Endocytic Properties of the M-type 180-kDa Receptor for Secretory Phospholipases A\(_2\)*

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Endocytic properties of the M-type 180-kDa receptor for secretory phospholipases A\(_2\) (sPLA\(_2\)) were first investigated in rabbit myocytes that express it at high levels. Internalization of the receptor was shown to be clathrin-mediated, rapid (\(k_0 = 0.1\) min\(^{-1}\)), and ligand-independent. The signal sequence for internalization was then identified upon transient and stable expression of various receptor constructs with mutated cytoplasmic sequences. Analysis of the internalization efficiency of the mutants suggested that the NSMY motif encodes the major endocytic signal, with the distal tyrosine residue playing the key role. A chimeric protein composed of the extracellular and transmembrane domains of the rabbit sPLA\(_2\) receptor and of the cytoplasmic domain of the structurally homologous human macrophage mannose receptor retained the high affinity for sPLA\(_2\) and was internalization competent, exhibiting 50% endocytic activity of the M-type sPLA\(_2\) receptor. The results indicate the compatibility of the structural domains of the two parent proteins and provide evidence for the interchangeable character of their internalization signals.

Secretory phospholipases A\(_2\) (sPLA\(_2\)s)† are implicated in a number of crucial physiological and pathological processes in mammals (for recent reviews, see Refs. 1–3). The pancreatic-type sPLA\(_2\) (group I), besides its well-known digestive function, has been implicated in fertilization (4), cell proliferation (5), and contraction of vascular and airway smooth muscles (6, 7). Elevated levels of the inflammatory-type sPLA\(_2\) (group II) in serum, bronchial lavage, and synovial fluids are associated with propagation of inflammation and hypersensibilization of the organism (for a recent review, see Ref. 8).

Different types of membrane receptors for sPLA\(_2\)s have been recently identified using various types of venom sPLA\(_2\)s (9–12). The M-type receptor is a 180-kDa protein that was first identified in rabbit muscle cells in culture (10). It binds some venom sPLA\(_2\)s like OS1, one of the sPLA\(_2\)s from the Taipan snake venom with a \(K_d\) value of 38 pM, but it does not bind bee venom sPLA\(_2\) (10). The N-type receptor, which is abundant in the brain (9) and is present in most tissues (12), does not bind OS1, but it associates very tightly with bee venom sPLA\(_2\) (\(K_d = 100\) pM). It is made of polypeptide units of about 40–50 and 85 kDa (9). The M-type receptor binds the pancreatic-type sPLA\(_2\) as well as the inflammatory-type sPLA\(_2\) with \(K_d\) values of about 1–10 nM (13, 14). These sPLA\(_2\)s are probably the normal endogenous ligands of the M-type 180 kDa receptor. Experiments with pancreatic-type sPLA\(_2\) suggested some of its effects, i.e. induction of DNA synthesis and cell proliferation (5) contraction (6), cell migration (15), and eicosanoid production (16) to be mediated by the M-type receptor. However, the intracellular signal transduction mechanisms linking these multiple effects to the event of ligand-receptor interaction still remain obscure.

The high molecular weight M-type sPLA\(_2\) receptors were cloned from rabbit (13), bovine (17), mouse (18), and human (19) tissues. It is a single subunit, type I plasma membrane glycoprotein of 180–200 kDa. The molecule is made of a large highly glycosylated extracellular N-terminal portion, comprising a N-terminal cystein-rich region, a fibronectin-like type II domain, and a repeat of eight carbohydrate-recognition domains in tandem followed by a single transmembrane domain and a short intracellular C-terminal tail. The overall molecular organization of the M-type receptor is similar to that of the macrophage mannose receptor that mediates the uptake of mannose-glycosylated ligands and the phagocytosis of parasitic microorganisms (20, 21) and to the recently discovered DEC-205 membrane protein, which is implicated in antigen processing (22). The structural similarity is especially pronounced in the extracellular and membrane-spanning protein portions, while the C-terminal cytoplasmic domains seem to be more specific for each protein.

The mannose receptor and DEC-205 protein are known to be internalized with high efficiency (22–24). Endocytosis is intimately linked to their function as integral parts of the defense and clearance systems. Internalization of the M-type sPLA\(_2\) receptor has also been observed (19, 25), and it could play an important role in receptor function. The purpose of this work is to analyze in details the endocytic properties of the M-type sPLA\(_2\) receptor by determining its rate of internalization, the utilized endocytic mode (ligand-induced or constitutive), as well as the structural signals promoting the endocytic process.

