Sortilin/Neurotensin Receptor-3 Binds and Mediates Degradation of Lipoprotein Lipase*

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Lipoprotein lipase and the receptor-associated protein (RAP) bind to overlapping sites on the low density lipoprotein receptor-related protein/α2-macroglobulin receptor (LRP). We have investigated if lipoprotein lipase interacts with the RAP binding but structurally distinct receptor sortilin/neurotensin receptor-3. We show, by chemical cross-linking and surface plasmon resonance analysis, that soluble sortilin binds lipoprotein lipase with an affinity similar to that of LRP. The binding was inhibited by heparin and RAP and by the newly discovered sortilin ligand neurotensin. In 125I-labeled 3T3-L1 adipocytes treated with the cross-linker dithiobis(succinimidyl propionate), lipoprotein lipase-containing complexes were isolated by anti-sortilin antibodies. To elucidate function in cells, sortilin-negative type cells. The degradation was inhibited by unlabeled lipoprotein lipase, indicating a saturable pathway, and by RAP and heparin. Moreover, inhibition by the weak base chloroquine suggested that degradation occurs in an acidic vesicle compartment. The results demonstrate that sortilin is a multifunctional receptor that binds lipoprotein lipase and, when expressed on the cell surface, mediates its endocytosis and degradation.

Lipoprotein lipase (LpL) is rate-limiting in hydrolysis of triglycerides in circulating lipoproteins, and reduced levels or activity of the active dimeric species therefore lead to increased triglyceride levels. Even small changes in LpL function in man may have important consequences. Thus, common mutations in the LpL gene with marginal effects on the activity are may have important consequences. Thus, common mutations in the LpL gene with marginal effects on the activity are

The mechanisms for synthesis, folding, and secretion of LpL to the luminal side of the vascular epithelium are not known in detail. A large part of the synthesized LpL is normally degraded within the cell (4, 5), and trimming of sugar residues from oligosaccharide chains of LpL appears necessary for proper folding and expression of LpL activity (6). Moreover, dimerization is important for regulation of catalytic activity (7). At the endothelium, LpL is bound to heparan sulfate proteoglycans. LpL easily dissociates from these sites (8) and reassociates to other sites, including receptors that mediate its uptake into cells. Clearance occurs via multifunctional endocytic receptors of the LDL receptor family, notably LDL receptor-related protein/α2-macroglobulin receptor (LRP) (9, 10), aided by accumulation of LpL on cell surface proteoglycans (10, 11) as well as directly via transmembrane proteoglycans (12–15).

Sortilin is a ~95-kDa type-I receptor first isolated from human brain (16) and recently shown to be identical with the neurotensin receptor-3 (17). It consists of a luminal domain homologous to yeast vacuolar protein-sorting 10 protein, a single transmembrane domain, and a cytoplasmic tail with a C terminus strongly homologous to that of the mannose 6-phosphate/insulin-like growth factor-II (IGF-II) receptor (16). In mature sortilin, an N-terminal 44-residue propeptide has been cleaved off, and recent results show that furin-mediated propeptide cleavage conditions sortilin for ligand binding (18). Besides in neurons, sortilin is abundant in several cell types including skeletal muscle, heart, and adipocytes (16, 19). Although sortilin, like the IGF-II receptor, is mainly located in the Golgi compartment and vesicles (16, 17), it is also expressed on the cell surface. In differentiated 3T3-L1 adipocytes, sortilin colocalizes with the IGF-II receptor, and insulin causes a ~2-fold increase in the expression of both receptors on the plasma membrane (19). Thus, like the IGF-II receptor, sortilin has the potential of functioning both as a receptor like the Golgi compartment and as a clearance receptor on the cell surface.

