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REGULAR ARTICLE

Lipoprotein mediated lipid uptake in oocytes of polychaetes (Annelida)

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Abstract The uptake of the 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI)-labeled sex-unspecific *Nereis* lipoprotein was investigated in oocytes of the nereidid polychaetes *Nereis virens* and *Platynereis dumerilii*. The fluorescence label was first observed in endocytic vesicles (<1 μm diameter), which later fused to larger vesicles (2–3 μm); these were finally incorporated into existing unlabeled yolk granules (5–6 μm). In *Platynereis* oocytes, the fusion of endocytic vesicles was delayed in oocytes at their final stage of development compared with those at an early stage of development. Lipoprotein double-labeled with fluorescein isothiocyanate (FITC) and DiI revealed that both the protein and the lipid moiety remained co-localized during incorporation into the yolk granules of the oocyte. No labeling of the cytoplasmic lipid droplets was observed. In *N. virens*, unlabeled *Nereis* lipoprotein was effective as a competitive inhibitor of DiI-labeled *Nereis* lipoprotein. Ligand blot experiments demonstrated the presence of a lipoprotein receptor with an apparent molecular mass of 120 kDa, which is different from that of the known yolk protein receptor. This indicates the presence, in the polychaete oocyte, of two distinct receptors mediating yolk protein and lipoprotein uptake, respectively. Thus, the sex-unspecific lipoprotein contributes to the lipid supply of the growing oocyte in addition to the known uptake of the yolk-protein-associated lipids. The absence of label in the cytoplasmic lipid droplets, even after prolonged incubation with labeled lipoprotein, suggests that these lipids arise either by the breakdown and resynthesis of lipoprotein-derived lipids and/or by *de novo* synthesis within the oocyte.

Keywords Endocytosis · Lipoprotein receptor · Oocyte · *Nereis virens* · *Platynereis dumerilii* (Annelida, Polychaeta)

Introduction

The endocytosis of yolk protein (vitellogenin) is the major route for the supply of protein and lipids in developing oocytes of most oviparous animals. The female-specific vitellogenins belong to the family of the large lipid transfer proteins (Smolenaars et al. 2007), which are involved in the transport of lipids. The invertebrate vitellogenins described so far (mainly insect and crustacean vitellogenins) have lipid contents between 7% and 51% (Kunkel and Nordin 1985; Lee 1991). For the vitellin (the final storage form of vitellogenin in the oocyte) of two polychaetes, a lipid content of 16% and 50% has been reported (Baert et al. 1984; Lee et al. 2005). On the other hand, the non-sex-specific lipoproteins among the large lipid transfer proteins have also been shown to contribute to egg yolk, at least in crustacean (Lubzens et al. 1997; Ravid et al. 1999), insect (Kawooya and Law 1988; Kawooya et al. 1988; Telfer et al. 1991; Ximenes et al. 2008), and chicken eggs (Steyrer et al. 1990). In oocytes of some vertebrates (i.e., trout, frog, and hen) and in some insects, the incorporation of both yolk and lipoproteins is also evident from the presence of the corresponding receptors (Steyrer et al. 1990; Cheon et al. 2001 and references cited therein). The responsible vitellogenin and lipoprotein receptors belong to a common protein

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superfamily (Willnow 1999); they are membrane proteins with a single membrane-spanning domain and have been characterized from a variety of vertebrates and insects (Schneider et al. 1982; Hayashi et al. 1989; Röhrkasten et al. 1989; Tsuchida and Wells 1990; Hafer et al. 1992; Lee et al. 2003). Information on lower invertebrates is scarce. So far, only the vitellogenin receptor of the polychaete *Nereis virens* has been described (Hafer et al. 1992). Neither yolk protein receptors nor lipoprotein receptors exhibit a strict specificity for their ligands. The hen oocyte vitellogenin receptor has an affinity for the mammalian lipoproteins (Steyrer et al. 1990; Warrier and Subramoniam 2002 and references cited therein), and in a crab, the vitellogenin receptor has even been found to recognize mammalian lipoprotein (Warrier and Subramoniam 2002). In insects, both lipoproteins have been speculated to be incorporated by the same receptor, with the higher affinity of this receptor for the yolk protein possibly leading to a preferential uptake of vitellogenin as the main storage protein of the oocyte (Kulakosky and Telfer 1990). On the other hand, the incorporation of yolk protein into the oocyte of the polychaete *N. virens* is not inhibited by male coelomic fluid (Fischer and Rabien 1986), despite its known content of lipoprotein, which has been characterized as a discoidal high-density lipoprotein (see Schenk et al. 2006).