**EXPERIMENTAL PROCEDURES**

*Materials—OS1, one of the *Oxyuranus scutellatus* venom sPLA\(_2\), was prepared and radiactively labeled using Na\(^{125}\)I (Amersham Corp.) as described previously (10). The plasmid construct containing the full-length human macrophage mannose receptor cDNA was a kind gift of Dr. R. A. B. Ezekowitz (Harvard Medical School, Boston, MA). Guinea pig anti-sPLA\(_2\) receptor antibodies were raised against the purified rabbit M-type sPLA\(_2\) receptor (10). Porcine pancreatic PLA\(_2\), was from Boehringer Mannheim. Oligonucleotide primers were from Genosys Biotechnologies, Inc., Cambridge, United Kingdom. Hybrid nitrocellulose (pore size, 0.45 µm) was from Amersham Corp.

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The abbreviations used are: sPLA\(_2\), secretory phospholipase A\(_2\); OS\(_1\), toxin 1 from *O. scutellatus* scutellatus.
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**Table I**

Synthetic oligonucleotides used for mutagenesis

Mutated nucleotides are in boldface. a) and b), coding and anti-sense site-directed mutagenesis.

| Mutant | Mutagenic oligonucleotide |
|--------|---------------------------|
| TR2    | 5'-GAAATCCCTTGTGTACTGATAATAGG |
| TR11   | 5'-CAACCTTTAAGTCTCTTG |
| 16-22  | 5'-GTATGATGTAAGTCTCTCC |
| S17P   | 5'-GATTACCTAGACTAAAGGATAGG |
| S18A   | 5'-GTTGTACACGTCAGAATTTG |
| S19A   | 5'-GTTGTAAGACCTGAAATTTC |
| P20A   | 5'-GTTGGTGTGTCGTAAGAAG |
| L34G   | 5'-GATGAGAATATTTTCAGTG |
| S36E   | 5'-CAACCCC |
| PLA2/2MAN | a) 5'-CAATCAGCACAGTTCCTCCTACATATAACAGAAGAGACTG |
| PLA2/2MAN | b) 5'-CTGTGGCCTTCTTTGGCTTCATTATTTATTAGATGACCGAGTG |

**RESULTS**

**Endocytic Properties of the Endogenously Expressed M-type sPLA2 Receptor**—The expression level and internalization properties of the endogenous M-type sPLA2 receptor were assessed on the primary culture of confluent rabbit skeletal muscle cells. The radioactively labeled ligand used in binding and internalization experiments was a snake venom sPLA2, O S1 (10). The maximal binding capacity value ($B_{max}$) is estimated on total cell lysates was 0.35 pmol/mg of protein, while the number of binding sites expressed on the cell surface was 0.12 pmol/mg of protein (data not shown). The $K_d$ value for the OS1-receptor complex was 30–50 pM as reported previously (10).

Fig. 1 shows the results of a Western blotting of the rabbit skeletal muscle cells membrane proteins and the indirect immunofluorescence microscopy of cells using anti-sPLA2 receptor antibodies. In the blots, the antibodies recognized a single protein band corresponding to a molecular mass of about 180 kDa. This value is in a good agreement with earlier estimations of the molecular mass of the mature highly glycosylated M-type sPLA2 receptor (10, 11). In immunofluorescence experiments, the antibodies revealed an intensive cell-surface staining typical for protein expressed at the plasma membrane.

Fig. 24 shows the kinetics of $^{125}$I-Os internalization during the first 15 min after ligand addition. The internalization rate constant ($k_i$) was 0.09 ± 0.005 min$^{-1}$ ($n = 5$). Exposure of cells to a hypertonic medium causes disassembly of membrane-associated clathrin lattices and, thus, abolishes internalization mediated by clathrin-coated pits (33). Pretreatment of rabbit skeletal muscle cells with a hypertonic sucrose-containing med-
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FIG. 1. Expression of the M-type sPLA₂ receptor in rabbit skeletal muscle cells. A. Western blotting analysis of the endogenously expressed M-type sPLA₂ receptor. The membrane protein fractions were prepared from confluent primary cultures of rabbit skeletal muscle cells. Aliquots (50 μg of total protein) were subjected to SDS-PAGE (6.5% polyacrylamide) followed by Western blotting using guinea pig anti-PLA₂ receptor antibodies (lane 1) or preimmune serum (lane 2). The position of the molecular weight standards is shown at left. B, indirect immunofluorescence staining for the M-type sPLA₂ receptor in cultured rabbit skeletal muscle cells. Confluent cells on coverslips were fixed with 4% paraformaldehyde and then treated with anti-sPLA₂ receptor antibodies and then fluorescein isothiocyanate-conjugated goat anti-guinea pig IgG. Immunocomplexes were visualized by fluorescence microscopy at a 400-fold magnification.

