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Binding of concanavalin A to isolated hepatocytes and its effect on uptake and degradation of asialo-fetuin by the cells

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1. The binding of ^{125}I-labelled concanavalin A to isolated rat hepatocytes was studied at temperatures between 4°C and 37°C. At the latter temperature, concentrations of concanavalin A from 0.01 to 0.4 mg/ml were used. In all of these experiments, binding reached a plateau after 40–60 min, when 28–35% of the concanavalin A added was bound to the cells (cell density 8×10^6 cells/ml). 2. The rate of uptake of ^{125}I-labelled asialo-fetuin by the hepatocytes was lowered to 30% of control values when the cells were preincubated with 0.1 mg of concanavalin A/ml. This decrease could be accounted for by a decrease in the rate of binding of asialo-fetuin to the β-galactoside receptor of the cells. The binding capacity of the cells was not influenced by preincubation with concanavalin A. 3. Degradation of asialo-fetuin was decreased only if concanavalin A was present during the uptake of asialo-fetuin by the cells. Subcellular fractionation revealed that concanavalin A lowered the rate of entry of endocytosed asialo-fetuin into the lysosomes. The effect of concanavalin A on degradation is distinct from its effect on the rate of uptake of asialo-fetuin by hepatocytes.

When sialic acid is removed from any one of a number of plasma glycoproteins, so that the penultimate galactoside residues of their carbohydrate chains are exposed, the proteins are rapidly taken up by the liver (Ashwell & Morell, 1974). The uptake process is mediated by a protein that binds β-galactoside residues with high affinity (Sarkar *et al.*, 1979). The receptor is found only on hepatocytes (Tolleshaug *et al.*, 1977). The asialo-glycoproteins are rapidly taken into the cell, transported through the cytoplasm and degraded in the lysosomes (LaBadie *et al.*, 1975; Tolleshaug *et al.*, 1979). This process has important features in common with receptor-mediated endocytosis of many other proteins (Goldstein *et al.*, 1979), including lysosomal enzymes (Stahl *et al.*, 1978; Ullrich *et al.*, 1979) and peptide hormones (Shechter *et al.*, 1978; Carpentier *et al.*, 1979).

Concanavalin A is a lectin of mol.wt. 110000 that binds four α-mannoside or α-glucoside residues (So & Goldstein, 1968). It has been used extensively as a tool in studies of the turnover of the plasma membrane (Noonan & Burger, 1973; Sharon & Lis, 1972). In some cell types concanavalin A promotes endocytosis (Edelson & Cohn, 1974; Zweig & Singer, 1979). The lectin also promotes uptake of lysosomal enzymes, probably by acting as a bridge between carbohydrate chains on the enzyme and carbohydrate chains on the cell surface (Juliano *et al.*, 1978; Gonzalez-Noriega & Sly, 1979; Beeck *et al.*, 1979). In macrophages, it prevents the fusion between phagosomes and lysosomes (Edelson & Cohn, 1974). On hepatocytes, the binding sites for concanavalin A are distributed evenly all over the cell surface (Guillouzo & Feldmann, 1977; Roth, 1974).

The binding capacity of freshly isolated hepatocytes for asialo-glycoproteins is approx. 0.7 pmol/10^6 cells (Kolset *et al.*, 1979; Tolleshaug & Berg, 1979). The maximum rate of receptor-mediated uptake is about 0.2 pmol/min per 10^6 cells (Tolleshaug *et al.*, 1977). Taken together, these values mean that about one-third of the occupied receptors are internalized every minute. This very high rate of internalization implies some kind of a selective mechanism, probably involving clustering of the receptors in specialized areas of the plasma membrane (Goldstein *et al.*, 1979).

We have previously shown that treatment of hepatocytes with chloroquine, a membrane-modifying drug (DiDonato *et al.*, 1977), causes a diminution in the binding capacity of the plasma membrane for asialo-glycoproteins (Tolleshaug & Berg, 1979). The present study was initiated in order to examine the influence of concanavalin A on the turnover of the asialoglycoprotein receptors of hepatocytes. Furthermore, concanavalin A might influence the intracellular distribution and degrada-

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tion of asialo-fetuin in hepatocytes. These effects could throw some light on the mechanism and the kinetics of intracellular degradation of asialo-glycoproteins in hepatocytes.