In this study, we have investigated the uptake of the non-sex-specific lipoprotein previously found in the polychaete *N. virens* in oocytes of this and another nereidid, *Platynereis dumerilii*, as a possible route for the lipid supply of the growing oocyte. In the light of variable lipoprotein receptor specificities, we have also examined whether separate receptors for vitellogenin and lipoprotein are present at the level of lower invertebrates such as the polychaetes.

Materials and methods

Animals

Platynereis dumerilii came from a continuous culture at the Institute of Zoology, University of Mainz (for details, see Hauenschild and Fischer 1969; Fischer and Dorresteijn 2004). *Nereis virens* came from the Wadden Sea at Yerseke, The Netherlands and were obtained through a local bait supplier. The animals were kept as described by Schenk et al. (2006).

Oocyte isolation

P. dumerilii were anesthetized by using a 1:1 mixture of seawater and 7.5% (w/v) $MgCl_2 \times 6\ H_2O$ in distilled water. The body cavity was cut open, and the oocytes were flushed out by gentle agitation. *N. virens* were cooled on ice for 20–30 min, and oocytes were obtained by puncturing the body cavity with a fine glass capillary. All oocytes were suspended in *Nereis* balanced salt solution (NBSS) consisting of 110 mM NaCl, 12 mM $NaHCO_3$, 4.5 mM KCl, 1.2 mM $CaCl_2$, 74% (v/v) filtered natural seawater in distilled water, pH 7.6, 920–960 mOsm (Heacox et al. 1983) and allowed to settle by gravity in a microcentrifuge tube to separate them from other coelomic cells. This procedure was repeated once, and the cells were finally suspended in culture medium (see below). For lipoprotein receptor isolation, *N. virens* oocytes were *N. virens* oocytes were obtained after opening the body cavity, treated as above and finally stored at –20°C.

Lipoprotein purification

N. virens was immobilized by cooling to –1°C, and coelomic fluid was collected by puncturing the coelomic cavity with a glass capillary. The lipoprotein was isolated from coelomic fluid by ultracentrifugation after raising the density of the coelomic fluid to 1.16 g/ml by the addition of solid KBr by applying the parameters described by Schenk et al. (2006). Lipoproteins were recovered from the top of the tube, dialyzed, and stored as described by Stieb et al. (2008). The purity of the isolated lipoprotein was assessed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

Fluorescence labeling of lipoprotein

We used two fluorophores, fluorescein isothiocyanate (FITC) for labeling the protein moiety, and the membrane marker 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) for labeling the lipid portion of the *Nereis* lipoprotein, which consists almost exclusively of phospholipids (~70% of the lipid mass; S. Schenk unpublished). DiI is a phospholipid analog with slow lateral diffusion (Honig and Hume 1986; Hofmann and Bleckmann 1999). We therefore studied the initial events of endocytosis of this lipoprotein. For labeling with DiI, the lipoprotein was incubated with DiI at a concentration of 0.1 mg/mg of protein. The dye was added from a stock solution (3 mg/ml) in dimethylsufoxide, and the solution was gently stirred for 3 h at room temperature in the dark. Excess dye was removed by centrifugation (5 min at 14,000*g*) and subsequent filtration through a glass fiber filter. For labeling with FITC, the lipoprotein was dialyzed overnight against sodium carbonate buffer (100 mM, pH 8.5), after which FITC (absorbed to diatomaceous earth, Sigma) was added (1 mg/mg of lipoprotein). Following

incubation in the dark for 1 h, the reaction was stopped by the addition of 10% (v/v) 1 M TRIS-HCl, pH 7.5. Excess FITC was then either dialyzed overnight against NBSS with two buffer changes or removed by passing the solution over a Sephadex G25 fine column (Helmerhorst and Stokes 1980).

