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Solubilization of the locust vitellogenin receptor

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Oocytes of *Locusta migratoria* contain a vitellogenin receptor which can be solubilized from the cell membranes by incubation with the nonionic detergent, octyl β-D-glucoside. Optimum results were obtained with 40 mM octyl glucoside concentrations at which 62% of the [^{3}H]propionylvitellogenin binding activity associated with 28% of the total membrane proteins can be extracted. The solubilized receptor has a high affinity for vitellogenin ($K_d = 4.2 \cdot 10^{-8}$ M) and it contains one class of binding sites. The high specificity of the receptor for native vitellogenin is supported by its inability to bind vitellogenin chemically modified by maleylation or extensive propionylation. The solubilized vitellogenin-binding site appears to be the same as the functional vitellogenin receptor recently demonstrated in locust oocytes and oocyte membrane preparations.

Introduction

In the insect *Locusta migratoria* the yolk protein vitellogenin is the principal haemolymph protein incorporated by maturing oocytes. Vitellogenin, a large lipoglycoprotein [1,2], is extraovarially synthesized in the adult female fat body. Isolated intact locust oocytes selectively accumulate this yolk protein [3,4]. The incorporation process has all the characteristics of a receptor-mediated endocytosis [5]. Vitellogenin is apparently bound to coated pits on the oocyte surface. Internalization is accomplished by the invagination of the binding sites and of their bound vitellogenin molecules into the oocyte and subsequent pinching off to form endocytotic coated vesicles. This process was first described in mosquito oocytes [6]. In a previous study we isolated membranes from locust oocytes and demonstrated their ability to bind vitellogenin with high affinity [7]. The current studies set forth procedures for isolation and purification of the locust vitellogenin receptor from oocyte membranes to facilitate studies on the biochemical, physiological and immunological properties of the vitellogenin receptor. For the first time we describe the successful solubilization of a vitellogenin receptor with the detergent octyl glucoside, a binding assay for this solubilized receptor and some characteristics of it.

Materials and Methods

Animals

5th instar *L. migratoria* were obtained from Insektarium Dr. Frieshammer, Jaderberg, F.R.G. In the laboratory they were kept at 30°C with a daily photoperiod of 14 h and fed fresh grass or wheat shoots and oats.

Solubilization of oocyte membrane proteins

Ovarioles were collected from egg-maturing locusts and stored frozen at −70°C until use. Purified oocyte membrane material was prepared as described previously [7]. Homogenized membranes were suspended in Hepes-buffered saline (20 mM Hepes/150 mM NaCl/1 mM $CaCl_2$ (pH 7.4)) and octyl β-D-glucopyranoside (Serva) was

usually added at a final concentration of 40 mM. After incubation for 10 min at 4°C, unsolubilized material was removed by centrifugation at 100000 $\times g$ for 60 min in a Beckman L8 ultracentrifuge using the SW 50.2Ti rotor. The supernatant was diluted with Hepes-buffered saline to a final octyl glucoside concentration of 5 mM, at which the membrane proteins become insoluble. The precipitated protein-lipid aggregates were collected by repeating the ultracentrifugation. The protein pellet was resuspended in Hepes-buffered saline by aspiration through a 22-gauge needle. The protein concentration was determined using the microassay procedure of the Bio-Rad (Munich) protein assay. Remaining octyl glucoside concentrations up to 0.5 μmol/assay do not affect the protein determinations. Locust vitellogenin was purified and labelled with *N*-succinimidyl [2,3-^{3}H]propionate (Amersham-Buchler) as described previously [4,7].