Mature sortilin binds the receptor-associated protein, RAP (16), a specialized chaperone that interacts with LDL receptor family members, notably the multifunctional receptors LRP, megalin, and the very low density lipoprotein receptor (for reviews, see Refs. 20–22). RAP consists of three homologous domains of which domain D3 binds to sortilin (23). We have previously shown that LpL and segments of RAP containing D3 cross-compete for binding to LRP, and these ligands are therefore thought to bind to the same or overlapping sites on the receptor (24). As an approach to elucidate the function of sortilin as a putative endocytic and sorting receptor, we therefore investigated whether sortilin binds LpL. We show that the soluble luminal domain of mature sortilin (s-sortilin) binds LpL with an affinity comparable with that of LRP and that the binding is inhibited by RAP and by neurotensin. Moreover, LpL is associated with sortilin in 3T3 adipocytes and is specifically

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§The abbreviations used are: LpL, bovine lipoprotein lipase (dimeric); LDL, low density lipoprotein; LRP, LDL receptor-related protein/α2-macroglobulin receptor; IGF-II, insulin-like growth factor-II; PAG, polycrylamide gel electrophoresis; RAP, receptor-associated protein; s-sortilin, soluble sortilin/neurotensin receptor-3; CHO, Chinese hamster ovary; CMV, cytomegalovirus; RU, response units; D23, domains 2 and 3.
degraded in stably transfected CHO cells that express mature full-length sortilin on the cell surface. We propose that sortilin, in addition to performing not yet elucidated sorting functions in the Golgi compartment, scavenges a diverse set of extracellular ligands such as LpL and neurotensin.

MATERIALS AND METHODS

Sortilin, LpL, and RAP—The extracellular domain of mature sortilin (s-sortilin), comprising residues 45–725 of full-length sortilin, was expressed in stably transfected Chinese hamster ovary (CHO-K1) cells and purified from the culture medium by RAP affinity chromatography as described (23). The propeptide (residues 1–44) was expressed in Escherichia coli BL21(DE3) cells as a glutathione S-transferase fusion protein using the pGEX4T vector (Amerham Pharmacia Biotech) and purified on a glutathione-agarose column (18). Full-length sortilin was subcloned from the cloning pBK-CMV vector (16) into the pCDNA3.1/Zeo(+) vector using XbaI and Smal restriction enzymes and transfected into CHO-K1 cells (18). LpL was purified from bovine milk as the enzymatically active dimeric enzyme (∼600 units/mg) as described previously (25). LpL and s-sortilin were 125I-iodinated to specific activities of about 0.5 mol of 125I/mol of protein using chloramine T as the oxidizing agent. The iodinated LpL preparation was applied to a Sepharose C16B column (Amersham Pharmacia Biotech). Labeled material was eluted at 1.5 M NaCl, representing the dimeric form of the lipase, was used for experiments. In some cases, LpL was made monomeric by incubation with 1 M guanidinium hydrochloride for 3 h at 20 °C and dialyzed immediately before use (26). The construct LpL-(347–389/394–448) was produced in E. coli as described (27). The construct consists of the hexahistidine- Factor X substrate sequence Met-Gly-Ser(His)6-SerIle-Glu-Gly-Arg and amino acid residues 347–389 and 394–448 of human LpL. Deletion of the sequence Trp390-Ser-Asp-Trp393 from the construct LpL-(347–448) increases the solubility of the peptide. The construct contains the sites for binding to LRP and has about the same affinity as the uninterrupted stretch LpL-(347–448) and LpL-(313–448), which constitutes the C-terminal folding domain of LpL (27). Human RAP and RAP constructs containing domain D1 (residues 18–112) or domains 2 and 3 (D23) (residues 113–323) were produced in E. coli as hexahistidine-tagged peptides (28).

Antibodies—Rabbit anti-s-sortilin IgG used for immunoprecipitation of sortilin and for Western blotting and rabbit antiserum against a synthetic peptide containing residues Pro10-Arg9 of the sortilin propeptide have been described (18). For detection of LpL in mouse 3T3-L1 adipocytes, we used IgG isolated from egg yolk of chickens immunized with bovine LpL (29).

Cross-linking of Soluble Components and Solid Phase Assay—125I-Labeled s-sortilin (10–16 cpm) was incubated with LpL in 140 mM NaCl, 10 mM CaCl2, 10 mM Hepes, pH 7.8, for 16 h at 4 °C followed by incubation with the bifunctional reagent BS3 (Pierce) at a final concentration of 10 μM for 30 min at 20 °C. The reaction was quenched by the addition of SDS sample buffer with 20 mM b-mercaptoethanol followed by SDS-PAGE and autoradiography. For solid phase assay, LpL was immobilized in Polysorb microtiter wells (NUNC, Denmark), the wells were blocked with 2% Tween 20 for 2 h at 20 °C (24), and incubations with 125I-s-sortilin were in the above buffer. After 16 h at 4 °C, the wells were washed, and bound radioactivity was eluted with 10% SDS and counted.