Uptake experiments

Oocytes were incubated in 96-well micro-titer plates by using a culture medium based on NBSS with the following additions (modified after Taki and Dhainaut 1988): 10% (v/v) medium 199 (10×; with Hanks' salts; Sigma M9163), 2% (v/v) hen egg ultrafiltrate, 45 mM NaCl, 0.125 mg/ml penicillin, and 0.5 mg/ml streptomycin. Before incubation, the wells were coated with bovine serum albumin (BSA; 0.1% in NBSS) for 1 h at 37°C to prevent protein adsorption and then rinsed with distilled water. Oocytes were incubated for 3–20 h in 200 μl medium containing lipoprotein (25–100 μg/ml). To each well, 20 μl of the oocyte suspension containing 20–50 oocytes was added. After 3 or 6 h, the medium was replaced by lipoprotein free medium, and the oocytes were incubated at 18°C under gentle agitation and examined after 20 h. For competition experiments with *Nereis* oocytes, unlabeled and DiI-labeled lipoprotein were added in concentrations of 50 μg/ml each.

Lipoprotein receptor purification

Frozen *N. virens* oocytes were homogenized in 10–20 times their volume in HEPES-buffered saline (HBS; 20 mM HEPES-NaOH, pH 7.8, 150 mM NaCl, 2 mM $CaCl_2$) containing 1:100 protease inhibitor cocktail (Sigma P8340) by ultrasonic disintegration on ice (5×30 s). The suspension was centrifuged for 15 min at 15,000*g* and 4°C to remove cell debris. The supernatant was collected and centrifuged for 60 min at 100,000*g* and 4°C to pellet the membranes. The membrane pellet was solubilized in HBS containing 40 mM octyl-glucopyranoside (OGP) for 30 min on ice and finally sonicated on ice (3×30 s). The membrane extract was recentrifuged for 60 min at 100,000*g* and 4°C to pellet insoluble material. The supernatant containing the membranes was dialyzed overnight against HBS to remove excess OGP. The membrane extract was then applied to a column containing CNBr-coupled *Nereis* lipoprotein (the column was prepared according to the manufacturer's recommendations) and recycled over the column at 4°C for 60 min. The column was washed with HBS, and the receptor fraction was eluted with 50 mM TRIS-maleic acid, pH 6.0, containing 5 mM EDTA, 5 mM suramin, and 0.1% (w/v) CHAPS. The eluted proteins were then precipitated by making up the solution to 50% in acetone (ice-cold), and the precipitate was collected by centrifugation for 20 min at 20,000*g* and 0°C. The pellet was reconstituted in HBS and used for the ligand blot experiments.

SDS-PAGE and ligand blotting

Lipoproteins and receptors were separated on 4%–20% gradient gels with 4% stacking gels (Laemmli 1970). For ligand blotting, the receptor proteins were run without reducing agents in the sample buffer and transferred to a nitrocellulose membrane following the method described by Kyhse-Andersen (1984). The membrane was subsequently blocked with 5% BSA in HBS for 1 h at room temperature. Ligand blotting was carried out essentially as described by Daniel et al. (1983). Briefly, the membrane was incubated at room temperature for 60 min with 25 μg/ml *Nereis* lipoprotein in buffer A (50 mM TRIS-HCl, pH 8.0, 2 mM $CaCl_2$, 90 mM NaCl, and 5% BSA) and then washed with buffer B (buffer A with 0.5% instead of 5% BSA) for 40 min (one rapid wash, two 20-min washes, one rapid wash). The blot was then incubated for 2 h at room temperature with an affinity-purified α-*Nereis* lipoprotein antibody (see below) at a 1:7,500 dilution in HBS containing 1% BSA. Subsequently, the blot was washed with HBS for 25 min (4–5 changes) and incubated with an alkaline-phosphatase-coupled goat α-rabbit antibody (1:20,000 in HBS) for 60 min. Following a 20-min wash with HBS (3–4 changes), a final wash for 5 min with distilled water, and a 1-min incubation in equilibration buffer (100 mM TRIS-HCl, pH 9.0, 100 mM NaCl, 50 mM $MgCl_2$), the blot was developed with 5-bromo-4-chloro-3-indolyl-phosphate (BCIP)/nitroblue tetrazolium (NBT) as substrate (final concentration 0.015% BCIP and 0.03% NBT).