Standard assay conditions

Aliquots of the solubilized membrane protein preparation were mixed with Hepes-buffered saline containing 0.5% bovine serum albumin (final concentration) and [^{3}H]propionylvitellogenin was added at the concentrations indicated. Incubations at 24°C were stopped after 80 min by collecting the receptor-[^{3}H]propionylvitellogenin complexes by filtration through 0.2 μm poly(vinylidene fluoride) membrane filters (Millipore GVWP 02500) which had been extensively pre-incubated with Hepes-buffered saline containing 0.2% bovine serum albumin and 0.01% vitellogenin to reduce unspecific binding. Each filter was then washed with 6 ml Hepes-buffered saline containing 0.2% bovine serum albumin. After drying the filters at 50°C for 60 min they were cut into small pieces and dissolved in 0.5 ml 0.5 M NaOH (120 min, 60°C). This solution was neutralized with acetic acid and finally taken up in 4 ml Unisolve 1 (Zinsser). The samples were counted in a Packard Prias PL liquid scintillation counter at an efficiency of 30%.

Chemical modification of vitellogenin

Vitellogenin was purified as described previously [4]. Maleylation was performed as described by Glazer et al. [8]. Propionylation was carried out using the method of Asher et al. [9]. The *N*-succinimidyl propionate needed for this reaction was prepared according to Rappoport and Lapidot [10].

Results

Solubilization of oocyte membrane proteins with octyl β-D-glucopyranoside

The amount of vitellogenin receptor protein extractable from oocyte membranes depends on the concentration of the detergent. Octyl glucoside concentrations exceeding 20 mM efficiently remove proteins from oocyte membranes (Fig. 1B). 40 mM octyl glucoside solutions solubilize 28% of the total membrane proteins; 42% of the total membrane proteins cannot be extracted. [^{3}H]Propionylvitellogenin-binding activity extractable

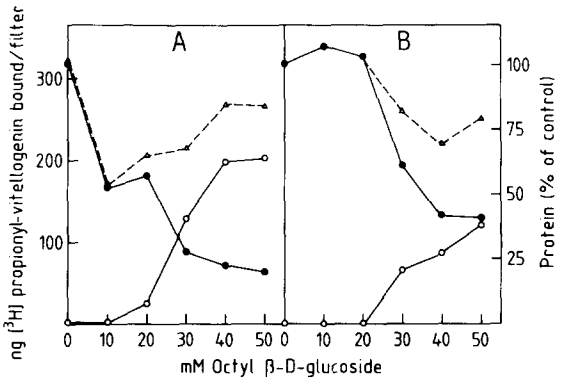


Fig. 1. Solubility of the vitellogenin receptor from locust oocytes. Locust oocyte membrane preparations containing 120 μg protein were dissolved in the presence of various octyl glucoside concentrations and centrifuged at 100000 $\times g$ for 60 min. Resulting supernatants were suspended in Hepes-buffered saline and the octyl glucoside concentration was lowered to 5 mM or less. The soluble membrane proteins were sedimented by centrifugation at 100000 $\times g$ for 60 min. (A) Aliquots of the membranes and soluble membrane proteins suspended in Hepes-buffered saline were incubated with 10 μg of [^{3}H]propionylvitellogenin for 80 min at 24°C and the amount of specifically bound labelled vitellogenin was determined. Unspecific binding was measured in the presence of 750 μg of unlabelled vitellogenin. ●, specific vitellogenin binding to membrane material; ○, specific vitellogenin binding to soluble membrane proteins; △, total vitellogenin binding as the numerical sum of binding to the membranes and the binding to the soluble extract. (B) Membranes were treated as in (A) and the protein finally recovered was determined.

from the oocyte membranes increases with increasing octyl glucoside concentrations (Fig. 1A). At 40 mM octyl glucoside 62% of the [^{3}H]propionylvitellogenin binding activity is associated with the extracted membrane proteins. Only 22% remains bound to the unsolubilized membranes. Some of the membrane proteins and the vitellogenin-binding activity are lost from the oocyte membrane preparations when extracted with octyl glucoside. Membrane solubilization was routinely performed with a 40 mM octyl glucoside solution.

Using the filter assay method described we compared the binding capacity of untreated oocyte membrane material with the octyl glucoside-solubilized membrane proteins of the same membrane preparation. As Table I illustrates, the solubilization does not negatively affect the binding process. The observed increase of specific binding by about 20% indicates that the vitellogenin receptor protein is efficiently removed from the membranes and that it fully retains its binding properties.

