Receptor-mediated vitellogenin binding to chicken oocytes

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The specific binding of vitellogenin to chicken oocyte membranes was characterized. This major hen serum phospholipoglycoprotein and one of its lower-molecular-weight components, phosvitin, bound to oocyte membranes with $K_D$ values of approx. $6 \times 10^{-7}$ m. The optimum pH for binding was 6.0, the same as the pH of yolk contents. Phosvitin and vitellogenin compete with each other for binding; other proteins tested do not compete to the same degree. Phosvitin, which contains 10% phosphate by weight, appears to be the polypeptide recognized by the receptor. RNA failed to compete with either vitellogenin or phosvitin for binding, suggesting that the binding specificity may require more than polymeric phosphate. The binding was tissue-specific in that phosvitin and vitellogenin bound to oocyte surfaces (at both pH 6.0 and 7.5), but not to chicken erythrocytes (at either pH).

Specific transport of proteins into cells involves receptors localized in the membrane. Thus far, most such instances of specific protein uptake appear to involve binding to or localization in coated pits, which subsequently pinch off and enter the cytoplasm as coated vesicles (Roth & Porter, 1964; Roth et al., 1976; Goldstein et al., 1979; Brown & Goldstein, 1979). This mechanism was first studied in the mosquito oocyte (Roth & Porter, 1964; Cutting & Roth, 1973), and subsequent studies in a variety of oocyte systems have shown that many proteins are specifically taken into the developing oocyte to form the yolk. More recently, this coated-vesicle-mediated uptake of proteins has gained further credence as a general mechanism, in large measure because of the studies of Goldstein et al. (1979) and Brown & Goldstein (1979). In their work, the serum LD lipoprotein is shown to be specifically sequestered by fibroblasts. A variety of mutant cell lines have permitted them to demonstrate unequivocally that the LD-lipoprotein receptors are membrane-bound, be become localized in the coated pits and are then moved to lysosomal systems, where the cholesterol released from the LD lipoprotein becomes regulatory in cholesterol metabolism. A variety of other examples of coated-pit/coated-vesicle uptake have been reviewed (Goldstein et al., 1979; Brown & Goldstein, 1979).

Abbreviations used: LD lipoprotein, low-density lipoprotein; IgG, immunoglobulin G.

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In the chicken, vitellogenin is the principal serum protein sequestered as a high-molecular-weight complex (500 000 mol.wt.). After uptake, it becomes processed by the oocyte into the two glycoprotein phosvitins (34 000 and 28 000 mol.wt.) (Clark, 1970; Shainkin & Perlmann, 1971), and the lipoprotein lipovitellin (Bergink et al., 1974; Deeley et al., 1975). These proteins are subsequently broken down during embryogenesis to provide the needed synthetic components during development. In addition, IgG, transferrin, LD lipoprotein and very-low-density lipoprotein are other proteins known to be transported.

The chicken oocyte system is in some ways an ideal system in which to investigate the specific uptake of certain serum proteins, for more than 1 g of serum protein is sequestered per day per oocyte during the last 5 days of oocyte maturation. Morphologically, the surface of the oocyte is highly convoluted, and is characterized by being covered on its cytoplasmic surface by coat-like material and numerous coated pits (Bellairs, 1967; Roth et al., 1976; Perry & Gilbert, 1979). Thus, both chemically and morphologically, the oocyte is in a state of rapid uptake.

We undertook this study of oestrogen-stimulated vitellogenin binding to the oocyte membrane in order to detail more carefully the binding of a lipoprotein other than LD lipoprotein. In the present study we show the binding of vitellogenin to be specific, saturable and pH-dependent. Importantly, the receptor is a protein that has a fucose residue that is
required for specific binding of the vitellogenin. The phosvitin portion of the vitellogenin appears to be the region of the molecule recognized by the receptor.

Materials and methods

Materials

Protease type V from *Streptomyces griseus*, neuraminidase type V from *Clostridium perfringens*, β-glucosidase from almonds, β-galactosidase from bovine liver. RNA and α-glucosidase type I from yeast were purchased from Sigma: α-L-fucosidase was purchased from Boehringer Mannheim and Siliclad was from Clay–Adams.

