Multifunctional Activity of the Extracellular Domain of the M-Type (180 kDa) Membrane Receptor for Secretory Phospholipases A_2[†]

Philippe Ancian, Gérard Lambeau, and Michel Lazdunski*

Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France

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ABSTRACT: M-type (180 kDa) receptors for secretory phospholipases A_2 ($sPLA_2$s) are thought to mediate some of the physiological effects of group I $sPLA_2$, including smooth muscle contraction and cell proliferation. The M-type $sPLA_2$ receptor is a large glycoprotein composed of several distinct extracellular domains which belongs to the C-type lectin superfamily. This receptor binds with high affinity both pancreatic group I and inflammatory group II $sPLA_2$s as well as various $sPLA_2$s purified from snake venoms. This paper shows that the rabbit M-type $sPLA_2$ receptor is a multifunctional protein which is able to promote cell adhesion on type I and IV collagens most probably via its N-terminal fibronectin-like type II domain. It also shows that binding of $sPLA_2$s to a recombinant soluble form of this receptor is associated with a noncompetitive inhibition of phospholipase A_2 activity.

Secretory phospholipases A_2 ($sPLA_2$s)[1] are structurally related enzymes which catalyze the hydrolysis of the acyl−ester bond at the *sn*-2 position of the glycerophospholipids (Dennis, 1983). These enzymes are found in mammals as well as in insect and snake venoms (Waite, 1987; Mayer & Marshall, 1993; Dennis, 1994).

Snake venom PLA_2s ($svPLA_2$s) produce neurotoxic, myotoxic, anticoagulant, and proinflammatory effects (Kini & Evans, 1989; Hawgood & Bon, 1991). This diversity of effects seems to be linked to the existence of very high affinity receptors for $svPLA_2$s that have been previously identified in brain (Lambeau et al., 1989) as well as in other tissues (Lambeau et al., 1990, 1991a,b).

Two main types of mammalian $sPLA_2$s have been extensively studied. The inflammatory group II $sPLA_2$ is considered as a potent mediator of the inflammatory process. It is found in plasma and synovial fluids of patients with various inflammatory diseases (Vadas & Pruzanski, 1986; Mukherjee et al., 1992; Kudo et al., 1993; Pruzanski et al., 1993) and has been proposed to play a key role in the pathogenesis of these diseases. This enzyme is up-regulated by proinflammatory cytokines like interleukin-1, interleukin-6, and tumor necrosis factor (Vadas & Pruzanski, 1986; Mukherjee et al., 1992; Kudo et al., 1993; Pruzanski et al., 1993) and is implicated in the production of potent lipid mediators of inflammation (Barbour & Dennis, 1993; Murakami et al., 1993; Suga et al., 1993; Fourcade et al., 1995). The pancreatic-type $sPLA_2$ has been first implicated in the digestion of dietary phospholipids (De Haas et al., 1968) and more recently in cell proliferation (Arita et al., 1991) and smooth muscle contraction (Nakajima et al., 1992; Sommers et al., 1992). These latter effects are thought to be linked to the existence of high affinity receptors with molecular masses of 180−200 kDa (M-type $sPLA_2$ receptors) which have been characterized in rabbit (Lambeau et al., 1994), rat (Lambeau et al., 1991a; Hanasaki & Arita, 1992), bovine (Ishizaki et al., 1994), and human tissues (Ancian et al., 1995). The rabbit M-type $sPLA_2$ receptor binds with a high affinity both the porcine pancreatic and the human inflammatory group II $sPLA_2$s, as well as OS_1 and OS_2, two $svPLA_2$s purified from the venom of the Taipan snake *Oxyuranus scutellatus scutellatus* (Lambeau et al., 1994). Structure−function relationship studies with the porcine pancreatic $sPLA_2$ have identified the $sPLA_2$s residues which are crucially involved in the binding to the rabbit M-type $sPLA_2$ receptor (Lambeau et al., 1995).