Time-course of vitellogenin binding

[^{3}H]Propionylvitellogenin binds rapidly to the solubilized receptor. At 24°C equilibrium is reached within 40 min (Fig. 2). Nonspecific binding was measured in parallel experiments after the addition of a 75-fold surplus of unlabelled vitellogenin. Only some 10% of the labelled vitel-

TABLE I

COMPARISON OF SPECIFIC BINDING OF [^{3}H]PROPIONYLVITELLOGENIN TO OOCYTE MEMBRANES AND SOLUBILIZED MEMBRANE PROTEINS

An oocyte membrane homogenate was prepared as described previously [7]. An aliquot of this preparation was extracted as mentioned in Materials and Methods. Aliquots of both preparations containing 30 μg membrane protein were subjected to binding tests using the reported filter assay method.

Material	ng [^{3}H]propionylvitellogenin bound to 30 μg membrane protein		
	total	unspecific	specific
Oocyte membrane	170	16	154
Membrane proteins (solubilized)	206	13	193

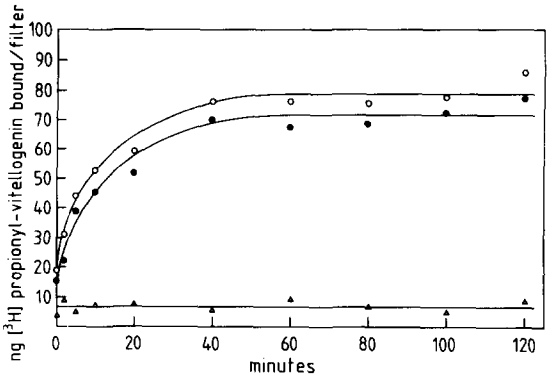
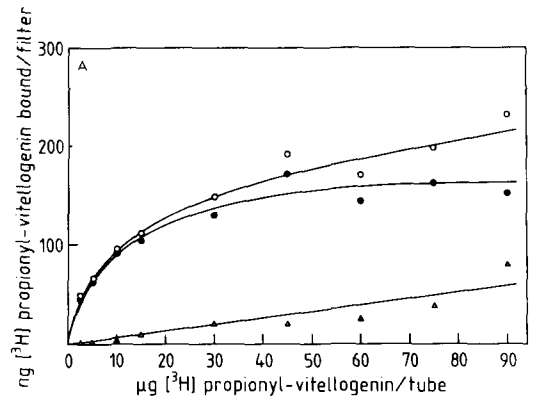


Fig. 2. Time-course of [^{3}H]propionylvitellogenin binding to solubilized oocyte membrane proteins. 14 μg of membrane protein were incubated with 10 μg [^{3}H]propionylvitellogenin at 24°C and the bound [^{3}H]propionylvitellogenin determined with the filter assay method. ○, total binding; ●, specific binding; △, unspecific binding.



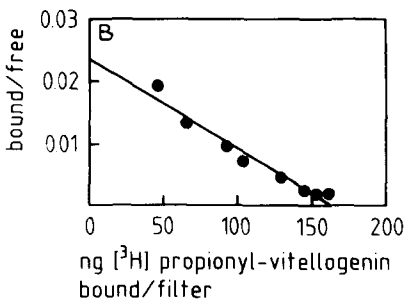


Fig. 3. (A) The binding of [^{3}H]propionylvitellogenin to soluble oocyte membrane proteins with increasing [^{3}H]propionylvitellogenin concentrations. 14 μg soluble oocyte membrane proteins were incubated with various amounts of [^{3}H]propionylvitellogenin. For estimation of the unspecific binding, unlabeled vitellogenin was added at a final concentration of 4.5 mg in the appropriate incubations. Symbols as in Fig. 2. (B) Results of the same experiment are plotted according to Scatchard analysis. The straight line was calculated using linear regression analysis (correlation coefficient, 0.972).

logenin are unspecifically bound by the solubilized membrane proteins. All binding studies reported were carried out at 24°C for 80 min.