FIG. 2. Internalization properties of the M-type sPLA₂ receptor in rabbit skeletal muscle cells. A, kinetics of 125I-OS₁ internalization. Cells kept in the normal (open circles) or hypertonic sucrose-containing (closed circles) medium were exposed to 125I-OS₁ (60 pm) for 3–15 min at 37 °C. The quantities of surface-bound and internalized ligand were determined as described under “Experimental Procedures.” The results are mean values of three independent experiments, each performed in quadruplicate. B, saturation plots for specific cell-surface 125I-OS₁ binding at 4 °C in control (closed triangles) and monensin-treated (open triangles) rabbit skeletal muscle cells. Cells were pre-treated with 10 μM monensin and processed as described under “Experimental Procedures.” All points were done in duplicate. Nonspecific binding was subtracted from all experimental values. Protein concentrations were determined on total cellular lysates.

The endocytosis of the sPLA₂ receptor occurs via clathrin-coated pits. The effect of the long term exposure to the ligand was investigated on cells pretreated for 30 min, 1, 2, and 24 h with 100 nM porcine pancreatic sPLA₂ (Kᵣ = 10 nM) (13). Following preincubation, the cells were chilled on ice, washed with ligand-free medium, and subjected to binding analysis using 125I-OS₁ (Kᵣ = 38 pm) (10) to evaluate the number of remaining sPLA₂ binding sites. The large difference in affinity of these respective ligands for the M-type receptor ensured the quantitative substitution of the pancreatic enzyme from the cell surface exposed ligand-receptor complex by OS₁. At all time points tested, the number of cell-surface ligand binding sites in the sPLA₂-pretreated samples was 0.1–0.13 pmol/mg of total protein, essentially the same as in controls (data not shown). The results, thus, argue against receptor sequestration and/or down-regulation upon prolonged exposure to the pancreatic enzyme.

The possibility of a sequential uptake and recycling of the unoccupied M-type sPLA₂ receptor was analyzed by comparing the quantity of the cell-surface PLAX₂ binding sites in controls and in cells pretreated with a carboxylic ionophore monensin, an inhibitor of trans-Golgi endosomal trafficking (34). Fig. 2B shows that monensin-pretreatment caused a substantial, up to 57%, decrease in the number of M-type sPLA₂ receptor molecules exposed at the cell surface. The affinity for the ligand and the B₅₀ values estimated on total cell lysates were unaffected by the inhibitor (not shown), ruling out any nonspecific inhibitory or modifying effect of monensin on the sPLA₂ binding.

Mapping of the Encoded Internalization Signals—The signals governing membrane receptor internalization are typically located in the cytoplasmic portions of proteins. The 42-amino acid sequence of the cytoplasmically exposed C-terminal tail of the rabbit M-type sPLA₂ receptor is presented in Fig. 3. Visual inspection of the sequence and analysis of its secondary structure predicted by Chou-Fasman algorithm outlined three peptide regions as potential internalization signals. The first region, NKGF (residues 4–8 in the cytoplasmic tail) is predicted to form a helix-parallel β-turn conformation and resembles the QQQFF internalization motif of the epidermal growth factor receptor (35). The second region, NSYY (residues 16–19) is highly conserved in M-type sPLA₂ receptors from different mammalian species (13, 17–19). The identical amino acid residues are shaded. The cytoplasmic sequences of the rabbit, human, and bovine M-type sPLA₂ receptors are given in full. * the cytoplasmic portion of the mouse receptor has 15 additional C-terminal residues that are not shown.
rabbit, and mouse (Fig. 3D) and were then assumed to be the most plausible candidates for directing internalization.

Three mutants were first designed for a preliminary mapping of endocytic signals (Fig. 3A). The mutant TR2 lacks all but two residues of the cytoplasmic portion. The extramembrane dipeptide was left in the sequence to ensure the anchoring of the expressed protein in the lipid bilayer. In the mutant TR11, the carboxyl-terminal truncation was introduced three amino acid residues downstream from the putative internalization signal NKGFF. The deletion mutant D16–22 lacks the peptide sequence NSYYPTT of the receptor tail.