Protein purification

Phosvitin was purified by the method of Mecham & Olcott (1949) and Beuving & Gruber (1971). For ease of calculations, we are assuming that phosvitin is only a single glycopolypeptide of 32000 daltons (Clark, 1970; Shainkin & Perlmann, 1971). Vitellogenin was prepared by standard methods (Deeley et al., 1975; Mecham & Olcott, 1949) and further purified by passage over a molecular-sieve column of Bio-Rad A-1.5 agarose. Only vitellogenin preparations showing no evidence of proteolysis were used (Deeley et al., 1975). Chicken-egg IgG was purified as previously described (Roth et al., 1976).

Iodination of proteins

Phosvitin and vitellogenin were iodinated with $^{125}$I by using chloramine-T as described previously (Roth et al., 1976).

Isolation and fixation of oocyte cortex

The oocyte cortex tissue was isolated as previously described (Roth et al., 1976). In some experiments the tissue was fixed for 20min in 6% (w/v) formaldehyde at 0°C in Hanks balanced salts solution, pH 6, without bicarbonate and Phenol Red dye (Hanks & Wallace, 1949) (hereafter called buffer). Membranes were then washed in the following sequence: twice in buffer, 30ml each; once in 20ml of buffer containing 0.1M-glycine; then twice in buffer. Unless stated otherwise, membranes were then finely diced with scissors so that individual pieces were less than 1 mm$^2$ in area.

Binding assays

The composition of the reaction mixtures is presented with each Figure or Table. All solutions were adjusted with NaOH or HCl to the appropriate pH before addition to the reaction tubes. The reactions were started by adding membrane tissue to the tubes. Tubes were incubated in a shaking water bath at 25°C for the indicated times. Tubes incubated for several hours were covered with Parafilm to prevent evaporation. Glass tubes (12 mm x 74 mm) treated with Siliclad were used throughout. In most experiments the reaction was ended by diluting the 250 µl reaction mixture with 4.5ml of buffer containing 5mg of bovine serum albumin/ml, then centrifuging the solution at low speed to sediment the tissue slices. The supernatant solution was removed by suction and the membranes were washed with $3 \times 4.5$ ml of buffer containing 1mg of serum albumin/ml. Before the last centrifugation step the solution was transferred to a new tube, centrifuged and the radioactivity of the membranes determined in a Nuclear–Chicago gamma scintillation counter.

In the time-course experiment (Fig. 1), the reaction was stopped by 100-fold dilution, followed by suction filtration through Whatman GF/C glass-fibre filters. Membranes were subsequently washed on the filters with $4 \times 5$ ml of buffer solution containing 1mg of albumin/ml as described previously (Roth et al., 1976).

In all cases the specific binding that we examined was defined as the difference between the amount of labelled protein bound in the presence and the absence of 50–100 times the approximate $K_D$ of the protein being studied. The labelled protein that remained bound in the presence of excess unlabelled protein may be due to entrapment and/or non-specific binding. Corresponding tubes without membranes (blanks) were incubated and washed in the same manner as the experimental tubes. The amount of labelled material that remained bound to these tubes was subtracted from that for the experimental tubes.

Isolation of chicken blood cells

Chicken blood was obtained by heart puncture, mixed with 15mg of sodium citrate/ml, then centrifuged for 30min at 1000g. Erythrocytes were dispersed in buffer at the desired pH, containing 25mg of bovine serum albumin/ml and 15mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid].

Homogenization of membranes

Membranes isolated from 12 oocytes were homogenized in 3ml of buffer with 15 strokes of a Teflon pestle for use in the binding assays after enzyme treatment. A more detailed description of the method is published elsewhere (Woods & Roth, 1980).

Enzyme treatment of membranes

Samples (200µl) of homogenized membranes were incubated with an equal volume of buffer containing various concentrations of the following enzymes: protease, neuraminidase, β-galactosidase, α-glucosidase, β-glucosidase and α-L-fucosidase. After incubation for 1h at 25°C, the membranes
were diluted 8-fold with buffer and centrifuged at 1200rev./min for 20min in a refrigerated IEC centrifuge. This wash was repeated three more times.

**Phosvitin binding to enzyme-treated membranes**

Treated membranes (200 μl) were incubated with 6 μM-125I-labelled phosvitin in buffer containing 1 mg of albumin/ml. After 1h the reaction mixture was diluted 4-fold with buffer containing 1 mg of albumin/ml and centrifuged in an IEC refrigerated centrifuge at 1200rev./min for 15min. After three more washes the membrane pellets were counted for radioactivity in a Nuclear–Chicago gamma spectrometer. All binding was normalized against parallel samples of untreated membranes in each experiment. As in previous experiments, specific phosvitin binding was determined.