Surface Plasmon Resonance Analysis—All measurements were performed on a BIACore 2000 instrument (Biosensor, Uppsala, Sweden) equipped with CM5 sensor chips. The carboxylated dextran matrix of the sensor chip (flow cells 1 and 2) was activated by the injection of 240 μl of solution containing 0.2% N-ethyl-N’-(3-dimethylaminopropyl)carbodiimide and 0.05 M N-hydroxysuccinimide in water, and s-sortilin was immobilized to an estimated density of 64 fmol/mm2. Samples for binding (40 μl) were injected at 5 μl/min (4 °C) in 10 mM Hepes, 150 mM NaCl, 1.5 mM CaCl2, 1 mM EGTA, 0.005% Tween 20, pH 7.4 (running buffer), and binding was expressed in terms of relative response units (RU), e.g. the response obtained from the flow cell with immobilized sortilin minus the response obtained using an activated but uncoupled cell. Degeneration of the chip was performed by injection of 10 μl of Tripreel (Baxter) in 4 mM trehalose, 0.05% Tween 20, pH 8.0. Kinetic parameters were determined using the BIASolution 3.0 software. The number of LpL molecules bound/mol of immobilized sortilin was estimated by dividing the ratio RUligand/massligand with RU_sortilin/mass_sortilin.

Culture and Incubation of Cells—3T3-L1 fibroblasts were grown in Dulbecco’s modified Eagle’s medium/P-12 (1:1 mix; Bio-Wittaker, Bel-gium), 10% donor calf serum, and 50 μg/ml gentamycin. To induce their differentiation to adipocytes, donor calf serum was replaced with fetal calf serum, and the medium was supplemented with 10 μg dexamethasone for 48 h and subsequently with insulin (10 μg/ml) for 8 days. Wild-type CHO-K1 cells and cells stably transfected with sortilin (18) were grown to near confluency in HyQ-CCM5 medium (HyClone, Utah; about 3 × 106 cells/well). Degradation of 125I-LpL at was measured as 12% trichloroacetic acid-soluble radioactivity in the medium.

Metabolic Labeling—3T3-L1 adipocytes were washed, preincubated for 10 min in cysteine- and methionine-free modified Eagle’s medium (Sigma) before overnight incubation in the same medium supplemented with 250 μM (35) cysteine and (35) methionine (pro-mix, Amerham Pharmacia Biotech). 5% full medium, and 10 μg/ml insulin. For identification of LpL-sortilin complexes, labeled 3T3-L1 cells were incubated with 1 mM cell-permeable and reducible cross-linker dithio- bis(succinimidyl propionate) (Pierce). After 30 min at 4 °C, the reaction was quenched in 20 mM Tris, and following washes, the cells were lysed in 400 μL of 1% Triton X-100 containing 20 mM Tris and protease inhibitors (Complete Mini, Boehringer Mannheim). After centrifugation, the lysate supernatant was diluted in 2 ml of a Tris-balanced salt solution and incubated with Sepharose-coupled anti-s-sortilin IgG for 16 h at 4 °C. The beads were washed, and bound material was eluted in 300 μL of 100 mM glycine, pH 2.7, neutralized with Tris buffer and subjected to reducing SDS-PAGE either before or after an additional step of pre-clearing using protein A-agarose coupled to anti-LpL IgG. Diphenylkoxide-fluoromethyl chloroform was added, and gels were exposed at −70 °C.

Quantitation of Cell Surface-expressed Sortilin—The transfected CHO cells were washed three times in ice-cold phosphate-buffered saline, pH 8.0, and incubated with 0.5 mg/ml membrane-impermeable reagent sulfo-N-hydroxysuccinimidobiotin (Pierce, IL) for 90 min at 4 °C. After washes in phosphate-buffered saline with 50 mM Tris to quench any unreacted reagent, the cells were lysed for 10 min at 4 °C in 1% Triton X-100, 20 mM Tris, 10 mM EDTA, 150 mM NaCl, pH 8.0, with protease inhibitors, and biotinylated proteins were precipitated with streptavidin-coupled Sepharose 4B beads (Zymed Laboratories Inc., CA). The fractions of streptavidin-bound (i.e. surface-biotinylated) and unbound sortilin were detected by Western blotting using horseradish peroxidase-conjugated swine anti-rabbit IgG as secondary antibody (Dako, Denmark) and enhanced chemiluminescence (ECL; Amerham Pharmacia Biotech). Quantitation was performed by laser scanning densitometry using a 2200 Ultrascan instrument (LKB, Sweden).