Wild-type and mutant M-type sPLA2 receptors were transiently expressed in COS cells. Cell surface expression of each construct was analyzed by indirect immunofluorescence microscopy (Fig. 4). All constructs exhibited an evenly dispersed pattern of staining with high fluorescence intensity at cell borders characteristic for a plasma membrane-delivered protein. Western blotting analysis of membrane proteins (Fig. 5A) revealed similar levels of receptor expression for all constructs. The receptors were recognized by antibodies as single protein bands of the expected electrophoretic mobilities (apparent molecular mass, 170–180 kDa). No sign for proteolytic degradation of the expressed mutants was detected. The expression level of receptor constructs (Bmax) estimated on total cell lysates was typically 1–2 pmol/mg of protein, while control COS cells had no OS1 binding sites (not shown). The affinity of 125I-OS1 for the different mutant receptors was essentially the same as that of the wild-type receptor (Kd = 40 ± 10 pM) and of the endogenous receptor in rabbit skeletal muscle cells. Taken together, all of these results indicate that deletions in the receptor cytoplasmic domain do not affect the plasma membrane delivery and the binding properties of the M-type sPLA2 receptor.

Fig. 5B represents a diagram of specific internalization rate constants (ke) for the wild-type and mutant M-type sPLA2 receptors. The 125I-OS1 internalization by the wild-type receptor was 0.02 ± 0.003 min⁻¹. The Δ16–22 mutant internalized the ligand with a 33% efficiency as compared with the wild-type receptor (ke = 0.006 ± 0.002 min⁻¹). The TR11 and TR2 receptor mutants were essentially internalization deficient (ke = 0.001 ± 0.001 min⁻¹ and 0.0003 ± 0.0002 min⁻¹, respectively). The significantly lowered internalization rate constant of the Δ16–22 mutant indicated receptor endocytosis to be mainly dependent on the deleted NSYYPTT sequence.

A series of point amino acid substitutions in the putative signaling region was then introduced to define more precisely the determinants required for efficient M-type sPLA2 receptor endocytosis. The list of residues mutated in the region
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NSYYPPTT is shown in Fig. 3B. Western blotting and immunofluorescence analyses revealed that all mutants were expressed, processed, and delivered to the plasma membrane (not shown). The affinity of all the mutants for $^{125}$I-OS$_1$ was essentially the same as that of the wild-type receptor. A diagrammatic representation of internalization rate constants for the wild-type and mutant receptors is shown in Fig. 6. Substitution of any of the two tyrosine residues in the NSYYPPTT sequence by an alanine (mutants Y18A and Y19A) dramatically impaired receptor internalization. Internalization rate constants ($k_e$) were determined as described under “Experimental Procedures.” The results are the mean values of three independent experiments, each performed in triplicate.

M-type sPLA$_2$ receptor. Internalization experiments were performed on two independent cell clones for each receptor construct, expressing 0.5–2 pmol of M-type receptor/mg of total protein. Fig. 7 shows a diagram of internalization rate constants of the wild-type and mutant receptors in HEK 293 cells. The full-length receptor internalized $^{125}$I-OS$_1$ as efficiently ($k_e = 0.067 \pm 0.003 \text{ min}^{-1}$) as in rabbit skeletal muscle cells. The rate constant of the $\Delta 16$–22 mutant was relatively high ($k_e = 0.03 \pm 0.005 \text{ min}^{-1}$), representing 45% of the wild-type receptor efficiency. The TR11 mutant was internalization deficient ($k_e = 0.01 \pm 0.002 \text{ min}^{-1}$). All these experiments confirmed the results obtained in the corresponding studies with COS cells, although the latter have significantly lower internalization efficiency.

Mutational Analysis of the Putative Casein Kinase II Phosphorylation Site—The cytoplasmic portion of the receptor contains a consensus sequence for casein kinase II phosphorylation, with Ser-36 as a possible phosphorylation target. To investigate the possible implications of the phosphorylation-dephosphorylation process in the regulation of the receptor endocytic activity, two mutants with point amino acid substitutions at Ser-36 were prepared (Fig. 3C) and transiently expressed in COS cells. Substitution of Ser-36 by an alanine (S36A) was expected to mimic a dephosphorylated state of the receptor. Mutation of Ser-36 to an aspartate (S36D mutant) was made to mimic a putative phosphorylated state. The internalization rate constants of these receptor mutants ($k_e = 0.022 \pm 0.002$ and $0.019 \pm 0.003 \text{ min}^{-1}$, respectively; Fig. 6B) were not significantly different from that of the wild-type receptor ($k_e = 0.02 \text{ min}^{-1}$) arguing against an implication of Ser-36 in endocytosis.