Molecular cloning of M-type $sPLA_2$ receptors (Lambeau et al., 1994; Ancian et al., 1995) has revealed that they share a common structural organization with the macrophage mannose receptor, a transmembrane C-type lectin involved in the endocytosis of glycoproteins and the phagocytosis of pathogenic microorganisms bearing mannose residues on their surface (Ezekowitz et al., 1990; Taylor et al., 1990). The extracellular domain of the M-type $sPLA_2$ receptor is large (≈1400 residues) and composed of several distinct domains, including a N-terminal cystein-rich domain, a fibronectin-like type II domain, and eight carbohydrate recognition domains (CRDs) in tandem, followed by a single transmembrane segment and a short cytoplasmic tail. A transcript encoding for a soluble M-type $sPLA_2$ receptor lacking the transmembrane segment and the cytoplasmic tail has been identified in human kidney (Ancian et al., 1995).

This paper shows that the rabbit M-type $sPLA_2$ receptor is a multifunctional protein with an extracellular domain which is able to promote cellular adhesion on type I and type IV collagens and to inhibit the $sPLA_2$ catalytic activity upon binding.

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* To whom correspondence should be addressed at the Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, Sophia Antipolis, 06560 Valbonne, France. Telephone: 33 93 95 77 02 or 00. Fax: 33 93 95 77 04. E-mail: douy@unice.fr.

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[1] Abbreviations: PLA_2, phospholipase A_2; $sPLA_2$, secretory phospholipase A_2; $svPLA_2$, snake venom phospholipase A_2; OS_1, toxin 1 from *Oxyuranus scutellatus scutellatus*; OS_2, toxin 2 from *Oxyuranus scutellatus scutellatus*; CRD, carbohydrate recognition domain.

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MATERIALS AND METHODS

Materials. *O. scutellatus scutellatus* toxins (OS_1 and OS_2) were purified as previously described (Lambeau et al., 1989). The porcine pancreatic $sPLA_2$ (group I) was purchased from Boehringer Mannheim. The human inflammatory $sPLA_2$ (group II) was a generous gift from Dr. Ruth Kramer (Eli Lilly Co., Indianapolis, IN). It is a recombinant protein expressed in Syrian Hamster AV 12 cells. Human type I and type IV collagens (catalog no. C7774 and C7521, respectively) were purchased from Sigma.

Construction of Rabbit M-type $sPLA_2$ Receptor Mutants. Mutants of the rabbit M-type $sPLA_2$ receptor were obtained by site-directed mutagenesis according to the methyl-dCTP method (Vandeyar et al., 1988). For this purpose, the cDNA encoding for the rabbit M-type $sPLA_2$ receptor was shortened by removing 713 bp in the 3′ noncoding region, between two *Sex*A1 sites located at positions 4556 and 5269 (Lambeau et al., 1994), and subcloned into the expression vector pRc/CMV (Invitrogen). Single-stranded DNA was produced using R408 helper phage in *Escherichia coli* JM 101 cells. Deletions were obtained by the gapped duplex method (Kramer et al., 1984) with 40-mer oligonucleotides mutagenic primers. ΔNF is a modified form of the rabbit M-type $sPLA_2$ receptor which lacks the N-terminal cystein-rich domain as well as the fibronectin-like type II domain [residues 1−218 (Lambeau et al., 1994)]. ΔNF was obtained with the oligonucleotide 5′-GGGCAGAATCCCCACTTCTCCCACT-TGAGGACCCGCTCGG-3′. ΔTSM is a form of the receptor which lacks the transmembrane segment [residues 1394−1416 (Lambeau et al., 1994)] and then corresponds to a soluble rabbit M-type $sPLA_2$ receptor. It was constructed using the oligonucleotide 5′-GCAAGTCTCCTGAAGAATC-CGTGAATATCTGCCTTCATTT-3′.

