Cloning and Expression of a Membrane Receptor for Secretory Phospholipases A₂*  
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Gérard Lambeau, Philippe Ancian, Jacques Barhanin, and Michel Lazdunski†  
From the Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France  

Snake venom and mammalian secretory phospholipases A₂ are structurally related enzymes that have been associated with several toxic (neurotoxicity, myotoxicity, etc.), pathological (inflammation, hypersensitivity, etc.), or physiological (contraction, proliferation, etc.) processes. We have previously shown that snake venom PLA₂s have specific high affinity receptors. Here, we report the molecular cloning of one of these PLA₂ receptors (molecular mass ~ 180 kDa), previously purified from rabbit skeletal muscle. It is a membrane protein with a N-terminal cysteine-rich domain, a fibronectin type II domain, eight repeats of a carbohydrate recognition domain, a unique transmembrane domain, and an intracellular C-terminal domain. The 1468-residue PLA₂ receptor, expressed in transfected cells, binds svPLA₂ with very high affinities (Kₐ values ~ 10⁻¹⁰ ps). It also tightly binds the two structural types of msPLA₂s, i.e., pancreatic PLA₂ and synovial PLA₂ (Kₐ ~ 1⁻¹⁰ nm). This receptor might have a key role in normal and pathological actions of secretory PLA₂s.

Snake venom (svPLA₂s) and mammalian secretory (msPLA₂s) phospholipases A₂ are structurally related enzymes that specifically catalyze the hydrolysis of the 2-ester bond of 3-sn-phosphoglycerides (1⁻³). These enzymes have been purified from a variety of sources including mammalian pancreas, spleen, lung, platelets, serum, and synovial fluid, as well as reptile and insect venoms. svPLA₂s display different types of toxicities including neurotoxicity, myotoxicity, anticoagulant, and proinflammatory effects (4⁻⁷). These different types of effects are apparently linked to the existence of a variety of very high affinity receptors (Kₐ values as low as 1.5 pm) for these toxic enzymes (8⁻¹¹). msPLA₂s are now implicated in many biological functions besides digestion, such as airway and vascular smooth muscle contraction (12, 13), fertilization (14), and cell proliferation (15). Moreover, clinical studies have identified elevated levels of PLA₂ activity in synovial fluid, serum, and bronchial lavage fluid of patients with arthritis, endotoxin shock, peritonitis, psoriasis, acute pancreatitis, respiratory distress syndrome (reviewed in Refs. 16⁻²⁰), and malaria (21). These high levels of msPLA₂s have often been associated with high levels of tumor necrosis factor and/or interleukin-1. Moreover, tumor necrosis factor and/or interleukin-1 action on osteoblasts, endothelial cells, chondrocytes, synovocytes, vascular smooth muscle, and mesangial cells results in msPLA₂ production (reviewed in Refs. 17⁻²⁰). After the identification of several types of PLA₂ receptors using svPLA₂ (8⁻¹¹), it has been shown that the expression of msPLA₂ also has a receptor of ~ 190⁻²⁰⁰ kDa (15) probably similar to the receptor of 180 kDa initially identified in rabbit skeletal muscle with svPLA₂ (9). This particular receptor is thought to be involved in the proliferative effect of pancreatic PLA₂ on fibroblasts (15), as well as on PLA₂-induced contraction of lung smooth muscle (22). To enhance our understanding of the structure and function of this high molecular weight PLA₂ receptor type, the cDNA encoding the PLA₂ receptor previously purified from rabbit skeletal muscle (molecular mass of 180 kDa) has now been cloned and expressed. The sequence of the PLA₂ receptor is homologous to the recently determined sequence of the macrophage mannose receptor, a membrane protein involved in the endocytosis of glycoproteins and pathogenic microorganisms (23). Very interestingly, the expressed receptor recognizes svPLA₂ and both types of msPLA₂ (group I and II; see Refs. 3 and 16⁻²⁰) as well as some glycosylated ligands that bind to the mannose receptor.

EXPERIMENTAL PROCEDURES

Materials—Oxynur sexatellus secatellus toxin (OS, and OS₂) were purified as described previously (5). Pancreatic porcine PLA₂, group I, N-glycosidase F, and O-glycosidase were purchased from Boehringer Mannheim. Human non-pancreatic PLA₂, group II was a generous gift from Dr. N. D. Jones (Eli Lilly Co., Indianapolis, IN). It is a recombinant protein expressed in Syrian hamster AV12 cells. The protein was > 99% single component by reverse phase high performance liquid chromatography, showed a single band on polyacrylamide gel electrophoresis, and was a single protein within experimental error by amino acid and N-terminal analysis. Bovine serum albumin (BSA, catalog no. A7665), D(+)-glucose (G8270), a-L(-)-fucose (A2102), D-glucosamine (A8625), D(+)-glucosamine-BSA (cat no. A7665), D(+)-glucose (G8270), D(-)-fucose (A2102), and N-acetylgalactosamine-BSA (A8625) were purchased from Sigma.