Internalization Activity of a M-type sPLA$_2$/Mannose Receptor Chimera—The chimeric cDNA construct was prepared by substitution of the nucleotide sequence coding for the entire cytoplasmic domain of the rabbit M-type sPLA$_2$ receptor by the
corresponding region of the human macrophage mannose receptor (Fig. 8A). The junction site was at the YK peptide sequence located immediately downstream the putative intramembrane segments of both receptors. The chimera transiently expressed in COS cells did not show any structural abnormalities. Indirect immunofluorescence analysis with anti-sPLA₂ receptor antibodies revealed that it was normally delivered to the plasma membrane (not shown). Moreover, its affinity for 125I-OS₁ was essentially the same as that of the wild-type sPLA₂ receptor (Kᵦ = 30 ± 5 pM). 125I-OS₁ internalization driven by the chimera was about 50% less efficient (kₑ = 0.01 ± 0.003 min⁻¹) than that of the wild-type sPLA₂ receptor (Fig. 8B).

DISCUSSION

This paper describes the endocytic properties of the M-type sPLA₂ receptor and characterizes its structurally encoded internalization signal(s). The endocytic receptors studied to date can be subdivided into two main groups. Receptors of the first group recycle constitutively and function as shuttles delivering their ligands to the cell interior. Their internalization is independent on ligand binding. It can be rather fast, i.e. 10–60%/min (corresponding to a kₑ of 0.1–0.6 min⁻¹) for the LDL, asialoglycoprotein (38), or transferrin receptors (30) or slow, i.e. 1–2%/min for the Fc and fibronectin receptors (37, 39). Internalization of the other group of receptors (such as hormone and growth factor receptors) is clearly ligand-dependent. It is negligible in the absence of their ligands and can be increased more than 50-fold (kₑ of 0.3–0.6 min⁻¹) in the presence of corresponding ligands (40, 41). In many cell types, a long term exposure of this latter group of receptors to their ligands leads to rapid intracellular sequestration and degradation (down-regulation) of the receptors.

The calculated internalization rate constant of the M-type sPLA₂ receptor in a primary culture of rabbit skeletal muscle cells (kₑ = 0.1 min⁻¹) was that of efficiently internalized receptors but was significantly lower than that reported for the structurally homologous mannose receptor (kₑ = 4.12 min⁻¹) in sinusoidal endothelial cells from rat liver (24). The blockade of 125I-OS₁ uptake observed under hypertonic conditions, which causes a disassembly of plasma membrane-associated clathrin lattices (33), proved receptor internalization via clathrin-coated pits (Fig. 2A). Long term exposure to a high concentration of a possible endogenous ligand, pancreatic-type sPLA₂, had no effect on the number of the cell surface-exposed 125I-OS₁ binding sites arguing against ligand-induced sequestration and/or down-regulation of the sPLA₂ receptor in rabbit skeletal muscle cells. In the absence of the added ligand, monensin, a classical inhibitor of the trans-Golgi endosomal trafficking (31, 34), produced a large, more than 57% reduction in the number of cell surface OS₁-binding sites, indicating the rapid uptake and recycling of the ligand-free sPLA₂ receptor. Similar extent of the monensin-induced protein trapping within the cells was reported earlier for the constitutively recycling transferrin receptor (34). Taken together, the above results suggest that internalization of the M-type sPLA₂ receptor in rabbit skeletal muscle cells is rapid, clathrin-coated pit-mediated, and ligand-independent. These properties allowed us to classify the M-type sPLA₂ receptor to the group of constitutively recycling receptors, the same as for its structural homologue, the mannose receptor (20, 21, 23). The classification of the M-type receptor into this group is in contrast with previously suggested role of the sPLA₂ receptor in cell proliferation (5) and eicosanoid production (16). Indeed, such biological effects are generally supposed to be mediated by receptors of the other group, i.e. by ligand-activated receptors. This apparent inconsistency is presently difficult to explain. Noteworthy, the mannose receptor, which also belongs to the group of constitutively recycling receptors, was shown to be involved in cell proliferation (42).