Note on nomenclature

In cell biology, the term 'receptor' is commonly used to denote the binding site in all instances of binding of a molecule to a limited number of sites on the surface of a cell (Langley, 1905). If we were to follow this usage in the present paper, we would have to speak on one hand of a 'concanavalin A receptor' (a carbohydrate moiety that is bound on the cell surface), and on the other hand of an 'asialoglycoprotein receptor' (a protein in the cell membrane that binds carbohydrate side chains of asialo-glycoproteins). To avoid unnecessary ambiguity, we here use the term 'binding site for concanavalin A', recognizing that the term 'binding site' may legitimately be used in other connections to denote those parts of the concanavalin A molecule that actually bind the sugar residues.

Materials and methods

Liver cells were prepared by a modification (Seglen, 1976) of the collagenase perfusion method of Berry & Friend (1969). Hepatocytes were separated from non-parenchymal cells by differential centrifuging (Nilsson & Berg, 1977). They were incubated in a minimal salt medium containing 1% charcoal-treated bovine serum albumin (Tolleshaug *et al.*, 1977) in a shaking water bath.

Concanavalin A and fetuin were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Fetuin was desialylated by treatment with neuraminidase (Tolleshaug *et al.*, 1979). Concanavalin A and asialo-fetuin were labelled with ^{125}I by the lactoperoxidase method of Frantz & Turkington (1972) as described previously (Tolleshaug *et al.*, 1977). Both labelled proteins had specific radioactivities of about 0.5 μCi/μg. In some experiments with very low concentrations of asialo-fetuin, the protein was iodinated by the sodium hypochlorite oxidation method of Redshaw & Lynch (1974). To minimize the oxidation of the carbohydrate side chains, the reaction was stopped by the addition of 10 μl of a solution (20 mg/ml) of $NaBH_4$ in 10 mM-NaOH. The specific radioactivity was 50 μCi/μg, or about one ^{125}I atom per asialo-fetuin molecule. Uptakes of asialo-fetuin labelled by the lactoperoxidase method and the hypochlorite method were indistinguishable.

Degradation (acid-soluble radioactivity) was followed by mixing a 250 μl portion of the cell suspension with an equal volume of ice-cold 4% (w/v) phosphotungstic acid in 2 M-HCl (LaBadie *et al.*, 1975; Tolleshaug *et al.*, 1977), followed by centrifuging. Uptake of labelled proteins by the cells was determined by placing a 250 μl portion on top of an equal volume of dibutyl phthalate and centrifuging immediately in a Beckman bench centrifuge (Tolleshaug *et al.*, 1977). In this manner the cells were separated from the medium. The amount of radioactivity in the cell pellet is referred to as 'cell-associated radioactivity'. This amount includes an amount of acid-soluble radioactivity that is approx. 2% of the extracellular acid-soluble radioactivity (Tolleshaug *et al.*, 1979). To provide a complete picture of the uptake of asialo-fetuin by the cells, the amounts of cell-associated and acid-soluble radioactivity at a given time point were added, and the sum was called 'total uptake'.

Isopycnic centrifuging in a sucrose density gradient has been described in detail previously (Tolleshaug *et al.*, 1979). Briefly, the washed hepatocytes were homogenized in a Dounce homogenizer, the nuclei were removed by differential centrifuging, and a portion of the resulting 'cytoplasmic extract' was layered on top of the sucrose gradients, which were spun at 25000 rev./min in a Beckman SW-27 rotor for 4 h at 4°C.