Stable Expression in 293 Cells. cDNAs encoding for wild-type and mutated forms of the rabbit M-type $sPLA_2$ receptor were stably transfected into 293 cells (American Type Cell Collection) by the $CaPO_4$ procedure (Graham & Van der Erb, 1973). Fifty percent confluent cells were transfected with 20 μg of DNA/75 cm^2 Petri dish on day 1. On day 2, the cells were trypsinized and replated. Transfected cells were selected in a medium supplemented with 1 mg/mL G418. Individual resistant colonies were selected and assayed for [^{125}I]OS_1 binding as previously described (Lambeau et al., 1990). All the binding experiments were performed in a buffer containing 140 mM NaCl, 50 mM Tris, pH 7.4, 1 mM $CaCl_2$, and 0.1% BSA. For binding experiments on the soluble recombinant rabbit receptor, the serum-free medium from transfected cells was centrifuged at 10000*g* for 10 min. Aliquots of the supernatant were assayed for [^{125}I]OS_1 binding as described above and filtered through Whatman GF/F glass-fiber filters. The concentration of the soluble M-type $sPLA_2$ receptor has been determined with [^{125}I]OS_1 as a ligand. For the inhibition of the $sPLA_2$ activities, serum-free medium containing the soluble M-type $sPLA_2$ receptor was concentrated by centrifugation using Centricon 30 (Amicon). Mock-transfected cells were obtained by transfection with the parent vector pRC/CMV.

Immunoblotting. Transfected 293 cells were rinsed three times with PBS, scraped in 20 mM Tris-HCl, pH 7.5, and 2 mM EDTA and sonicated. Cell homogenates and serum-free extracellular medium were analyzed by SDS−PAGE in 7.5% acrylamide gels. Protein samples were transferred to nitrocellulose (Hybond C-extra, Amersham). Nitrocellulose sheets were blocked with 3% nonfat drymilk in PBS and incubated with guinea pig polyclonal antibodies raised against the purified whole rabbit M-type $sPLA_2$ receptor (working dilution 1:5000), followed by peroxidase-conjugated goat anti-guinea pig IgG (Cappel Research Products; working dilution 1:10000). After extensive washings in PBS containing 0.1% Tween 20, blots were revealed with the BM chemiluminescence western blotting reagent (Boehringer Mannheim) and exposed to X-OMAT AR films (Eastman Kodak Co.).

Cell Adhesion Assays. 96-well plates were coated for 1 h at 37 °C with laminin, type I collagens from rat tail or from human placenta, or type IV collagen from human placenta at 20 μg/mL in PBS. Wells were washed twice with PBS and blocked for 1 h at 37 °C with 0.1% BSA in PBS. Wells were washed again twice with PBS. Stably transfected 293 cells expressing the wild-type or the mutant ΔNF rabbit M-type $sPLA_2$ receptor were harvested by mild trypsinization in the presence of EDTA, centrifuged, and resuspended in serum-free medium containing 0.1% BSA. Immunoblotting experiments have shown that the M-type $sPLA_2$ receptor was not affected by the mild trypsinization procedure (not shown). To measure inhibition of the attachment, cells were resuspended in serum-free medium containing 100 nM recombinant soluble M-type $sPLA_2$ receptor or the equivalent amount of serum-free medium of mock-transfected cells. A total of 5×10^4 cells were added to each well and allowed to attach during 15 min at 37 °C. Nonadherent cells were removed by gentle washing once with 200 μL of PBS. Adherent cells were stained overnight with PBS containing 1% toluidine blue and 3% paraformaldehyde. After extensive washings with PBS, stained cells were dissolved in 50 μL of 2 M, HCl and the optical density of each well was measured at 570 nm. A linear relationship was observed between the optical density and the number of fixed stained cells. This relationship was identical for each cellular clone.

$sPLA_2$ Activity. $sPLA_2$ activity was measured by the hydrolysis of autoclaved [^{3}H]oleate-labeled *E. coli* membranes as substrate (Franson et al., 1974). Briefly, 1 mL of a overnight culture of *E. coli* XL_1 strain (Stratagene Inc.) was incubated for 4 h at 37 °C in 75 mL of LB medium containing 500 μCi of [^{3}H]oleic acid (NEN). Cells were pelleted for 10 min at 3000*g*, resuspended in 50 mL of LB medium, and allowed to chase for 30 min at 37 °C. After centrifugation, the cell pellet was washed in 1 mL of 0.1 M Tris, pH 8, and 1% free fatty acid BSA, centrifuged, resuspended in 2 mL of 140 mM NaCl and 20 mM Tris, pH 7.4, and autoclaved.