Antibody

A *Nereis* lipoprotein-specific antibody was raised in rabbit (Dr. Pineda Antikörperservice, Berlin) by using lipoprotein isolated by ultracentrifugation. The antibody was affinity purified from the serum on a CNBr-coupled *Nereis* lipoprotein-containing column. After application of the serum to the column, unbound proteins were removed by exhaustive washing with 10 mM Na_2HPO_4, pH 7.4, 150 mM NaCl. The bound antibody was eluted with 100 mM glycine, pH 2.7, 0.5 M NaCl. The eluted fractions (500 μl) were neutralized with 50 μl 1 M TRIS-HCl, pH 9. The protein-containing fractions were pooled.

Protein determination

Protein was determined by using a bicinchoninic acid assay (Kaushal and Barnes 1986).

Microscopy

Oocytes were mounted on microscopic slides by using strips of coverslips (thickness 200 μm) as spacers and observed under an epifluorescence microscope (Axioskop, Zeiss) with an Hg HBO-50 lamp and Plan-Neofluar 20×, 40×, and 100× (oil) lenses. FITC/tetramethylrhodamine isothiocyanate (TRITC) filters were used to acquire digital images on a Color View 2 digital camera (Olympus) at an exposure time of 250 or 500 ms. Images taken with both TRITC and FITC filter settings were overlaid and processed by using Photoshop CS.

Quantitation of fluorescent images

For the competition experiment, the incorporated fluorescence was quantified in *Nereis* oocytes. Images of oocytes were taken at 20× magnification after focusing in the equatorial plane. The digital images were converted to grayscale images by using Image J (http://rsb.info.nih.gov/ij/). The oocyte area was outlined, and its average brightness was calculated by using the "mean gray value" function of the program as a relative measure of lipoprotein incorporation after subtraction of the background fluorescence. The brightness of 10 oocytes was compared in incubations with DiI lipoprotein only and in those with additional unlabeled lipoprotein (see above) by using an unpaired *t*-test. The camera settings were kept constant for each series of measurements.

Chemicals

FITC adsorbed to diatomaceous earth was obtained from Sigma, whereas DiI was from Invitrogen/Molecular Probes. All other reagents and chemicals were from Sigma, AppliChem, or Fluka and were of reagent grade quality.

Results

Lipoprotein uptake in oocytes

Both DiI- and FITC-labeled lipoproteins were incorporated by oocytes of both *N. virens* and *P. dumerilii*. However,