Receptor Purification and Sequencing—The 180-kDa PLA₂ receptor was purified from rabbit skeletal muscle cells as previously described (9), except that elution from the OS₂ affinity column was done in the presence of 0.8% N-acetyl-D-glucosamine, 50 mm citrate-Na⁺, pH 2.5, and 8 µM urea. The N-terminal sequence was determined by Edman degradation after electroblotting of the purified receptor (~ 200 pmol) onto Problott membranes (Applied Biosystems) or dialysis through a microspin cartridge (Applied Biosystems). Tryptic peptides were obtained from ~ 300 pmol of purified protein according to the described procedure (24, 25) with minor modifications. Cyanogen bromide peptides were prepared with the probe design peptide separation system (Promega). Purified receptor (~ 1 nmol) was digested with cyanogen bromide in solution and then treated according to the manufacturer's protocol.

Isolation of cDNA Clones for the PLA₂ Receptor—Degenerate oligonucleotide primers encoding the 5' and 3' parts of amino acid sequences of peptides C₁, C₂, and C₃ shown in Fig. 1A were synthesized in both

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‡ To whom correspondence should be addressed. Tel.: 33-93-35-77-00; Fax: 33-93-96-77-04.

The abbreviations used are: svPLA₂, snake venom phospholipase A₂; PLA₂, phospholipase A₂; msPLA₂, mammalian secretory phospholipase A₂; OS₁, O. scutellatus scutellatus toxin 1; OS₂, O. scutellatus scutellatus toxin 2; CRD, carbohydrate recognition domain; BSA, bovine serum albumin; PCR, polymerase chain reaction; bp, base pair(s); kb, kilobase pair(s).
**FIG. 1.** Rabbit PL₂ receptor protein. **A,** alignment of the amino acid sequence of the PL₂ receptor with the human mannose receptor (23). Peptide sequences derived from purified receptor preparations are overlined and numbered according to the order in which they were isolated (C₁ to C₁₆ correspond to peptides obtained after cyanogen bromide cleavage and T₁ to T₁₆ to tryptic peptides). The signal peptide segment has the characteristic features of a cleavable signal sequence, including a hydrophobic region (13 residues) and a small (9 residues) polar C-terminal region with the expected most probable site of signal peptide cleavage after the tripeptide Ala-Ala-Ala (40). This prediction is supported by the N-terminal amino acid sequence obtained from the purified protein. *, potential sites of N-linked glycosylation; Δ, consensus site for casein kinase II phosphorylation. Comparison of these putative sites of N-glycosylation with the peptide sequences obtained revealed that at least asparagine residues 1121 and 1311 are likely to be glycosylated. R, putative structural model of the PL₂ receptor. The amino acid lengths of each domain are indicated in parentheses. Potential sugar attachment sites to asparagine (Y), tyrosine residue 1435, and potential site of regulation by casein kinase II (Δ) are indicated.

**RESULTS AND DISCUSSION**

This paper reports the cloning and expression of the 180-kDa PL₂ receptor that was previously purified from rabbit skeletal muscle cells (9). This receptor binds very tightly two PL₂s (OS₁ and OS₂) isolated from the Taipan snake *O. scutellatus* (8). Partial sequences were obtained from this receptor and were then used for cDNA cloning. The open reading frame of the longest cloned cDNA sequence encodes a protein of 1458 amino acids (Fig. 1), with a predicted molecular mass of 167 kDa. Hydrophobicity analysis reveals only one major hydrophobic segment (23 residues, positions 1394–1418), which is probably the unique transmembrane anchor of the receptor. The PL₂ receptor appears as having more than 95% of the molecule exposed to the extracellular space (Fig. 1). The purified receptor is a glycoprotein (9) and indeed 15 potential sites of N-glycosylation are present in the extracellular domain (Fig. 1).

The PL₂ receptor has a significant homology (overall 29%) with the macrophage mannose receptor (Fig. 1A), a protein implicated in the endocytosis of glycoproteins bearing terminal mannose, fucose, N-acetylglucosamine, or glucose residues (23) which might also mediate the phagocytosis of yeast and other pathogenic microorganisms (27). Both receptors present the same general structural features (Fig. 1) including a N-terminal cysteine-rich domain (24% identity), a fibronectin-like type I domain (43% identity), eight repeats of carbohydrate recognition domains (CRDs, 24–38% identity), a unique transmembrane glycosylation domain (CRDs, 24–38% identity), a unique transmembrane glycosylation domain (CRDs, 24–38% identity).
brane domain (42% identity), and an intracellular C-terminal domain (17% identity).