The reaction mixture (100 μL) contained 140 mM NaCl, 50 mM Tris, pH 7.4, 1 mM $CaCl_2$, 0.1% BSA, various amounts of [^{3}H]oleate-labeled *E. coli* membranes, and $sPLA_2$s as described in the legends to figures. Reaction mixtures were incubated for 2 min at 25 °C and then stopped by adding 300 μL of a solution containing 0.1 M EDTA, pH 8, and 1% free fatty acid BSA. After centrifugation at 10000*g* for 3 min, aliquots of supernatants containing hydrolyzed phospholipids were counted.

For the inhibition of catalytic activity, $sPLA_2$s were preincubated for 90 min at 25 °C with various concentrations of the recombinant soluble rabbit M-type $sPLA_2$ receptor, after which substrate was added in a small volume (10 μL) and $sPLA_2$ activity measured as above.

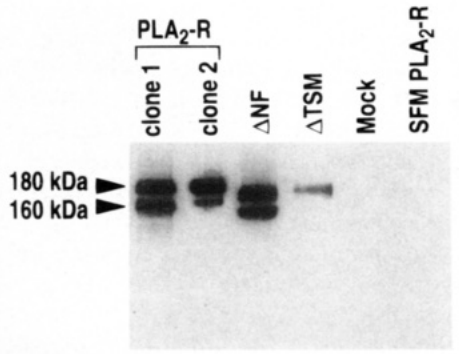

FIGURE 1: Immunoblot of native and truncated rabbit M-type $sPLA_2$ receptors. Protein samples were separated on a 7.5% acrylamide gel, electroblotted, and subjected to an immunolabeling using an anti-rabbit M-type $sPLA_2$ receptor guinea pig antiserum. PLA_2-R clone 1 and clone 2: membranes of cell stably transfected with the wild-type rabbit M-type $sPLA_2$ receptor (see text). ΔNF: membranes of cell stably transfected with a truncated rabbit M-type $sPLA_2$ receptor lacking the N-terminal cystein-rich domain as well as the fibronectin-like type II domain. ΔTSM: serum-free medium of cells stably transfected with a truncated soluble receptor lacking the transmembrane segment. Mock: Membranes of mock-transfected cells. SFM PLA_2-R: serum-free medium of PLA_2-R clone 2.

To ensure that all kinetics measurements were conducted under initial rate conditions, time course studies were carried out using [^{3}H]oleate-labeled *E. coli* as substrate. Using human group II $sPLA_2$ at a final concentration of 0.3 nM, results indicate that phospholipids hydrolysis was linear through 2 min at 25 °C and that the reaction velocity became constant after a lag period of 20 s. Hydrolysis after 2 min was less than 10% of the total phospholipids. Similar results were obtained using OS_2 at 5 nM and the porcine pancreatic and the bee venom $sPLA_2$s at 0.5 nM (not shown).