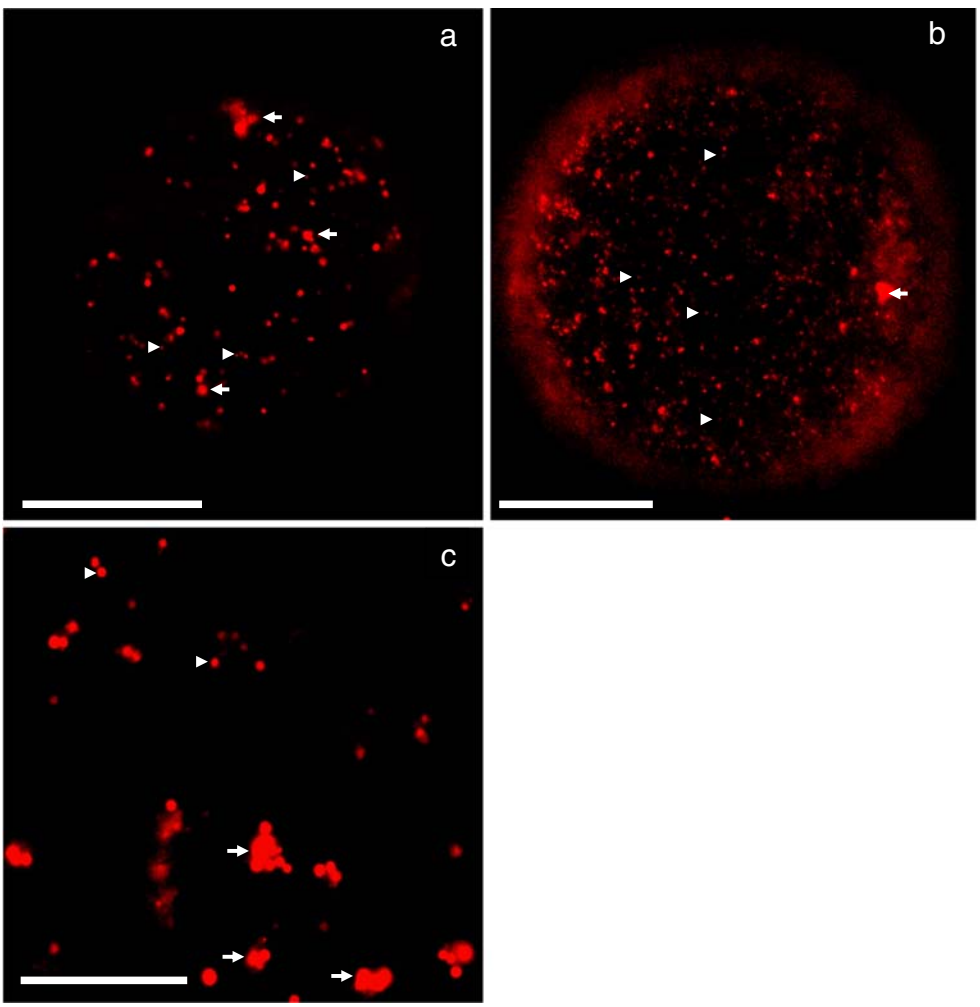

Fig. 1 Surface of oocytes of *Platynereis dumerilii* after incubation (3-h pulse, 20-h chase) with DiI-labeled *Nereis* lipoprotein (25 μg/ml). **a** Young oocyte (107 μm in diameter) showing small (≤1 μm in diameter; *arrowheads*) and larger (4–5 μm in diameter; *arrows*) endocytic vesicles. **b** Full-grown oocyte (170 μm in diameter). Note the presence of predominantly small vesicles (*arrowheads*), aggregations of small vesicles (*arrows*), and the absence of larger vesicles compared with **a**. Magnification: 40×. *Scale bars* 50 μm. **c** Surface of a full-grown oocyte (as in **b**) at higher magnification. Note the aggregations (*arrows*) of small vesicles (1–1.5 μm in diameter; *arrowheads*), which do not fuse to larger vesicles as seen in **a**. Magnification: 100×. *Scale bar* 20 μm

large differences were found between FITC-labeled and DiI-labeled lipoproteins. DiI-labeled lipoprotein was incorporated much faster, and endocytic vesicles were visible after 3 h and 6 h for *Platynereis* and *Nereis*, respectively, whereas FITC-labeled lipoprotein had to be applied for a much longer time (12–19 h) before endocytosis could be visualized. By taking the mean oocyte brightness as a relative measure of lipoprotein incorporation (see Materials and methods), the uptake of DiI-labeled lipoprotein at 50 μg/ml was shown to be significantly reduced by 44% (unpaired *t*-test, $P<0.001$, $n=10$) in *N. virens* oocytes when an equal concentration (50 μg/ml) of unlabeled lipoprotein was included in the medium. Further observations at higher magnification were carried out on *Platynereis* oocytes because of their better transparency.

After incubation (3-h pulse, 20-h chase), the endocytosis of lipoprotein was visible as vesicles between 1 and 5 μm in diameter (Fig. 1a, b). The larger vesicles were rarely found in large oocytes. At higher magnification, small vesicles were found clustered in large oocytes (Fig. 1c), but not in small oocytes.

Fate of discoidal *Nereis* lipoprotein in *P. dumerilii* oocytes

The fate of the discoidal *N. virens* lipoprotein was followed in *Platynereis* oocytes by using a lipoprotein preparation labeled with both FITC (labeling of the protein moiety) and DiI (labeling of the lipid portion; Fig. 2). For this experiment, incubation was carried out for 6 h and at a higher lipoprotein concentration (100 μg/ml) compared with that normally used in order to compensate for the slower uptake rate of FITC-labeled lipoprotein. When focusing on the yolk granules, the lipoprotein was found in crest-like structures at the surface of existing unlabeled yolk granules (see circles in Fig. 2a, b, d). The lipoprotein maintained both its red and green fluorescence when examined after 20 h (Fig. 2a, b), and both labels were co-localized (Fig. 2d) indicating that the lipoprotein particles had been incorporated completely without significant unloading of lipids during or after endocytosis. The large cytoplasmic lipid droplets present in the oocytes (stars in Fig. 2a-d) were not labeled indicating that they were not a primary site of lipid delivery.