RESULTS

Soluble Sortilin Binds LpL—To test for binding, we first incubated LpL with 125I-labeled s-sortilin followed by the addition of the cross-linker BS3. As shown in Fig. 1A, a complex was formed when the labeled receptor was incubated with 125 nM LpL (lane 2) but not without LpL (lane 1). The labeled complex was increased by unlabeled soluble sortilin at low concentration (9 nM) (lane 3). However, the formation of complex was inhibited by 180–540 nM unlabeled soluble sortilin (lanes 4 and 5) and by heparin (lane 6). In addition, 500 nM LRP or megalin, which are both known to bind LpL, 10, 11, 30, abolished the formation of complex between sortilin and LpL (not shown).

We next examined if RAP inhibits the binding. Because the cross-linked complex with RAP is not easily distinguished from that with LpL (Fig. 1B, lanes 3 versus 2), we used the smaller construct comprising RAP D23, which binds to sortilin (23) and shares binding sites with LpL on LRP (24). At 5 μM, D23 markedly inhibited sortilin-LpL complex and caused the formation of a smaller complex (lane 4), whereas 125 nM D23 caused partial inhibition (lane 5). To further analyze the inhibition, 125I-s-sortilin was incubated with LpL immobilized in microtiter wells. Fig. 2 shows that RAP and RAP D23, but not D1, inhibited the binding reaction. This agrees with the previous result that sortilin binds RAP D3 and D23 but not the individual domains D1 and D2 (23).

The binding of LpL was further substantiated by plasmon resonance analysis using s-sortilin immobilized on the sensor chip. Fig. 3A shows that LpL binds readily and demonstrates a marked decrease in binding when LpL was made monomeric by treatment with guanidinium hydrochloride. The calculated capacity was about 0.4 mol of LpL/mol of s-sortilin, suggesting
that the binding stoichiometry might be 1:1. The $K_d$ for binding of LpL to s-sortilin was calculated at about 26 nM from these and a series of similar curves obtained at LpL concentrations ranging from 9 nM to 454 nM. The affinity for binding of monomeric LpL was about 100-fold lower. For comparison, LpL binding was also analyzed using LRP immobilized to the chip. The $K_d$ value was calculated at about 11 nM for binding of LpL to LRP and was in the micromolar range when LpL had been made monomeric (not shown), which is in broad agreement with previous observations using LRP immobilized in microtiter wells (11). To further test the specificity of LpL binding, the chip with immobilized sortilin was perfused with the fragment LpL-(347–389/394–448), which contains the two LRP binding segments in the C-terminal folding domain of human LpL (27). Fig. 3B shows that this peptide (20 μM) binds to sortilin, suggesting that the same segments in LpL are required for interaction with the two receptors. We also probed whether the 13-residue neuropeptide neurotensin, recently identified as a sortilin ligand (17), interferes with LpL binding. As shown in Fig. 3B, neurotensin at 20 μM essentially abolished the binding of LpL to sortilin, suggesting that the two ligands bind to overlapping sites on sortilin. Finally, we tested the sortilin propeptide (as glutathione S-transferase fusion protein), which binds to s-sortilin as well as to mature full-length sortilin and inhibits binding of RAP and neurotensin (18). The results demonstrated that sortilin, when first incubated with LpL at a near-saturating concentration (200 nM, cf. panel A), was unable to bind propeptide (not shown).
Thus, LpL binds to soluble sortilin with an affinity comparable with that for binding to LRP, presumably via its C-terminal folding domain. Moreover, the binding is competed for by the sortilin ligands, RAP (via D3) and neurotensin and by the sortilin propeptide.