Saturation of [^{3}H]propionylvitellogenin binding

The specific binding of [^{3}H]propionylvitellogenin to the solubilized vitellogenin receptor is a saturable process (Fig. 3A). At saturation concentrations about 11.9 μg [^{3}H]propionylvitellogenin binds to 1 mg of detergent-solubilized vitellogenin receptor protein. Unspecific binding was determined in a parallel experiment by addition of 4.5 mg of unlabelled vitellogenin. Fig. 3B shows the result of an analysis of the binding data according to Scatchard [11]. From the slope of the regression line an apparent equilibrium dissociation constant (K_d) of $4.2 \cdot 10^{-8}$ M can be calculated. Obviously, only one population of vitellogenin-binding compounds is present in locust oocyte membranes.

Binding as function of membrane protein concentration

Incubating a constant amount of [^{3}H]propionylvitellogenin (10 μg) with increasing amounts of octyl glucoside-solubilized oocyte membrane proteins (0–78 μg) results in a nearly linear increase of vitellogenin binding (Fig. 4). The unspecific binding, determined by the addition of 750 μg unlabelled vitellogenin, remains very low.

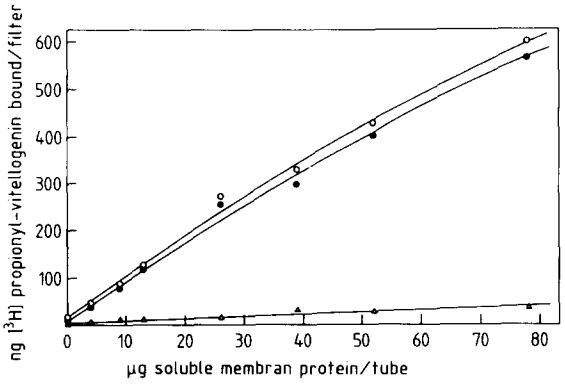


Fig. 4. Binding of [^{3}H]propionylvitellogenin as a function of soluble membrane protein concentration. Symbols as in Fig. 2.

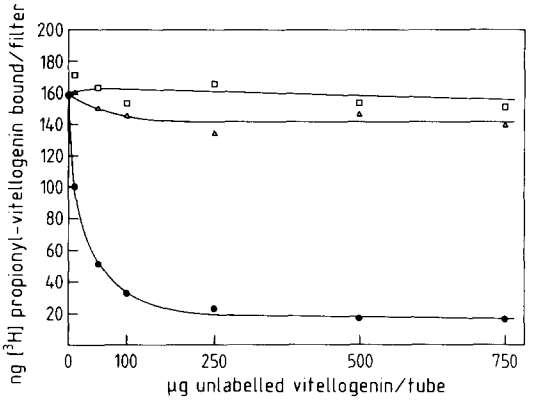


Fig. 5. Competition by unlabelled vitellogenins for binding of [^{3}H]propionylvitellogenin to soluble oocyte membrane protein. Each assay tube contained 24 μg soluble oocyte membrane proteins and 10 μg [^{3}H]propionylvitellogenin and the indicated concentrations of unlabelled, native vitellogenin (●), unlabelled maleylvitellogenin (□) or unlabelled propionylvitellogenin (△).

Binding of chemically modified vitellogenin

Propionylation or maleylation of vitellogenin results in a change of the positive netto charge of the amino acid, lysine. Modifying the lysine residues of the vitellogenin molecule causes altered

TABLE II

EFFECT OF HEAT TREATMENT OF THE SOLUBILIZED MEMBRANE PROTEINS AND OF EDTA ADDITION ON TOTAL BINDING OF [^{3}H]PROPIONYLVITELLOGENIN

Prior to incubation aliquots of the solubilized membrane proteins were heated for 5 min and rapidly cooled to 24°C and the binding assay started by addition of 10 μg [^{3}H]propionylvitellogenin.