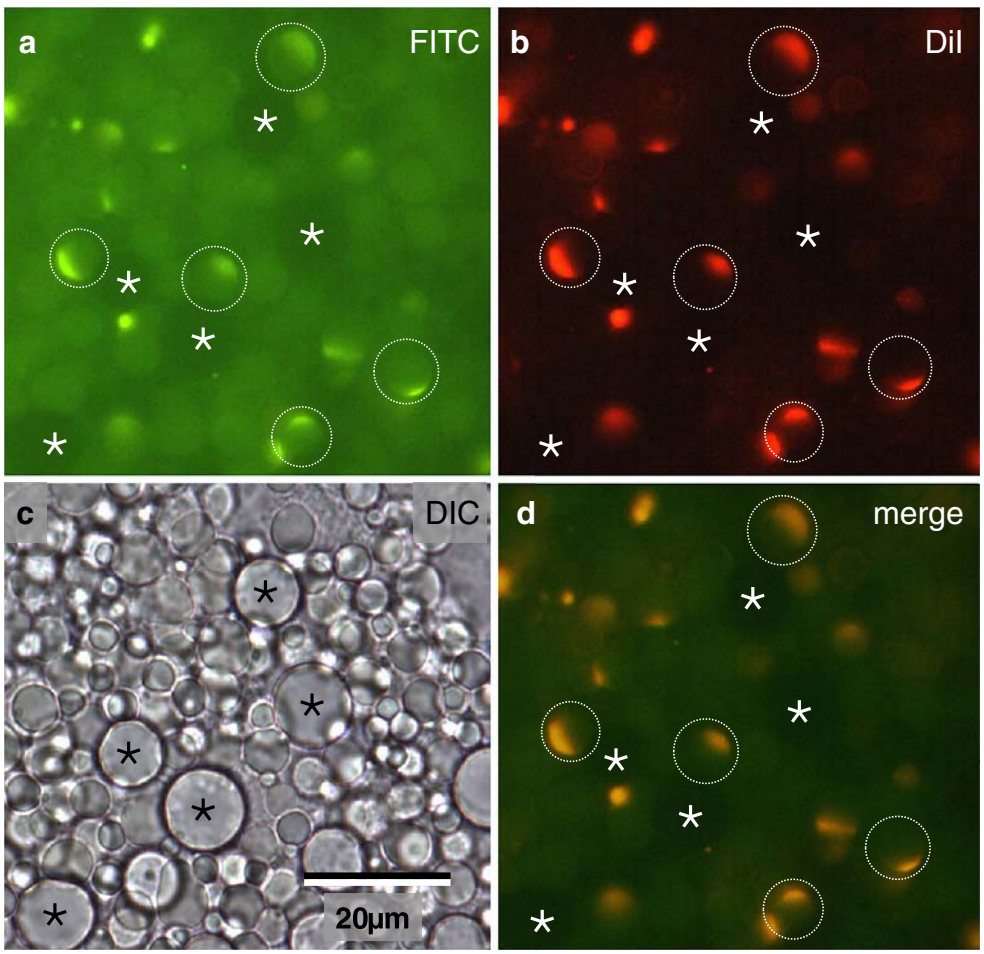

Fig. 2 Image of yolk granules and lipid droplets of a young oocyte (110 μm in diameter) of *P. dumerilii* after incubation (6-h pulse, 20-h chase) of both lipid (DiI)- and protein (FITC)-labeled *Nereis* lipoprotein (100 μg/ml). Labeled lipoprotein is seen as crest-like structures in the yolk granules (*circles* in **a**, **b**, **d**) as a result of the fusion of endocytic vesicles with existing yolk granules (5–6 μm in diameter; *faint green spheres*, see *circles* in **a**). Note that the larger lipid droplets (*stars*, **a-d**) are not labeled. **a** FITC fluorescence. **b** DiI fluorescence. **d** Merged picture indicating no separation of lipids from the protein upon fusion with the yolk granules. **c** Bright field. Magnification 100×

Ligand blot experiment

The ligand blot of the purified receptor protein showed a single band with an apparent molecular mass of ~120 kDa (Fig. 3, lanes b, c). Upon separation of the receptor preparations under reducing conditions, no lipoprotein binding was found, indicating the requirement of intact intramolecular disulfide bridges for ligand binding.

Discussion

The present study demonstrates the efficient uptake of the sex-unspecific lipoprotein of *N. virens* in the oocytes of this species and those of another nereidid, *P. dumerilii*. This indicates both a female-specific (vitellogenin) and sex-unspecific (lipoprotein) supply route for exogenous lipids, which we have demonstrated for the first time in oocytes of two lower invertebrates.

