (1976).

2.3. Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Samples of partially purified (5 μg) and purified (0.8 μg) soluble and particulate PKC were suspended in SDS-sample buffer under reducing conditions (1 mM dithiothreitol). The samples were electrophoresed on a 10% SDS-PAGE (Hoefer Tall Small Elec-