RESULTS AND DISCUSSION

One of the characteristic features of the M-type $sPLA_2$ receptor is the presence in the N-terminal extracellular region of a fibronectin-like type II domain. This domain is also found in other proteins such as fibronectin (Skorstengaard et al., 1986), the bovine seminal protein PDC-109 (Esch et al., 1983), and the type IV collagenase (Collier et al., 1988). In all these proteins, this domain has been shown to contain collagen-binding determinants (Banya et al., 1990; Banya & Patthy, 1991; Skorstengaard et al., 1994). In order to check whether the fibronectin-like type II domain of the M-type $sPLA_2$ receptor can fulfill the same function, we produced stable transfectants expressing the native full-length M-type $sPLA_2$ receptor or a truncated receptor lacking the N-terminal cystein-rich and the fibronectin-like type II domains (called ΔNF). Western-blot analysis using guinea pig anti-rabbit M-type $sPLA_2$ receptor antiserum revealed that one major band of 180 kDa and one minor band of 160 kDa were labeled in the membranes of cells stably transfected with the full-length cDNA (PLA_2-R clones 1 and 2, Figure 1). Two bands of 170 and 150 kDa were labeled in membranes of cells stably transfected with the truncated cDNA (ΔNF, Figure 1). All these bands were specifically labeled since no signal was obtained with nonimmune serum antibodies (not shown). The nature of the 160 and 150 kDa bands is presently unknown. One possibility is that they correspond to incompletely processed M-type $sPLA_2$ receptors, with different degrees of glycosylation. Further experiments are needed to address this question. PLA_2-R clones 1 and 2 expressed the full-length rabbit M-type $sPLA_2$ receptor with maximum binding capacity (B_{max}) values of 0.16 and 7 pmol/mg of protein, respectively, for [^{125}I]OS_1 (a specific ligand of the M-type $sPLA_2$ receptor) (not shown). The truncated M-type $sPLA_2$ receptor retained the same binding properties as the full-length receptor (not shown). The ΔNF clone expressed the mutated receptor with a B_{max} value for [^{125}I]OS_1 of 1.1 pmol/mg of protein (not shown). Figure 2 shows that cells expressing the full-length M-type $sPLA_2$ receptor attach to collagen while mock-transfected cells and ΔNF do not. Adhesion to collagens seems to be related to the expression level of the M-type $sPLA_2$ receptor as the PLA_2-R clone 2 which expresses higher levels of the M-type $sPLA_2$ receptor (a higher B_{max} value) than PLA_2-R clone 1 attaches better on the different types of collagen. Cellular attachment is specific to type I and type IV collagens. A negative control was made on plates precoated with laminin. On this substrate, no cells were seen to adhere after 15 min.

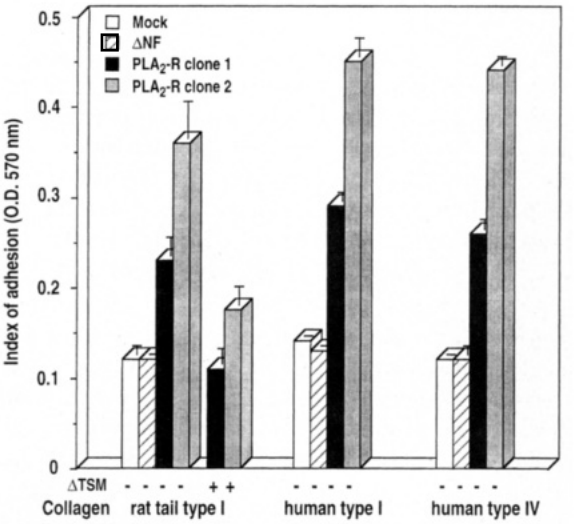

FIGURE 2: Adhesion of cell clones expressing the native or a truncated M-type $sPLA_2$ receptor. A total of 5×10^4 cells were seeded in 96-well plates precoated with various collagens at 20 μg/mL in PBS. Mock shows the attachment of mock-transfected 293 cells, and ΔNF represents the adhesion of a 293 cell clone expressing a truncated rabbit M-type $sPLA_2$ receptor lacking the N-terminal cystein-rich domain as well as the fibronectin-like type II domain with a B_{max} value of 1.1 pmol/mg of protein for [^{125}I]OS_1. PLA_2-R clone 1 and clone 2 show the adhesion of 293 cell clones expressing the full-length receptor with B_{max} values of 0.16 and 7 pmol/mg of protein for [^{125}I]OS_1, respectively. Signs − and + on the bottom of the figure indicate the absence or the presence of 100 nM of the soluble M-type $sPLA_2$ receptor in the incubation medium. Each measure is the mean ± SEM for $n = 3$.