Internalization of the membrane receptors is dependent on their cytoplasmic signal sequences selectively recognized by adaptor proteins of clathrin-coated pits (for a recent review, see Ref. 37). The internalization signals identified to date fall into three main groups. The first group is represented by four to six-amino acid sequences containing an aromatic residue, usually a tyrosine, in a tight β-turn structure (43–47). This type of signal is found in the vast majority of endocytic receptors, a typical example being a consensus NPXY motif (36), first detected in the structure of the LDL receptor (43). The dileucine (or leucine-isoleucine) dipeptide (48–53) and the recently identified KKXXF/KXF sequence (54) represent two other groups of internalization signals. Importantly, analogous motives were shown to be involved in other intracellular sorting events, such as lysosomal targeting (48, 55–59), transcytosis, and basolateral sorting in polarized cells (53, 60–62), and in the endoplasmic reticulum protein retrieval (54, 63, 64).

The cytoplasmic portion of the rabbit sPLA₂ receptor contains three stretches of potential signal sequences (Fig. 3): (i) the tight turn-forming region NSYY; (ii) the LI dipeptide in the distal part of the tail; and (iii) the juxtamembrane NKGF motif, being poorly conserved in mammalian sPLA₂ receptors (Fig. 3D). A mutant (25% of the wild-type rate) suggested that in the signal sequence motif of the rabbit M-type sPLA₂ receptor, the amino acid residue occupying the consensus X position can be a subject for certain structural restraints. Substitution of Ser-17 by a proline was made to reconstitute the canonical NPXY motif.
The mutation increased internalization, but only slightly (25%), indicating that both serine and proline residues are permissible at this position. Indeed, both residues are known to be turn-promoting (66). However, the M-type sPLA2 receptor is to our knowledge the first reported example of an endocytic receptor in which this substitution has naturally occurred. Alanine substitution of the highly conserved Pro-20 immediately adjacent to the critical tyrosine residues (Fig. 3D) was of no significant effect on endocytosis, suggesting that this residue is not important for receptor internalization.

Interestingly, the internalization of the Δ16–22 mutant, although significantly impaired, was still clathrin-coated pit-mediated, and considerably higher than that of the C-terminally truncated mutants TR11 and TR2 (Fig. 5). Substitution of Leu-34 by a glycine (ΔL34G) abolished internalization and proved the endocytosis of the Δ16–22 mutant to be dependent on the LI motif (residues 34–35). In the full-length receptor, however, the same glycine substitution had no effect on internalization (R/L34G mutant, Fig. 6). These results suggest that the LI motif, although recognized as an internalization signal in the receptor mutant with the deleted NSYY region, does not play the same role in the context of the full-length sPLA2 receptor. The data on the presence of “cryptic” internalization (sorting) signals in the cytoplasmic portions of the proteins are not unprecedented. Analogous findings were made for the lysosomal acid phosphatase, the interleukin 6 signal transducer gp130, and the cation-independent mannose 6-phosphate/insulin-like growth factor II receptor (51, 57, 67).

The negatively charged region surrounding the LI motif of the sPLA2 receptor is highly conserved from mouse to human (Fig. 3D) and contains a consensus casein kinase II phosphorylation site, with Ser-36 as a putative target residue (13). Similar structures were found in the cytoplasmic domains of many recycling receptors (68) and are supposed to be implicated in intracellular sorting events, such as basolateral transport (60), sorting into Golgi-derived clathrin-coated pits (57), and/or to regulate the efficiency of receptor internalization (69). Amino acid substitutions at Ser-36 mimicking putative phosphorylated and dephosphorylated sPLA2 receptor states (Fig. 6B) were of no significant effect on the rate of endocytosis, arguing against implication of the region in internalization of the M-type sPLA2 receptor.

The M-type sPLA2 receptor and the macrophage mannose receptor are predicted to share the same structural organization (13), although the amino acid sequence identity of these two receptors is quite low (28% identity in the extracellular region and only 17% in the cytoplasmic tail). The cytoplasmic NTLY motif of the macrophage mannose receptor resembles the NSYY signal sequence of the rabbit M-type sPLA2 receptor and was previously shown to be implicated in internalization (65). It was, thus, interesting to analyze the internalization properties of a chimeric composed of the predicted extracellular and transmembrane domains of the rabbit M-type sPLA2 receptor and of the cytoplasmic portion of the human macrophage mannose receptor. The chimeric receptor was properly delivered to the plasma membrane and displayed the normal binding affinity for 125I-OS1, proving the correct assignment of the topological domains. The internalization efficiency of the chimeric receptor was only 50% less efficient than that of the wild-type sPLA2 receptor, suggesting that the cytoplasmic domains and the encoded internalization signals of these two proteins are interchangeable.