**Results**

**Time course of binding**

We first determined how rapidly specific binding reached an apparent equilibrium, so that under the conditions of a particular experiment sufficient time would have elapsed to ensure maximal binding. As shown in Fig. 1, specific binding reaches an apparent equilibrium by 30min. There is a small additional component of binding that continues after this time, but its contribution to specific binding appears to be negligible.

**Binding as function of vitellogenin concentration**

The specific binding of vitellogenin was examined as a function of ligand concentration below the apparent K_D. In part we wished to determine if there were sites (in the range of 0.3 μM–8 nm) that were markedly different in affinity from those obtained when phosvitin binding was assayed. It proved difficult to carry out binding experiments at higher concentrations, owing to the difficulty of obtaining high concentrations of highly purified vitellogenin. Over the range tested, a Scatchard plot of the data (Fig. 2) yields a straight line which gives a K_D of 6 × 10^{-7}M. Although the limited range of this data

![Fig. 1. Time course of vitellogenin binding](image)

**Fig. 1. Time course of vitellogenin binding**

Tubes were incubated at 25°C with shaking for the indicated times, after which the reaction was stopped by 100-fold dilution, followed by vacuum filtration and four 5 ml washes. Each tube contained 5 mg of bovine serum albumin/ml. 15 mM-Hepes. 50 μg of 125I-labelled vitellogenin (3 × 10^6 c.p.m./μg)/ml and 9.6 ± 0.06 mg of formaldehyde-fixed membranes in 250 μl of buffer, pH 6.0. Tubes with excess of unlabelled competing protein contained 2.9 mg of phosvitin/ml. Values obtained from tubes containing no membranes were subtracted from the radioactivity in tubes containing membranes. Error bars show the S.E.M. for triplicate determinations at each point.

![Fig. 2. Scatchard plot of vitellogenin binding](image)

**Fig. 2. Scatchard plot of vitellogenin binding**

The abscissa is the concentration of bound vitellogenin and the ordinate is the ratio of bound to free vitellogenin. Incubation was for 6h at 25°C with shaking, with fixed membranes. The reaction mixtures contained 5 mg of bovine serum albumin/ml. 15 mM-Hepes, various concentrations of labelled vitellogenin (6.84 × 10^6 c.p.m./μg) and 6.5 ± 0.6 mg of fixed membranes in a total volume of 250 μl of buffer at pH 6.0. To determine specific binding, another set of tubes with labelled protein was incubated with excess unlabelled vitellogenin at 1.7 mg/ml. After correcting the data for labelled protein bound to tubes without membranes, the difference in binding between vitellogenin bound in the presence and absence of unlabelled vitellogenin was plotted. All reactions were terminated by a 20-fold dilution and centrifugation, followed by four more buffer washes containing 1 mg of serum albumin/ml.
set does not permit an accurate extrapolation of the line to the axis, the apparent intercept gives an estimated value of 60 nmol of vitellogenin bound per mg of membrane.

Phosvitin competition for vitellogenin binding

Since phosvitin is a cleavage product of vitellogenin, we wished to determine if phosvitin competed with vitellogenin for binding to the oocyte cortex. Lipovitellin, the other principal component of vitellogenin, could not be used because it is insoluble at physiological salt concentrations. As shown in Table 1, unlabelled phosvitin and vitellogenin both compete with labelled vitellogenin for binding to the oocyte tissue.

Specific displacement of bound protein

The effects of various ligands on the release of 125I-labelled phosvitin or vitellogenin from washed membranes are shown in Fig. 3. Of particular interest is the ability of vitellogenin to enhance the release of phosvitin, and vice versa. In the presence of IgG or RNA, approximately the same amount of labelled ligand is released as in buffer alone. IgG was

<table>
<thead>
<tr>
<th>Additions to 50 μg of labelled vitellogenin/ml</th>
<th>Vitellogenin bound (c.p.m.)</th>
<th>Binding (c.p.m./mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>18,868</td>
<td>50,738 ± 14,457</td>
</tr>
<tr>
<td>Unlabelled phosvitin (1.4 mg/ml)</td>
<td>3783</td>
<td>13,897 ± 3706</td>
</tr>
<tr>
<td>Unlabelled vitellogenin (2.03 mg/ml)</td>
<td>3352</td>
<td>12,376 ± 1252</td>
</tr>
</tbody>
</table>