Treatment	[^{3}H]Propionylvitellogenin bound	
	ng/21 μg membrane protein	%
Untreated control	185	100
55°C	139	75
80°C	56	30
100°C	32	17
5 mM EDTA added	33	18
750 μg vitellogenin added	11	6

TABLE III

EFFECT OF TRYPSIN ON SPECIFIC BINDING ACTIVITY OF THE SOLUBILIZED VITELLOGENIN RECEPTOR

Aliquots of the isolated membrane proteins (21 μg) were suspended in Hepes-buffered saline containing 20 μg trypsin (TPCK-treated, Sigma) or 20 μg trypsin and 100 μg soybean trypsin inhibitor (Sigma). Incubations were performed for 30 min at 24°C. Before introduction of the complete mixtures into the binding assay described above, 100 μg trypsin inhibitor was also added to the trypsin-treated sample.

Treatment	[^{3}H]Propionylvitellogenin bound	
	ng/21 μg membrane protein	%
Untreated control	185	100
Trypsin	10	5
Trypsin plus trypsin inhibitor	170	92

binding behaviour. The modified vitellogenins are unable to compete with [^{3}H]propionylvitellogenin for binding to the receptor even when present in a large surplus (maximum 75-fold) (Fig. 5). However, as little as a 10-fold surplus of unlabelled vitellogenin displaces labelled vitellogenin to background amounts.

Effect of heat and trypsin on the receptor protein

The solubilized receptor protein is heat-sensitive. When heated above 55°C the receptor protein loses its vitellogenin-binding capacity (Table II). The same effect has the addition of 5 mM EDTA to the binding assay. The protein character of the vitellogenin-binding component present in the solubilized membrane proteins is supported by its sensitivity to trypsin (Table III). A short incubation of the membrane proteins completely destroys the binding properties. However, in the presence of the soybean trypsin inhibitor, trypsin has no effect, and the vitellogenin receptor fully retains its activity.

Discussion

In the present study, the locust vitellogenin receptor has been solubilized from oocyte membranes by incubation with the nonionic detergent, octyl β-D-glucoside. A maximum amount of vitellogenin-binding activity can be recovered from the 100 000 × *g* supernatant with 40 mM octyl glucoside concentrations. Lowering the octyl glucoside concentration below its critical micellar concentration [12] results in a precipitation of the solubilized membrane proteins. In this way, most of the octyl glucoside can be removed and the membrane proteins can be collected. About 30% of the total membrane proteins can be solubilized, to which 62% of the total vitellogenin-binding activity is associated. Using the filter assay method described, it can be shown that the extract fully retains its ability to bind [^{3}H]propionylvitellogenin specifically and with high affinity. The [^{3}H]propionylvitellogenin-binding activity per mg membrane protein before and after octyl glucoside treatment is nearly identical. Octyl glucoside has proven to be of general utility and was recently used to solubilize the low-density lipoprotein receptor [13], the α_2-macroglobulin receptor, the insulin receptor [14] and the epidermal growth factor receptor [15]. Mild methods such as freeze-thawing or treatment with high salt concentration cannot remove the vitellogenin receptor proteins from the membranes (data not shown), indicating that the receptor is probably an intrinsic membrane protein penetrating the oocyte membrane. This observation is well in agreement with the general concept of receptor-mediated endocytosis, including the binding of clathrin during formation of coated vesicles [5,16].

The [^{3}H]propionylvitellogenin-binding properties of the soluble membrane proteins are quite similar to that of intact oocytes and of isolated oocyte membranes [4,7]. (a) It contains one high-affinity compound with an equilibrium dissociation constant (K_d) of $4.2 \cdot 10^{-8}$ M, which is comparable to that of isolated oocyte membranes (K_d $1.092 \cdot 10^{-7}$ M). (b) The binding process requires divalent ions such as Ca^{2+}. (c) The binding activity is sensitive to trypsin and it is abolished by boiling. (d) Vitellogenin is not bound when the lysine residues had been modified by maleylation or extensive propionylation. This observation corroborates previous results which show that the locust vitellogenin receptor apparently recognizes certain positive charges at the vitellogenin molecule (Ref. 4; unpublished data). Thus, we believe that the vitellogenin-binding activity solubilized

by octyl glucoside represents the physiological vitellogenin receptor. The vitellogenin receptor preparation obtained for the first time in soluble form can easily be used for further purification and characterization, as studies in progress demonstrate.

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