In conclusion, this work has revealed the mechanism of endocytosis of the M-type sPLA2 receptor and has identified the structural regions implicated in its internalization. The role of the M-type sPLA2 receptor internalization process remains, however, obscure. Internalization of the M-type sPLA2 receptor could first be an important component of the signal transduction system coupled to the binding of the sPLA2 to its specific receptor. It could serve to terminate the signals produced by sPLA2 on target cells. Alternatively, the endosomal vesicles could serve as a vehicle delivering sPLA2 to specific intracellular compartments, where the ligand, before being degraded in lysosomes, could manifest its enzymatic activity. Another possibility is that the internalization of the M-type sPLA2 receptor could serve a clearance function selectively withdrawing sPLA2 from the extracellular fluid. This could be of a crucial importance at various inflammatory disease states (such as rheumatoid arthritis, acute peritonitis, septic shock) when high levels of inflammatory type II sPLA2 are produced and secreted (8). Further investigations on the properties of the M-type sPLA2 receptor in various tissues and species should provide more evidence on its function and the role of internalization process, in particular.

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REFERENCES

1. Glaser, K. B., Mohilo, D., Chang, J. Y., and Senko, N. (1993) Trends Pharmacol. Sci. 14, 92–98
2. Dennis, E. A. (1994) J. Biol. Chem. 269, 13057–13060
3. Mukherjee, A. B., Miele, L., and Pattabiraman, N. (1994) Biochem. Pharmacol. 48, 1–10
4. Fry, M. R., Ghosh, S. S., East, J. M., and Franson, R. C. (1992) J. Biol. Reprod. 47, 751–759
5. Arita, H., Hanasaki, K., Nakano, T., Oka, S., Teraoka, H., and Matsumoto, K. (1991) J. Biol. Chem. 266, 19139–19141
6. Nakajima, M., Hanasaki, K., Ueda, M., and Arita, H. (1992) FEBS Lett. 309, 261–265
7. Sommers, C. D., Bobbitt, J. L., Beimis, K. G., and Snyder, D. W. (1992) Eur. J. Pharmacol. 216, 87–96
8. Vadas, P., Browning, J., Edelson, J., and Pruzan, W. (1993) J. Lipid Med. 8, 1–30
9. Lambeau, G., Barhanin, J., Schweitz, H., Qar, J., and Lazdunski, M. (1995) J. Biol. Chem. 270, 2655–2664
10. Lambeau, G., Barhanin, J., and Lazdunski, M. (1994) FEBS Lett. 339, 21–25
11. Lambeau, G., Barhanin, J., and Lazdunski, M. (1994) J. Biol. Chem. 269, 1785–1794
12. Lambeau, G., Lazdunski, M., and Barhanin, J. (1990) J. Biol. Chem. 265, 9526–9532
13. Ishizaki, J., Hanasaki, K., Higashino, K., Kishino, J., Kikuchi, N., Ohara, O., and Nussenzweig, M. C. (1995) J. Exp. Med. 181, 1195–1201
14. Lopata, M. A., Cleveland, D. W., and Sollner-Webb, B. (1984) J. Biol. Chem. 259, 1–10
15. Stein, B. S., and Sussman, H. H. (1986) J. Biol. Chem. 261, 10319–10331
16. Zvaritch, E., Vellani, F., Guerin, D., and Carafoli, R. (1995) J. Biol. Chem. 270, 2679–2688
17. Hansen, S. H., Sandvig, K., and Deurs, B. V. (1993) J. Cell Biol. 121, 61–72
18. Stein, B. S., Bensch, K. G., and Sussman, H. H. (1984) J. Biol. Chem. 259, 14763–14772