Fig. 3. Displacement of bound protein

(a) Displacement of bound 125I-labelled phosvitin. Pieces of oocyte cortex were diced and fixed as described. Membranes were incubated with 2 ml of buffer containing 66.7 μg of 125I-labelled phosvitin (163,000 c.p.m./μg)/ml for 1 h. They were washed once with 30 ml of buffer and incubated for 12 h with 30 ml of buffer and then washed seven more times, 30 min each. During the last wash membranes were divided into five portions, centrifuged, and supernatant solutions removed. Each portion was then eluted with 0.5 ml of one of the following non-radiolabelled solutions: 5.5 μM-phosvitin, 6.5 μM-vitellogenin, 5.5 μM-IgG, RNA (2 mg/ml), all at pH 6.0. The total radioactivity in each tube was counted at the beginning of the elution. After 3 h of incubation, solutions were centrifuged and the 125I-labelled phosvitin in the supernatant solutions was measured. Each tube contained about 13 mg wet wt. of oocyte cortex. In (a) each bar represents the amount of 125I-labelled phosvitin eluted from the membranes as a percentage of that eluted by buffer alone. (b) Displacement of bound 125I-labelled vitellogenin. The experimental conditions were the same as in (a), but membranes were incubated in 1 ml of buffer containing 400 μg of 125I-labelled vitellogenin (400,000 c.p.m./μg). The elution of bound 125I-labelled vitellogenin was performed as in (a). The scale is the same as in (a).
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selected since it is also transported from the circulation into the oocyte, and the putative vitellogenin receptor is thus normally exposed to this molecule. RNA was selected to test if a non-protein polyphosphate might display properties similar to those of phosvitin. The data shown in Fig. 3 suggest that phosvitin and vitellogenin may bind to the same site.

**Direct competition by vitellogenin for phosvitin-binding sites**

Vitellogenin inhibits binding of 125I-labelled phosvitin to the oocyte membrane (Fig. 4). The apparent $K_D$ for specific phosvitin binding was approx. 7.8 x 10^{-7} M, and vitellogenin inhibited binding with an apparent $K_I$ of 2.9 x 10^{-7} M. That the two lines meet at the same point on the ordinate suggests that vitellogenin competitively inhibits phosvitin binding.

A 12 h incubation was performed to determine if long incubation times would significantly alter the apparent $K_D$ for phosvitin binding (Fig. 5). The apparent $K_D$ measured after 12 h was 6.5 x 10^{-7} M, a value which is in close agreement with that determined at shorter incubation times.

The fact that phosvitin and vitellogenin bind to the same sites is of practical value, because vitellogenin is not stable at room temperature and is much more difficult to prepare and store than phosvitin. This meant that in subsequent experiments phosvitin could be substituted for vitellogenin for some purposes, such as when large molar excesses of unlabelled protein are added to a reaction mixture to determine what portion of the bound protein is specifically bound.

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Fig. 4. Competition by vitellogenin for phosvitin-binding sites

125I-labelled phosvitin specifically bound to membranes is shown as a function of phosvitin concentration. The reaction mixtures were incubated for 1.5 h at 25° C with shaking. Control tubes (●) contained 5 mg of bovine serum albumin/ml and 15 mM-Hepes in buffer, pH 6.0, 4.5 ± 0.4 mg of formaldehyde-fixed membranes per tube, and 125I-labelled phosvitin (2 x 10^4 c.p.m./μg) at concentrations from 1.3 to 6.25 μM (42.5–200 μg/ml) in a total volume of 250 μl. The concentration of competing vitellogenin (○) was 500 μg/ml (1 μM) in parallel assays. Binding in tubes with excess unlabelled phosvitin (2.26 mg/ml) was subtracted from the corresponding tubes containing only labelled protein to determine specific binding. The radioactivity in blank tubes with no membranes was subtracted from that of the experimental tubes, but not from those with excess unlabelled protein. The binding reaction was ended by 20-fold dilution, followed by centrifugation and washing of membranes. The correlation coefficients for the control and inhibited curves were 0.97 and 0.98 respectively.