Results

Binding of ^{125}I-labelled concanavalin A to hepatocytes at 37°C

Fig. 1 shows the time course of increase in the amount of cell-associated ^{125}I-labelled concanavalin A at different initial concentrations. The maximum percentage bound is about 35% at 0.01 mg/ml as well as at 0.4 mg/ml. After 40 min the reaction was assumed to be at equilibrium because the fraction of cell-associated ^{125}I-labelled concanavalin

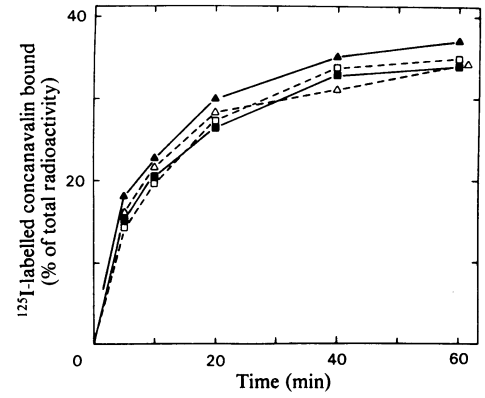

Fig. 1. *Binding of ^{125}I-labelled concanavalin A to hepatocytes at 37°C*

For experimental details see the text. The cell density was 8×10^6/ml, and the initial concentrations of ^{125}I-labelled concanavalin A were: ■, 0.001 mg/ml; □, 0.01 mg/ml; ▲, 0.1 mg/ml; △, 0.4 mg/ml.

A did not change with time. The equilibrium may be described by the familiar equation:

$$K_a = \frac{C_b}{(B_0 - C_b)(C_0 - C_b)} \quad (1)$$

where C_b is the concentration of bound concanavalin A, C_0 is the initial concentration of the lectin and B_0 is the initial concentration of binding sites. If C_b is very small in comparison with B_0, the equation may be written as:

$$\frac{C_b}{C_0 - C_b} = K_a \cdot B_0 = \text{constant} \quad (2)$$

In other words, the fraction of bound concanavalin A does not change with the amount of lectin added to the system if the amount bound is small compared with the total binding capacity of the cells in the suspension. In absolute values, 35% bound out of a total of 0.4 mg/ml added to a cell suspension containing 8×10^6 cells/ml implies that 96×10^6 molecules of ^{125}I-labelled concanavalin A are bound per cell.

At 37°C, 60% of the cell-associated ^{125}I-labelled concanavalin A was removed from cells by washing and re-incubation in a buffered iso-osmotic 0.1 M solution of methyl α-mannoside. This extent of dissociation is comparable with the results of similar experiments with cultured fibroblasts (Noonan & Burger, 1973).

Measurements of binding of ^{125}I-labelled concanavalin A were also obtained at 4, 10 and 15°C. The initial concentration of the lectin was 0.01 mg/ml. In all other respects the experimental conditions were identical with those stated in the legend to Fig. 1. At the low temperatures the results were very similar to those obtained at 37°C. The maximum percentage bound was between 28 and 32%.

As the binding capacity of the hepatocytes is evidently far from saturated even at high concentrations of concanavalin A, it did not seem fruitful to try to determine the total binding capacity of the cells and the association constant of the binding site for concanavalin A by means of a Scatchard plot.

Intracellular distribution of ^{125}I-labelled concanavalin A

The cells were incubated with a trace amount (0.01 mg/ml) of ^{125}I-labelled concanavalin A for 10 min at 37°C. A cytoplasmic extract was prepared from the homogenate (see the Materials and methods section) and fractionated by isopycnic centrifugation in a sucrose density gradient. The distribution of the radioactive label in the gradient was closely similar to that of the plasma-membrane marker enzyme 5′-nucleotidase (Fig. 2), indicating that all of the cell-associated ^{125}I-labelled concanavalin A is associated with the plasma membrane.