19. Chang, C. P., Lazar, C. S., Walsh, B. J., Kumuro, M., Collawn, J. F., Kuhn, L. A., Tainer, J. A., Trowbridge, I. S., Farquhar, M. G., Rosenfeld, M. G., and Diczfalusy, E. (1992) J. Biol. Chem. 267, 8963–8970
20. Stein, W. J., Goldstein, J. L., and Brown, M. S. (1990) J. Biol. Chem. 265, 3116–3123
21. Trowbridge, I. S., Collawn, J. F., and Hopkins, C. R. (1993) Annu. Rev. Cell Biol. 7, 129–161
22. Sharma, R. J., and Grant, D. A. (1986) Biochem. Biophys. Acta 862, 199–204
23. Almond, B. D., and Eide, L. (1994) J. Biol. Chem. 269, 26655–26641
24. Loh, K. F., Opresko, L. K., Starkov, C. C., Walsh, B. J., and Wiely, H. S. (1990) J. Biol. Chem. 265, 15713–15723
25. Carpenter, J. L., and Paceaud, J. P. (1994) Ann. N. Y. Acad. Sci. 733, 266–278
26. Lew, B. C., Sung-Mee, P., Bostw, S. E., Stahl, D. F., and Rattazzi, M. C. (1994) J. Biol. Chem. 266, 4658–4669
27. Davis, C. G., van Driel, I. R., Russell, D. W., Brown, M. S., and Goldstein, J. L. (1987) J. Biol. Chem. 262, 4075–4082
28. Flakimoto, H., Huber, C., Klaus, W., Schmidt, B., von Figura, K., and Peters, C. (1991) Cell 67, 1203–1209
29. Bansal, A., and Giersch, L. M. (1991) Cell 67, 1195–1201
30. Backer, J. M., Shoelson, S. E., Weiss, M. A., Hua, Q. X., Chester, R., Haring, C. H., and White, M. F. (1992) J. Cell Biol. 118, 811–839
31. Collawn, J. F., Stangel, M., Kuhn, L. A., Esekogu, V., Jing, S. Q., Trowbridge, I. S., and Tainer, J. A. (1990) Cell 63, 1061–1072
32. Letourneau, F., and Klauser, R. D. (1992) Cell 69, 1143–1157
33. Haft, C. R., Klauser, R. D., and Taylor, S. I. (1994) J. Biol. Chem. 269, 26286–26294
34. Civera, S., Chawla, A., Chakrabarti, R., Joly, M., Buxton, J., and Czech, M. P. (1994) J. Cell Biol. 126, 979–989
35. Dittrich, E., Rose-John, S., Gerhardt, C., Mullberg, J., Stoyan, T., Yasukawa, K., Heinrich, P. C., and Greaves, L. (1994) J. Biol. Chem. 269, 19114–19120
36. Auker, C., Konner, J., Landau, R. N., Lenburg, M. E., and Treso, D. (1994) Cell 76, 853–864
37. Hunziker, W., and Fumeau, C. (1994) EMBO J. 13, 2963–2967
38. Ein, C., Kappeler, F., Linstedt, A. D., and Hauri, H.-P. (1995) EMBO J. 14, 2250–2256
39. Williams, M. A., and Fukuda, M. (1990) J. Biol. Chem. 265, 129–161
40. Matter, K., Hunziker, W., and Sellmann, I. (1992) Cell 71, 741–753
41. Geffen, I., Fuhrer, C., Leitung, B., Weiss, M., Huggett, K., Griffiths, G., and Spiess, M. (1993) J. Biol. Chem. 268, 20772–20777
42. Eisen, M. B., Yamamoto, E. M., and Sellmann, I. (1994) J. Cell Biol. 126, 991–1004
43. Schindler, R., Ibin, C., Zerial, M., Lottespiech, F., and Hauri, H.-P. (1993) Eur. J. Cell Biol. 61, 1–9
44. Mallababrena, A., Jimenez, M. A., and Rico, M. (1994) J. Biol. Chem. 269, 6622–6625
45. Matter, K., Hunziker, W., and Sellmann, I. (1992) Cell 71, 741–753
46. Hauri, H.-P., and Sellmann, I. (1994) J. Cell Biol. 126, 1–39
47. Ktistakis, N. T., Thomas, D., and Roth, M. G. (1990) J. Cell Biol. 111, 1393–1407
48. Lehmann, R., Eberle, W., Krull, S., Prill, V., Schmidt, B., Sander, C., von Figura, K., and Peters, C. (1992) EMBO J. 11, 4391–4399
49. Goldstein, J. L., Brown, M. S., Anderson, R. W., Russell, D. W., and Schneider, W. J. (1985) Annu. Rev. Cell Biol. 1, 1–38
50. Okamoto, C. T., Sven, W., Bossel, M., and Mustov, K. E. (1994) J. Biol. Chem. 269, 15676–15682