The inhibition of adhesion by a recombinant soluble M-type $sPLA_2$ receptor has been assayed to ensure that the attachment of cells to collagen was mediated by the M-type $sPLA_2$ receptor. In order to produce this soluble form of the M-type $sPLA_2$ receptor, a mutant cDNA which lacks the sequence encoding for the transmembrane segment has been constructed by site-directed mutagenesis. 293 cells have been stably transfected with this modified cDNA, and one clone (called ΔTSM) has been isolated after G418 selection. Western-blot analysis using guinea pig anti-rabbit M-type $sPLA_2$ receptor antiserum revealed the presence of a 180 kDa band in the supernatant of ΔTSM (Figure 1). No band was detected in the medium of mock-transfected cells

or in the medium of cells stably transfected with the cDNA encoding for the transmembrane form of the rabbit M-type $sPLA_2$ receptor [mock and SFM PLA_2-R, respectively (Figure 1)]. When PLA_2-R clones 1 and 2 were incubated with 100 nM ΔTSM on plates precoated with collagen, cellular attachment was drastically reduced (Figure 2), to a level comparable to that of the mock-transfected cells. No inhibition was observed when PLA_2-R clones 1 and 2 were incubated with serum-free medium of mock-transfected cells (not shown). All these data taken together clearly indicate that the N-terminal region of the M-type $sPLA_2$ receptor has adhesive properties to type I and IV collagens. The functional significance of these properties in relation to the physiological role of the M-type $sPLA_2$ receptor still has to be determined. Of the two domains which have been deleted in the ΔNF mutant, the fibronectin-like type II domain is most probably directly involved in cellular adhesion to collagen since fibronectin-like domains in other proteins are known to bind to collagens (Banya et al., 1990; Banya & Patthy, 1991; Skorstengaard et al., 1994).

One of the key questions related to the M-type $sPLA_2$ receptor is to know whether it alters $sPLA_2$ activity. This problem has been solved using the cell clone expressing the truncated soluble ΔTSM receptor. This clone expressed the soluble M-type $sPLA_2$ receptor at a very high level [1.2 pmol/10^6 cells/24 h, measured with [^{125}I]OS_1 as a ligand (not shown)]. The soluble receptor accumulated in the medium until 10 days (not shown). Saturation curves with [^{125}I]OS_1 as a ligand revealed that the recombinant soluble M-type $sPLA_2$ receptor recognizes this $svPLA_2$ with a K_d value of 40 pM (Figure 3A). Competition experiments showed that OS_2 inhibits [^{125}I]OS_1 binding to the soluble M-type $sPLA_2$ receptor with a $K_{0.5}$ value of 30 pM (Figure 3B) and that the porcine pancreatic and the human group II $sPLA_2$s inhibit [^{125}I]OS_1 binding with $K_{0.5}$ values of 30 and 3 nM, respectively (Figure 3B). Conversely, the bee venom $sPLA_2$ is without effect on [^{125}I]OS_1 binding (Figure 3B). These $K_{0.5}$ values are very similar to those measured for the wild-type transmembrane rabbit M-type $sPLA_2$ receptor (Lambeau et al., 1994).

Figure 4 shows the inhibitory effects of the recombinant soluble M-type $sPLA_2$ receptor on the enzymatic activities of several $sPLA_2$s. Figure 4A shows that OS_2 associates stoechiometrically with the soluble M-type $sPLA_2$ receptor. Activities of the human group II and porcine pancreatic $sPLA_2$s are inhibited with IC_{50} values of 2.1 and 14.4 nM, respectively (Figure 4B). The bee venom $sPLA_2$, which does not bind to the rabbit M-type $sPLA_2$ receptor, is not inhibited by this receptor protein (Figure 4B).

Figure 4C shows double-reciprocal plots of kinetic data for the hydrolysis of [^{3}H]oleate-labeled *E. coli* by the human group II $sPLA_2$ at different soluble M-type $sPLA_2$ receptor concentrations. Lineweaver–Burk analysis reveals that the soluble M-type $sPLA_2$ receptor behaves as a noncompetitive inhibitor (V_{max} is changed, K_m is unchanged) of the human group II $sPLA_2$ with a calculated K_i value of 1.4 nM.