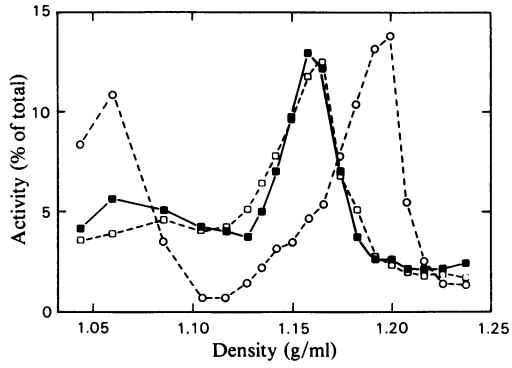

Fig. 2. *Distributions of ^{125}I-labelled concanavalin A and the marker enzymes 5′-nucleotidase (plasma membrane) and acid phosphatase (lysosomes) in a sucrose density gradient*

The cells (8×10^6/ml) were incubated with ^{125}I-labelled concanavalin A (0.01 mg/ml) for 10 min before the washing and fractionation (see the Materials and methods section). The recoveries of the various activities shown were between 95 and 105%: □, ^{125}I-labelled concanavalin A; ■, 5′-nucleotidase; O, acid phosphatase.

If the cells were incubated at 37°C for 60 min instead of 10 min, the distributions were identical (not shown). No peak was found under the peak of activity of the lysosomal marker enzyme acid phosphatase. The absence of any lysosome-associated ^{125}I-labelled concanavalin A even after 60 min of incubation may mean that lectin that enters the lysosomes is degraded rapidly, so that no accumulation occurs (Tolleshaug *et al.*, 1979). A more important factor is probably the inhibition of phagolysosome formation by concanavalin A (Edelson & Cohn, 1974).

Effects of concanavalin A on the uptake and degradation of asialo-fetuin

Figs. 3 and 4 show the results of an experiment in which the cells were preincubated with 0.05, 0.1 or 0.2 mg of unlabelled concanavalin A/ml (no addition to control cells) before labelled asialo-fetuin was added at time zero. Total acid-soluble radioactivity (Fig. 4) and cell-associated radioactivity were determined, and these two quantities were combined to give total uptake (Fig. 3) (see the Materials and methods section). After 20 min of incubation, the extent of degradation was small. Up to this time point, 'cell-associated radioactivity' and 'total uptake' were nearly the same. It is evident from Fig. 3 that the rate of uptake is lowered in the presence of concanavalin A. Nearly maximal effect is seen with

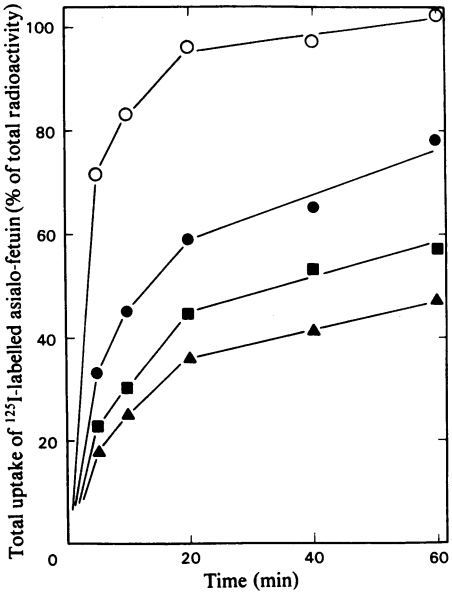

Fig. 3. *Effects of various concentrations of concanavalin A on the uptake of ^{125}I-labelled asialo-fetuin*
The cells (8×10^6/ml) were preincubated with various concentrations of unlabelled concanavalin A for 25 min at 37°C before the addition of 7 nM ^{125}I-labelled asialo-fetuin at time zero. The initial concentrations of concanavalin A were: ○, 0 (control); ●, 0.05 mg/ml; ■, 0.1 mg/ml; ▲, 0.2 mg/ml. Cell-associated radioactivity and degradation were determined, and these two percentages were added to give 'total uptake' at each time point (with a correction for the tiny amount of acid-soluble radioactivity in the cells; see the Materials and methods section).