Fig. 5. Double-reciprocal plot of phosvitin binding

Binding is plotted as the reciprocal of the amount of phosvitin (pmol) bound versus the reciprocal of phosvitin concentration. Incubation was for 12 h at 25° C, with shaking with formaldehyde-fixed membranes. The reaction mixtures contained 5 mg of bovine serum albumin/ml. 15 mM-Hepes, both labelled and unlabelled phosvitin and 4.5 ± 0.4 mg of fixed membranes in a total volume of 250 μl of buffer, pH 6.0. The concentrations of labelled phosvitin (1.07 x 10^4 c.p.m./μg) ranged from 5.1 μM to 1.56 μM (1.63–50 μg/ml). Tubes with excess unlabelled competing phosvitin contained 3 mg of phosvitin/ml. The reactions were terminated by 20-fold dilution and centrifugation, followed by four washes. The correlation coefficient was 0.99.
**pH-dependence of vitellogenin binding**

In vivo, the oocyte plasma membrane is bathed on one side by the developing egg yolk and on the other side by the plasma after it has percolated between several layers of cells. Since we had determined previously that the pH of the yolk is 6, it seemed appropriate to determine the pH at which maximal specific binding occurs. There is a distinct optimum for specific vitellogenin binding at pH 6.0 (Fig. 6). Specific binding decreases rapidly as the pH is increased and is 8-fold lower by pH 7.5.

**Tissue specificity**

To determine the tissue specificity of phosvitin binding, binding of $^{125}$I-labelled phosvitin to whole erythrocytes and oocyte membranes was compared, as shown in Table 2. Erythrocytes were chosen since they are exposed to vitellogenin during its transport from the liver to the ovary by the blood. Binding was measured at pH 7.5 (the approximate pH of blood) and pH 6.0, the pH of the yolk contents as well as the pH optimum for vitellogenin binding. Little specific binding to erythrocytes was found at either pH. What slight binding did occur was more pronounced at pH 7.5 that at pH 6.0, whereas specific vitellogenin binding to oocyte membranes was greater at pH 6 than at pH 7.5.

**Phosvitin binding to enzyme-treated membranes**

The previous experiments demonstrated that phosvitin and vitellogenin bind to a common receptor. In an attempt to characterize the receptor chemically, we compared phosvitin binding to enzyme-treated and untreated membranes. As shown in Fig. 7, a variety of enzymes were tested, but only protease and fucosidase were particularly effective in decreasing phosvitin binding to the putative receptor. Both enzymes were able to decrease binding significantly, and in each case binding decreased as the enzyme concentration was raised. Interestingly, low amounts of protease consistently enhanced phosvitin binding.

Once we noted the proteinase-sensitivity of phosvitin binding, we tested the other enzymes for contaminating proteinases by using the Calbiochem assay (Table 3). Of those enzymes that affect

![Fig. 6. pH-dependence of vitellogenin binding](image)

**Table 2. Membrane specificity of phosvitin binding**

<table>
<thead>
<tr>
<th>Tissues</th>
<th>pH 6.0</th>
<th>pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oocyte cortex</td>
<td>6120 ± 396 (0.464 mg)</td>
<td>1722 ± 43 (0.470 mg)</td>
</tr>
<tr>
<td>Whole chicken erythrocytes</td>
<td>-101 ± 89 (0.452 mg)</td>
<td>327 ± 225 (0.418 mg)</td>
</tr>
</tbody>
</table>

Each permutation in the experiment was run in triplicate and the average is presented ± S.E.M. The experiment was conducted at 25°C with shaking. Tissues were not fixed. Tubes contained 1 mg of serum albumin/ml in buffer with 15 mM-Hepes and 50 μg of vitellogenin (2300 c.p.m./μg)/ml. Tubes with excess unlabelled protein contained 2.5 and 3 mg of phosvitin/ml, respectively, at pH 7.5 and 6.0. Incubation was for 1 h and was ended by centrifugation (10,000 g-min), and membranes were washed four times at the pH of the respective reaction mixture. Specific binding was determined by subtracting the amount of iodinated vitellogenin bound in the presence of phosvitin (4.83 mg/ml) from that bound in the absence of the phosvitin. The amount of radioactive vitellogenin bound to tubes which contained no membranes was subtracted to correct for tube-bound vitellogenin. Error bars show the S.E.M.
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binding, proteinase and neuraminidase hydrolysed the diazo-collagen substrate to give a marked absorbance at 520nm. Fucosidase showed no detectable proteinase activity at the highest concentration used in our experiments.