The M-type receptor for $sPLA_2$s contains eight CRDs in tandem, and site-directed mutagenesis experiments have recently shown that $sPLA_2$s bind to the M-type receptor via its CRDs (article in preparation). The fact that the M-type receptor is inhibitory for $sPLA_2$s is to be put in parallel with the recent identification in snake plasma (Inoue et al., 1991; Ohkura et al., 1993) as well as in mammals (Fisher et al., 1994) of several proteins which also behave as noncompetitive inhibitors of $sPLA_2$ activity and which comprise a CRD-like domain. The inhibitory action of the M-type $sPLA_2$ receptor is not really surprising since residues that have been previously shown to be essential for binding of the pancreatic $sPLA_2$ to M-type $sPLA_2$ receptors such as Gly-30 and Asp-49 (Lambeau et al., 1995) are also essential for the catalytic activity of the enzyme (Verheij et al., 1980).

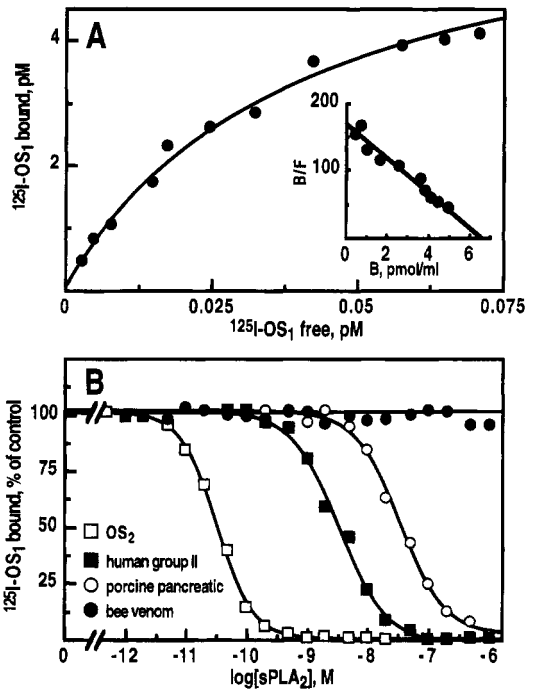

FIGURE 3: Expression of the recombinant soluble rabbit M-type $sPLA_2$ receptor in stably transfected 293 cells. (A) Equilibrium binding of [^{125}I]OS_1 to serum-free medium of cells stably transfected with a mutated rabbit M-type $sPLA_2$ receptor lacking the transmembrane segment (ΔTSM). (Main panel) Saturation curve with [^{125}I]OS_1 obtained by difference between total and nonspecific binding measured in the presence of 30 nM unlabeled OS_1. (Inset) Scatchard plot analysis of the specific binding. (B) Competition experiments with [^{125}I]OS_1 (10 pM) and different unlabeled $sPLA_2$s for the binding to the recombinant soluble rabbit M-type $sPLA_2$ receptor. Results are expressed as percentage of the maximal specific binding measured in the absence of competitor. 100% corresponded to 2 pM [^{125}I]OS_1 specifically bound. Nonspecific binding was measured in the presence of 30 nM unlabeled OS_1 and represented 20% of the total binding. No binding activity was detected either with the medium of mock-transfected cells or with the medium of cells transfected with the membrane-anchored receptor cDNA.

One of the functions of the $sPLA_2$ receptor is then clearly to neutralize the enzymatic activity of its ligand. Whether this property is an essential component of the transduction system associated with the $sPLA_2$/M-type receptor interaction is not known. On the other hand, soluble forms of the human $sPLA_2$ receptor have been identified (Ancian et al., 1995). Since there has been over the last years a long search of inhibitors of type II $sPLA_2$ with the idea that they could be used in inflammation and against rheumatoid arthritis (Wilkerson, 1990; Gelb et al., 1994), the production of recombinant forms of these receptors might then lead to useful therapeutic applications. Similarly, the use of the soluble forms of receptors for cytokines has been proposed as a possible way to prevent their action in several diseases (Rose-John & Heinrich, 1994).