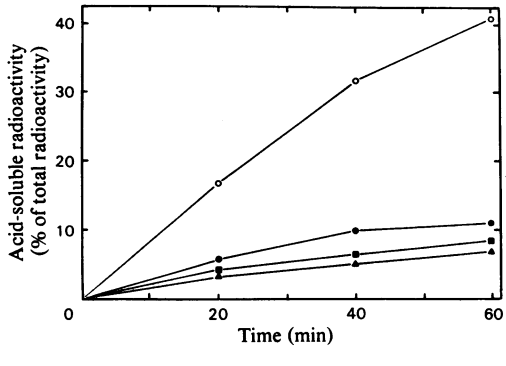

Fig. 4. *Effects of various concentrations of concanavalin A on the degradation of ^{125}I-labelled asialo-fetuin*
The data are from the same experiment as that shown in Fig. 3. The initial concentrations of concanavalin were: ○, 0 (control); ●, 0.05 mg/ml; ■, 0.1 mg/ml; ▲, 0.2 mg/ml.

a concentration of 0.1 mg/ml. At 20 min the amount of cell-associated radioactivity in the presence of concanavalin A (0.1 mg/ml) was 51% of that in the control cells, in which the cell-associated radioactivity actually decreased after 20 min because significant amounts of degradation products were produced (Fig. 4) and released from the cells. In the concanavalin A-treated cells, degradation of asialo-fetuin was sharply decreased at all concentrations of concanavalin A that were tested (Fig. 4). When interpreting this observation, it should be kept in mind that only asialo-fetuin that had been taken up by the cells was degraded, so that a smaller total uptake will also lead to a lower extent of degradation. Accordingly, a lower rate of degradation in the concanavalin A-treated cells could simply be a consequence of the smaller amount of asialo-fetuin available for degradation in these cells.

However, if the rate of degradation is seen in conjunction with the amount of cell-associated asialo-fetuin at 20 min, then it is readily seen that in cells treated with concanavalin A the decrease in rate of degradation (Fig. 4) is much more pronounced than would be expected from the decreased uptake. At 0.1 mg of concanavalin A/ml the rate of degradation is 15% of that in the untreated cells, whereas the percentage of cell-associated radioactivity after 20 min of incubation with asialo-fetuin is 51% of control cells. Thus concanavalin A has an effect on degradation in addition to its effect on uptake.

Next, an effort was made to establish whether concanavalin A has an effect on the degradation of asialo-fetuin that has been internalized before the cells are exposed to the lectin. The cells were allowed to take up labelled asialo-fetuin (initial concentration 6 nM) for 15 min at 37°C, and then the extracellular asialo-fetuin was removed by washing. The cell suspension was divided into three parts: one control flask (no addition), and two flasks to which were added concanavalin A to final concentrations of 0.2 and 0.4 mg/ml. The flasks were re-incubated at 37°C and the amount of acid-soluble radioactivity was determined at 10 min intervals for 40 min. There was no difference between the degradation in the control cells and that in the concanavalin A-treated cells. This experiment shows that concanavalin A has no effect on degradation unless it is present during the uptake of asialo-fetuin.

Intracellular distribution of ^{125}I-labelled asialo-fetuin in the presence of concanavalin A

To study the effect of concanavalin A on the intracellular distribution of asialo-fetuin, cells were allowed to take up asialo-fetuin in the presence of concanavalin A (0.2 mg/ml) or in the absence of lectin (controls). The time course of uptake and degradation of asialo-fetuin was followed during

70 min, with results identical with those shown in Fig. 3. After the end of the incubation, the suspension was put on ice, the cells were centrifuged down and a cytoplasmic extract was fractionated by isopycnic centrifuging in a sucrose density gradient (see the Materials and methods section). After 70 min of incubation, the distribution of radioactive label from asialo-fetuin was expected to show two distinct peaks in the gradient (Tolleshaug *et al.*, 1979), namely one that was associated with the lysosomal marker enzyme acid phosphatase at $d = 1.19$ and the other in the same region as the plasma-membrane marker enzyme 5'-nucleotidase at $d = 1.14$.

This expectation was fulfilled as regards the control cells. In the lectin-treated cells, however, the lysosome-associated peak was missing (Fig. 5), and much more radioactivity was found at the top of the gradient. Concanavalin A had no effect on the positions of the peaks of the marker enzymes in the gradients (Fig. 5). There is good evidence that the ^{125}I-labelled asialo-fetuin in the middle of the gradient at $d = 1.14$ actually represents endocytic vesicles (Tolleshaug *et al.*, 1979). Accordingly, it seems that concanavalin A prevents fusion between endocytic vesicles and lysosomes.