The endocytic pathway as exemplified by that of vitellogenin in the insect oocyte (see Sappington and Raikhel 1998) usually starts with the formation of coated vesicles that fuse to form transport vesicles and larger endosomes. From these, the receptor is sequestered for further recycling at the cell surface, whereas the endosome fuses with a yolk granule. The fusion process of small vesicles (<1 μm in diameter), probably representing transport vesicles, with larger vesicles has not been observed in smaller (younger) oocytes (Fig. 1). However, the aggregations of small vesicles present in large oocytes (Fig. 1c) are indicative of such a process. The predominance of smaller vesicles in large oocytes (Fig. 1b) compared with smaller oocytes under the same incubation conditions suggests that vesicle fusion is delayed in larger oocytes. In *Platynereis* oogenesis, oocytes grow in a precise pattern reaching a final diameter of 160–170 μm shortly before spawning as the terminal event of the life cycle (see Fischer 1984). Thus, whereas endocytic events still seem to take place, the further pathway leading to vesicle fusion and incorporation of the lipoprotein into the yolk granules might be slowed down or even blocked in oocytes at their terminal phase of development.

A receptor-mediated pathway of lipoprotein endocytosis is supported by the use of unlabeled lipoprotein, which is an effective competitor for DiI-labeled lipoprotein uptake. Furthermore, the occurrence of a lipoprotein-binding protein in the oocyte membrane fraction as demonstrated by ligand blotting indicates the presence of a corresponding receptor. The apparent molecular mass of the lipoprotein receptor of the *Nereis* oocyte (~120 kDa) is clearly different from that the yolk protein receptor described previously (~190 kDa; Hafer et al. 1992) and thus indicates the presence of two distinct receptors for the lipoprotein and

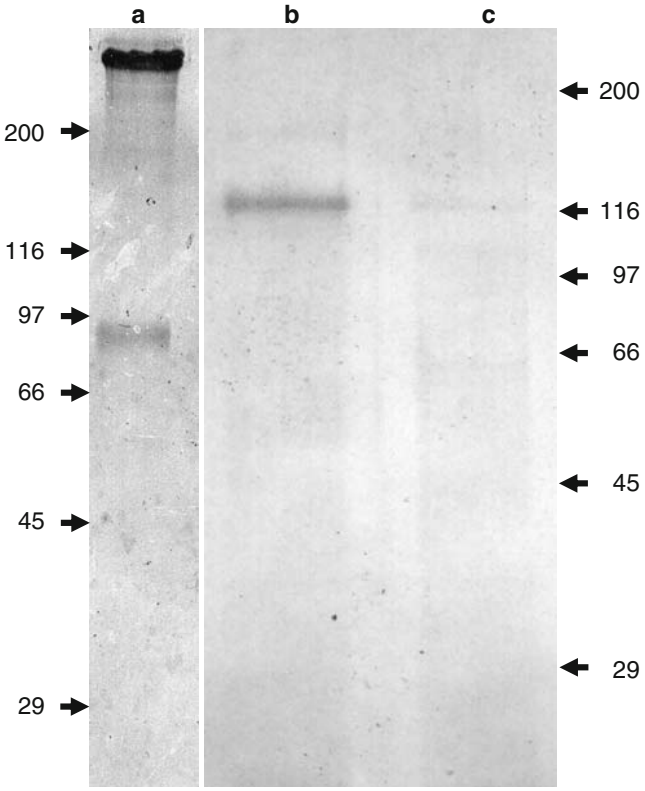

Fig. 3 SDS-PAGE and ligand blot of the purified *Nereis* lipoprotein and the *Nereis* lipoprotein receptor, respectively; *lane a* SDS-PAGE showing only the characteristic two apolipoproteins (Schenk et al. 2006) stained by the Coomassie method (*numbers* molecular mass markers), *lane b* ligand blot of the *Nereis* lipoprotein receptor under non-reducing conditions, *lane c* ligand blot of the *Nereis* lipoprotein receptor under reducing conditions. No ligand binding was found under reducing conditions (*numbers* molecular mass markers)

the yolk protein in the *Nereis* oocyte. The sensitivity of the ligand binding to reducing conditions indicates the requirement for intramolecular disulfide bridges for ligand binding. This is consistent with findings for other members of the low-density lipoprotein receptor family such as the insect vitellogenin receptors (see Telfer et al. 1991).