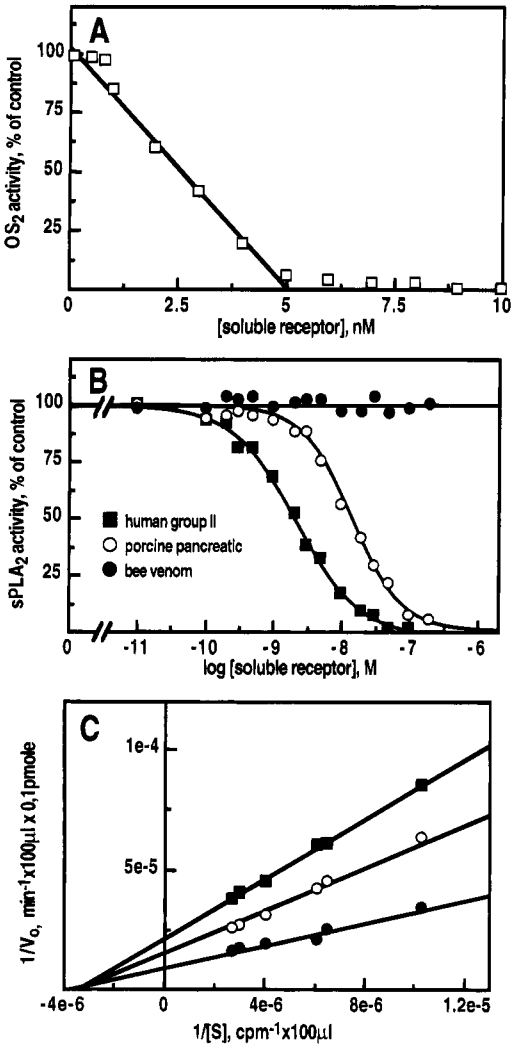

FIGURE 4: Effect of varying concentrations of the soluble rabbit M-type $sPLA_2$ receptor on the activity of different $sPLA_2$s. (A) Stoechiometric inhibition of OS_2 enzymatic activity by the soluble rabbit M-type $sPLA_2$ receptor. OS_2 (5 nM) was preincubated during 45 min with increasing concentrations of the soluble rabbit M-type $sPLA_2$ receptor after which [^{3}H]oleic acid labeled *E. coli* membranes were added in a negligible volume. (B) Inhibition of the enzymatic activities of different $sPLA_2$s. The porcine pancreatic, the bee venom $sPLA_2$s (0.5 nM), and the human group II $sPLA_2$ (0.3 nM) were preincubated for 90 min at 25 °C with increasing concentrations of the soluble rabbit M-type $sPLA_2$ receptor after which [^{3}H]oleic acid labeled *E. coli* membranes were added in a negligible volume. 100% corresponds to the activity of the different $sPLA_2$s measured without inhibitor. 0% represents the nonspecific hydrolysis of labeled phospholipids measured without $sPLA_2$s and represented less than 5% of the total phospholipids. Serum-free medium from mock-transfected cells was without effect on the porcine pancreatic and the human group II $sPLA_2$ activities (not shown). (C) Double-reciprocal analysis of the human group II $sPLA_2$ activity. Hydrolysis of [^{3}H]oleic acid labeled *E. coli* phospholipids was measured with the human group II $sPLA_2$ at 0.3 nM in the absence (●) or in the presence of 1 (○) or 2 nM (■) soluble M-type $sPLA_2$ receptor. The K_i value for this inhibition was found to be 1.4 nM. V_m and K_m values without inhibitor for this $sPLA_2$ were 1.14×10^5 cpm^{-1} min^{-1} (0.1 pmol)$^{-1}$ and 2.7×10^5 cpm/100 μL, respectively.

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