**Binding of LpL to Sortilin in 3T3-L1 Cells**—To elucidate interaction in cells, we used 3T3-L1 adipocytes because they express LpL as well as sortilin (31, 19). Initially we observed coexpression of LpL and sortilin upon differentiation, i.e. after 3–4 days in culture (not shown). Fully differentiated 35S-labeled 3T3-L1 adipocytes were incubated with the permeable and thiol-cleavable cross-linker dithiobis(succinimidyl propionate) and lysed, followed by incubation with Sepharose-coupled rabbit anti-sortilin IgG. Complexes were then released from the Sepharose beads and subjected to reducing SDS-PAGE. As shown in Fig. 4, lane 1, a dominating band corresponds to sortilin itself, and a marked band is compatible with LpL. Other labeled peptides may represent nonspecific binding reactions. To confirm the presence of LpL, we immunoprecipitated the complexes released from the anti-sortilin Sepharose beads using chicken anti-LpL IgG. Fig. 4, lane 2, shows that reducing SDS-PAGE identified labeled peptides compatible with LpL and sortilin. Thus, a fraction of LpL appears associated with sortilin in the 3T3-L1 cells.

**Sortilin Can Mediate LpL Degradation**—We used stably transfected CHO cells to elucidate functional consequences of LpL binding to sortilin. The transfectants expressed sortilin in contrast to the wild-type cells as seen from Western blotting of total lysates (Fig. 5A, inset, lane 2 versus lane 1). The receptor was predominantly in the mature form because no reaction was observed when using anti-serum directed against the N-terminal propeptide (not shown). This is in agreement with the results of pulse-chase experiments, demonstrating that all newly synthesized sortilin is cleaved (18). To assess the fraction of sortilin expressed on the cell surface, the transfectants were treated with the nonpermeable reagent sulfo-N-hydroxysuccinimidobiotin at 4 °C and lysed, and biotinylated proteins were recovered on streptavidin-Sepharose beads. Sortilin was then detected by SDS-PAGE followed by Western blotting of the bound material as compared with the sortilin content in the fraction of the lysate not bound to streptavidin-Sepharose. Fig. 5, inset, shows that biotinylated (lane 3) as well as nonbiotinylated sortilin (lane 4) were readily detected. Scanning densitometry of the Western blots (cf. legend to Fig. 5) revealed that about 8% sortilin in the transfected cells had been accessible to biotinylation and, thus, represents the fraction expressed on the cell surface.

We next aimed at determining LpL binding to the cell surface expressed sortilin. However, 125I-labeled LpL bound readily and equally well to wild-type and transfected CHO cells (not shown), presumably to cell surface proteoglycans as shown previously (12). Experiments were therefore performed to determine whether sortilin mediates uptake and subsequent degradation of the surface-associated LpL. As shown in Fig. 5A, the transfected cells degraded 125I-LpL much faster than wild-type cells. The cell-mediated degradation was inhibited by 100 units/ml heparin (not shown) and by 500 nM unlabeled LpL, indicating a saturable mechanism. As shown in Fig. 5B, the degradation of LpL in sortilin transfectants was inhibited by RAP and by the weak base chloroquine that raises pH in intracellular compartments. We conclude that sortilin expressed on the cell surface can interact with LpL and mediate its degradation.

**DISCUSSION**

The results show that sortilin is multifunctional because it binds LpL in addition to neurotensin, and they establish sortilin as an endocytic receptor on the cell surface because it can mediate LpL degradation. The affinity of LpL for binding to sortilin is similar to that for binding to LRP. As also shown for LRP (11), the LpL monomer has markedly reduced affinity, possibly because the dimeric state provides the right conformational constraints for binding to sortilin. The results show that sortilin is multifunctional because it binds LpL in addition to neurotensin, and they establish sortilin as an endocytic receptor on the cell surface because it can mediate LpL degradation. The affinity of LpL for binding to sortilin is similar to that for binding to LRP. As also shown for LRP (11), the LpL monomer has markedly reduced affinity, possibly because the dimeric state provides the right conformational constraints for binding to sortilin.
LpL Binds Sortilin

Sortilin is mainly located intracellularly in several cell types. In transfected COS-1 cells, full-length sortilin is mainly in the Golgi compartment, and chimeric receptors consisting of the C-terminal cytoplasmic tail of sortilin and the luminal domain of the interleukin-2 receptor colocalize with the IGF-II receptor (16). The molecular basis for the predominant retrieval of sortilin to the Golgi compartment is most likely the acidic cluster at the C terminus, because an identical cluster in the IGF-II receptor, containing a phosphorylatable serine residue, is important for the retrieval via interaction with the newly identified protein PACS-1 (32). In neurons, however, sortilin is up-regulated on the cell membrane following neurotensin-induced sequestration of the neurotensin receptor-1 (17, 33), and in adipocytes and 3T3-L1-cultured adipocytes, the fraction of sortilin expressed on the surface is increased 1.7-fold by insulin (19). Interestingly, both the localization and the insulin responsiveness of sortilin in adipocytes resembles that of the IGF-II receptor, presumably because of the homology of important signal sequences in their cytoplasmic tails (16). The two receptors are therefore likely to be similar in terms of cycling among the Golgi, endosomal, and plasma membrane compartments. The transfected CHO cells described in this report express about 8% of sortilin on the cell surface, in broad agreement with the fractional surface expressions of sortilin and IGF-II receptors in adipocytes and of IGF-II receptors in normal rat kidney cells (19, 34).