Except for its similarity with the mannose receptor (Fig. 1A), the N-terminal cysteine-rich domain displays no significant homology with other known protein sequences. The fibronectin-like type II domain is found not only in the mannose receptor, but also in fibronectin, collagenase, coagulation factor XII precursor, cation-independent mannose 6-phosphate receptor, and seminal plasma protein BSP-A3.

The PLA2 receptor contains eight domains consisting of 110-130 amino acids that are related to CRDs of C-type lectins (Fig. 2). These domains contain 21-29 residues out of the 37 residues of the consensus pattern for CRDs (28).

The C-terminal cytoplasmic tail of the receptor situated after the unique transmembrane domain (residues 1394-1416) has no significant homology with any other known protein sequence. However, this intracellular domain contains Qr-1435, which corresponds to Tyr-1429 in the mannose receptor sequence. This residue has been shown to be important for internalization of the mannose receptor (29). The C-terminal cytoplasmic domain contains a consensus site for casein kinase II phosphorylation (30) at Ser-1452, suggesting a possible role in regulation (Fig. 1).

Northern blot analysis shows that the PLA2 receptor is encoded by a major 5.3-kb mRNA and a minor 7.7-kb form (Fig. 3). The mRNA encoding the PLA2 receptor is found in lung, skeletal muscle, brain, heart, and kidney (Fig. 3). Highest levels of transcripts are expressed at embryonic stages. These results are entirely consistent with the previously observed distribution of this PLA2 receptor measured by binding experiments with 125I-OS2 (9, 10).

Expression of the PLA2 receptor cDNA in transfected cells is presented in Fig. 4. These cells express the PLA2 receptor at a high level (Fig. 4A) with a maximal binding activity of 0.4-1.2 pmol/mg protein, and a Kd value of 7.5 ± 2 pm, n = 5 for 125I-OS2. This Kd value is identical with the Kd value initially measured on muscle membranes (9). Competition experiments show that the expressed receptor, like the native receptor, has a high affinity for OS2 (Kd = 11 ± 3 pm, n = 3) and for OS1 (Kd = 19.5 ± 6 pm, n = 3) but it does not interact with bee venom PLA2 (Fig. 4B). The cloned receptor, like the native receptor present in skeletal muscle membranes (not shown), binds group I PLA2 from porcine pancreas with a Kd value of 10 ± 3 nm (n = 3) and group II human secreted PLA2 (from platelets and synovial fluid) with a Kd value of 0.8 ± 0.2 nm (n = 3). Cross-linking experiments on transfected cell membranes indicate that the expressed receptor has a molecu-
lar mass of 180 kDa (Fig. 4C) as the cross-linked native skeletal muscle PLA₂ receptor (9).

The PLA₂ receptor recognizes some of the glycosylated ligands that have previously been used to identify the mannos receptor (23, 31). Conjugates of bovine serum albumin (BSA) with mannos and N-acetylglucosamine were found to inhibit 125I-OS₂ binding to the PLA₂ receptor with K₀ values of -34 nm (Fig. 4D) similar to those found for the mannos receptor (31). Conversely, the BSA-galactose derivative inhibits 125I-OS₂ binding (K₀ = 5 nm, Fig. 4D) although it does not bind to the mannos receptor (31). Invertase, mannan, d-mannose, d-glucose, l-fucose, and N-acetylglucosamine, which bind to the mannos receptor (31), are without effect on 125I-OS₂ binding (not shown).

The structural domain of the PLA₂ receptor to which PLA₂,₀₅ₕ bind is presently unknown. Neither OS₁, OS₂, group I pancreatic PLA₂, nor group II mSPA₂₈s are glycosylated. No N-glycosylation site was found in the known sequences of OS₁, OS₂, and pancreatic PLA₂ (32). No sugar could be determined in OS₁ and OS₂ by the very sensitive technique of Kamerling et al. (33). No change in PLA₂ mobility and no change in affinity for the PLA₂ receptor was observed after N-glycosidase F and O-glycosidase treatments of OS₁ and OS₂. Therefore, elements of recognition of the receptor are clearly within the PLA₂ protein sequences. Since structures of numerous PLA₂₈s are available (34–39), site-directed mutagenesis of PLA₂ genes should rapidly provide information on that point. Site-directed mutagenesis will also be required to determine which part of the PLA₂ receptor is involved in PLA₂ recognition.

There are two subtypes of mSPA₂₈ (3, 16–20). The typical group I PLA₂ is from pancreatic origin, and the typical type II PLA₂ is found in platelets and synovial fluid. One particularly interesting aspect of this work is that mSPA₂₈s recognize the cloned receptor. The determination of the PLA₂ receptor structure should help determine the signaling pathway of PLA₂ after they bind to their receptors on target cells. It will also probably suggest new therapeutic directions against inflammation.

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