Discussion

Lipoproteins such as vitellogenin, in the present study, and LD lipoprotein, in particular, have been shown to enter cells by binding to receptors embedded in the plasma membrane (Roth & Porter, 1964; Cutting & Roth, 1973; Roth et al., 1976; Goldstein et al., 1979; Brown & Goldstein, 1979). Our work (Roth & Porter, 1964; Cutting & Roth, 1970, 1973; Yusko & Roth, 1976; Roth et al., 1976, 1977) and more recently that of Goldstein and co-workers (Goldstein et al., 1979; Brown & Goldstein, 1979) have amply demonstrated that the bound lipoproteins are internalized by coated pits and coated vesicles. Like specific uptake of proteins in a variety of cases, vitellogenin binding is saturable, specific and occurs at the plasma membrane as the initial event in the uptake process.

The pH optimum of 6 at which vitellogenin binds to the oocyte is of interest, since this is the pH of the oocyte yolk contents. It can be argued that, since the oocyte surface is several cells removed from the nearest blood sinus, any lactate released by glycolysis in the oocyte into the microenvironment near the plasma membrane will not be immediately swept away. Thus it is likely that a receptor with a pH optimum near 6 could have evolved as a means of increasing the specificity of the surface binding. If the vitellogenin receptor exists on tissues which are more directly bathed by the blood at or near pH 7.4, binding to these receptors would be low, since this is the pH of minimum binding. A similar mechanism was previously proposed for IgG binding and subsequent release in the rat intestine, as studied by Rodewald (1976).

It has become evident over the last few years that an increasingly wide variety of proteins, as well as proteins that contain sugars and/or lipids, enter cells via coated-pit/coated-vesicle mechanisms (Roth et al., 1976; Goldstein et al., 1979; Brown & Goldstein, 1979). The universality of the mechanism in all animal cells marks it as a major cellular process. In the present study, it is now evident that the chicken oocyte is a system that not only lends itself to quantitative analysis of the kinetic properties of receptor–lipoprotein interactions but promises to be one of the most enriched membrane systems available for examining the process. Our earlier studies had demonstrated that very large quantities of protein were sequestered, more than 1g of protein per day per oocyte (Cutting & Roth, 1973). They also showed that at certain developmental stages at least 50% of the membrane surface was covered with coated pits (Roth et al., 1976).

Other proteins are also specifically sequestered by

![Graph](image_url)

**Fig. 7. Effect of enzymes on receptor recognition**

Enzyme-treated and untreated membranes were incubated for 1h at 25°C with 125I-labelled phosvitin. The numbers within each bar represent the enzyme concentration in µg/ml. After washing, the specifically bound radioactivity was normalized as a percentage of that bound to untreated membrane. Separate controls were compared with enzyme treatments in each experiment. The controls were combined and composite s.e.m. values calculated.

<table>
<thead>
<tr>
<th>Azocoll added (mg)</th>
<th>Test solution</th>
<th>A_{520}</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.05 Buffer alone</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>4.95 Protease (10µg/ml)</td>
<td>1.297</td>
<td></td>
</tr>
<tr>
<td>5.04 Protease (1µg/ml)</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>5.01 Protease (1µg/ml)</td>
<td>0.364</td>
<td></td>
</tr>
<tr>
<td>4.96 Neuraminidase (100µg/ml)</td>
<td>0.154</td>
<td></td>
</tr>
<tr>
<td>5.05 Fucosidase (100µg/ml)</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. **Proteolytic activity of fucosidase**

Azocoll assay was used for proteolytic activity in fucosidase and neuraminidase. Calbiochem Azocoll was incubated with 1ml of buffer or buffer plus enzyme for 1h at 37°C. The solution was then centrifuged for 15000g-min and the A_{520} of the supernatant measured.
the chicken oocytes, as shown by our studies of binding very-low-density lipoprotein, LD lipoprotein and IgG (Roth et al., 1976; Krumins & Roth, 1981). Studies demonstrating the isolation of the phosvitin receptor and its properties are published elsewhere (Woods & Roth, 1980).

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