LpL is taken up by members of the LDL receptor family, particularly LRP (9, 11), through contacts in the C-terminal folding domain of the LpL (27). The molecular basis for the interaction with sortilin appears to be similar because a construct of LpL containing the segments important for binding to LRP also bound to sortilin. In addition, RAP domains D23 and LpL cross-compete for binding to sortilin, indicating that the two ligands have the same or overlapping sites for binding like LpL and RAP domains (24). Previous studies using cultured cells have shown that LpL, independent of its catalytic activity, can enhance lipoprotein uptake by providing a bridge between lipoproteins and endocytic receptors of the LDL receptor family (Refs. 11, 35, and 36; for review, see Ref. 22), and LpL-facilitated lipoprotein uptake has recently been confirmed in vivo (37). Future studies should show whether sortilin, via interaction with LpL, participates in receptor-mediated lipoprotein uptake.

The result that cell surface-expressed sortilin can mediate degradation of LpL may apply to other ligands, e.g. neurotensin (17, 33). However, sortilin may also exhibit important functions in the Golgi compartment in analogy with the established dual functions of the IGF-II receptor as an endocytic and a sorting receptor. Because sortilin is abundantly expressed in cell types that secrete LpL, future studies should elucidate whether it is involved in sorting of newly synthesized LpL to specialized compartments.

In conclusion, we find that sortilin binds LpL with an affinity comparable with that of LRP and that the receptor can mediate uptake and degradation of the ligand when expressed on the cell surface.

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REFERENCES

1. Gerdes, C., Fischer, R. M., Nicaud, V., Boer, J., Humphries, S. E., Talmud, P. J., and Fargenman, G. (1992) Circulation 85, 768–775
2. Wittrup, H. H., Thybjerg-Hansen, A., Abildgaard, S., Steffensen, R., Schnohr, P., and Nordestgaard, B. G. (1997) J. Clin. Invest. 99, 1606–1613
3. Jukema, J. W., van Boven, A. J., Groeneveld, B., Zwinderman, A. H., Reiber, J. H., Brusche, A. V., Henneman, J. A., Moestrup, S. K., Brunzell, J. D., and Madsen, P. (1998) J. Clin. Invest. 102, 2627–2634
4. Eriksson, J. S., Bengtsson-Olivecrona, G., and Olivecrona, T. (1999) J. Biol. Chem. 274, 26271–26276
5. Osbourne, J. C., Jr., Bengtsson-Olivecrona, G., Lee, N. S., and Olivecrona, T. (1985) Biochemistry 24, 5606–5611
6. Nielsen, M. S., Brejnning, J., Garcia, R., Zhang, H., Hayden, M. R., Viliers, S., and Gliemann, J. (1997) J. Biol. Chem. 272, 5821–5827
7. Elggaard, L., Holst, T. L., Nielsen, P. R., Eriksen, M. J., and Gliemann, J. (1997) Eur. J. Biochem. 244, 544–551
8. Vilella, E., Joven, J., Fernandez, M. V., Bruckdorfer, W., and Gliemann, J. (1992) J. Lipid Res. 33, 1521–1541
9. Beisiegel, U., Weber, W., and Bengtsson-Olivecrona, G. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8342–8346
10. Chappell, D. A., Inoue, I., Fry, G. L., Pladet, M. W., Bowen, S. L., Iverius, P. H., Lulow, J. M., and Strickland, D. K. (1994) J. Biol. Chem. 269, 18001–18006
11. Merkel, M., Kako, Y., Radner, H., Cho, I. S., Ramasamy, R., Brunzell, J. D., Goldberg, I. J., and Breslow J. L. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13841–13846