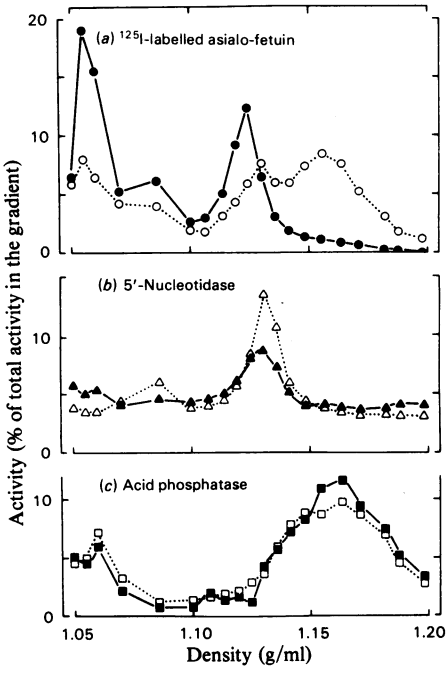

Fig. 5. *Effects of concanavalin A on the distributions of ^{125}I-labelled asialo-fetuin (a), the plasma-membrane marker enzyme 5'-nucleotidase (b) and the lysosomal marker enzyme acid phosphatase (c) in a sucrose density gradient*

Two flasks were used, each containing 10 ml of cell suspension with 8×10^6 cells/ml. Both flasks were preincubated for 25 min at 37°C, one of them in the presence of 0.2 mg of unlabelled concanavalin A/ml (●, ▲ and ■); there was no addition to the control flask (○, △ and □). At zero time, 17 nM ^{125}I-labelled asialo-fetuin was added to both flasks. After 70 min the cells in both flasks were washed and fractionated (see the Materials and methods section).

Binding of asialo-fetuin concanavalin A-treated hepatocytes at 10°C

Two types of experiments were performed: first, the influence of concanavalin A on the rate of the binding reaction between asialo-fetuin and hepatocytes was investigated, and, secondly, the association constant and number of binding sites in the presence and absence of concanavalin A were determined.

In order to measure the influence of concanavalin A on the rate of the binding reaction, the hepatocytes were first preincubated with concanavalin A at 37°C at two different concentrations, then the temperature was lowered to 10°C, a trace amount of asialo-fetuin was added and the amount of cell-associated asialo-fetuin was determined at suitable intervals. Fig. 6 shows that a moderate concentration (0.05 mg/ml) of concanavalin A has a definite effect on the rate of uptake, and that a high concentration (0.2 mg/ml) lowers the rate markedly.

With the use of essentially the same experimental protocol (except that only one concentration of

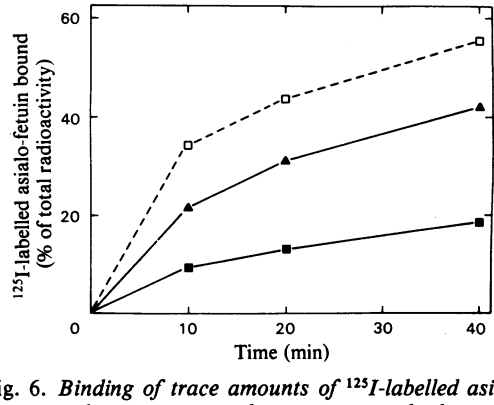

Fig. 6. *Binding of trace amounts of ^{125}I-labelled asialo-fetuin to hepatocytes in the presence and absence of concanavalin A at 10°C*

The cells (8×10^6/ml) were incubated with 0.05 mg (▲) or 0.2 mg (■) of concanavalin A/ml for 25 min at 37°C; there was no addition to control cells (□). Then the temperature was lowered to 10°C, and 0.14 nM ^{128}I-labelled asialo-fetuin was added to each flask.