The situation found in the polychaete oocyte contrasts with that of the insect oocyte. In mature *Manduca sexta* eggs, most of the lipids are delivered by the low-density (higher lipidated) form of the insect lipoprotein (lipophorin) without internalization of the protein moiety (Kawooya and Law 1988). Only a small fraction (10% of egg lipids) has been found to originate from the combined cellular uptake of vitellogenin and the high-density (less lipidated) form of lipophorin (Kawooya and Law 1988). Similarly, the uptake of lipophorin by developing oocytes has been demonstrated in the saturniid moths *Philosamia cynthia* (Chino et al. 1977) and *Hyalophora cecropia* (Kulakosky and Telfer 1990; Telfer et al. 1991). The competition for cellular uptake between vitellogenin and high-density lipophorin in *H. cecropia* was initially explained by the presence of a

common receptor for these two lipoproteins (Kulakosky and Telfer 1990). However, recent research in insects (Cheon et al. 2001; Roosendaal et al. 2009) has demonstrated the presence of independent receptors for vitellogenin and lipophorin, including oocytes. Experiments by Roosendaal et al. (2009) have indicated a higher affinity of the lipophorin receptor for a partially delipidated form of lipophorin compared with its fully lipidated form. In this light, the complete internalization of the *Nereis* lipoprotein may be favored as it is a high-density lipoprotein (Schenk et al. 2006).

The primary target of the lipoprotein-bound lipids in polychaete oocytes are the yolk granules, the site of yolk protein storage. Incubation of oocytes in the presence of both FITC-labeled yolk protein and DiI-labeled lipoprotein results in the co-localization of the labels in endocytic vesicles, thus indicating the same endocytic pathway is employed (data not shown). The cytoplasmic lipid droplets in the oocyte have not been seen to display any labeling during our experiments. In insect oocytes, endocytosed lipophorin is also stored in the yolk (Telfer et al. 1991). In contrast, no evidence for the storage of lipoprotein has been found in the *Nereis* oocyte. Western blots of oocyte extracts have failed to demonstrate the characteristic apoprotein bands (Schenk et al. 2006) by using an antibody against *N. virens* lipoprotein, and only degradation products have been found (S. Schenk, unpublished). This indicates that the lipoprotein is degraded after uptake into the yolk granules, in contrast to vitellogenin, which is well known to undergo only a limited proteolytic cleavage after uptake, as found in the polychaetes *Perinereis cultrifera* (Baert 1986; Baert and Slomianny 1992) and *N. virens* (García-Alonso et al. 2006) and in other invertebrates such as insects (Kunkel and Nordin 1985) and the crayfish *Cherax quadricarinatus* (Abdu et al. 2002).

The further fate of the lipoprotein-derived lipids in the oocyte has not been investigated, not even in the comparatively well-studied insect oocyte (see Ziegler and van Antwerpen 2006). However, the absence of lipid droplets in the yolk granules and the apparent degradation of the lipoprotein after uptake suggest the hydrolysis of the lipoprotein-derived lipids followed by the export of fatty acids, their utilization for *de novo* synthesis of lipids within the oocyte, and their final deposition in the lipid droplets. This could explain the finding that neutral lipids are the main storage lipids in the oocyte of the polychaete *Perinereis*; these lipids account for the bulk of 20%–40% of the dry weight (Fontaine et al. 1984a, 1984b), whereas phospholipids are the main lipids transported by the *Nereis* lipoprotein (Schenk et al. 2006). However, as vitellogenin comprises 43% of the oocyte proteins (~0.1 μg/oocyte; Fischer and Schmitz 1981) and incorporates ~16% lipids (Baert et al. 1984), this would result in a contribution of 4%–8% to the total lipids of the oocyte, leaving 92%–96% of the total lipids to be derived from the lipoprotein and/or *de novo* synthesis. The uptake of free fatty acids either by direct import from the coelomic fluid or after hydrolysis of lipoprotein-associated lipids by a membrane-bound lipoprotein lipase, as proposed for the insect oocyte (see Ziegler and van Antwerpen 2006), cannot be ruled out. However, this aspect awaits further investigation.

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