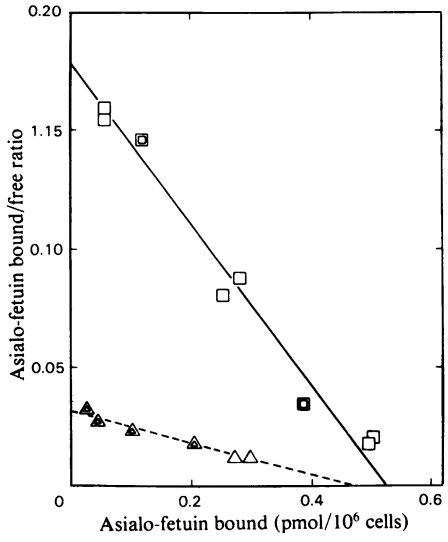

Fig. 7. *Determination of the association constant and the total binding capacity in the presence or absence of concanavalin A*

The cells (8×10^6/ml) were incubated with 0.2 mg of concanavalin A/ml (△) for 25 min at 37°C; there was no addition to control cells (□). The temperature was lowered to 10°C, and each cell suspension was divided into five parts. ^{125}I-labelled asialo-fetuin was added to make the final cell suspensions 1–30 nM with respect to labelled glycoprotein. The incubation was continued for 3 h at 10°C. Duplicate uptake samples were taken in the usual manner to determine total cell-associated radioactivity. To the remainders of the cell suspensions was added the chelator EGTA to make the final concentration 3 mM (50% excess over free Ca^{2+} ions). The cells were briefly warmed to 37°C. This procedure removes surface-bound asialo-fetuin (Tolleshaug *et al.*, 1979), so that uptake samples from EGTA-treated suspensions represent internalized asialo-glycoprotein. In calculating the amount of surface-bound asialo-fetuin, the amount of internalized radioactivity was subtracted from the amount of total radioactivity.

concanavalin A was used) the amount of plasma-membrane-bound asialo-fetuin at equilibrium was determined at several different concentrations of asialo-fetuin, and the results were plotted in the form of a Scatchard (1949) plot. The binding capacities of the two batches (concanavalin A-treated and untreated) of cells were the same within the experimental error, namely 0.6 pmol/10^6 cells (Fig. 7). The association constant was, however, 5-fold higher in the control cells (0.34 ± 0.02 versus 0.066 ± 0.007 nM^{-1}; means ± S.D.) Although this difference between the respective association constants is highly statistically significant, it does not actually indicate a drastic effect by concanavalin A on the affinity of the hepatocytes for asialo-fetuin (see the Discussion section).

Discussion

In sucrose density gradients distributions of labelled concanavalin A and the plasma-membrane marker enzyme are very similar when the cells have been incubated at 37°C. This is strong indication (but not proof) that most of the cell-associated concanavalin A is bound to the surface of the cells. Further evidence comes from a comparison of the extent of binding at 10°C and at 37°C. The cell membrane is rather rigid at 10°C (Gordon *et al.*, 1978), allowing only a very low rate of endocytosis (Duncan & Lloyd, 1978). If concanavalin A is endocytosed at a significant rate at 37°C, more of the lectin would be expected to be cell-associated at this temperature than at 10°C. This is not the case. From the measurements of binding at different initial concentrations of concanavalin A, it does not appear that the binding of the lectin to hepatocytes at 37°C is truly saturable.

In contrast with other workers who have been using concanavalin A to influence the rate of phagocytosis or adsorptive endocytosis (see the introduction), we found a decrease in the rate of uptake of asialo-fetuin by isolated rat hepatocytes at 37°C. At this temperature the increase in cell-associated radioactivity reflects binding to the receptor on the plasma membrane, followed by internalization.

Concanavalin A lowered the rate of binding of asialo-fetuin to the cell surface at 10°C. This decrease could reflect either a decrease in the rate constant of the reaction or in the number of receptors. Analysis of the total binding capacity for asialo-fetuin in the presence or absence of concanavalin A revealed that the binding capacity was unaltered, but that the association constant was decreased 4-fold. This finding indicated that a decrease in the rate constant for binding to the receptor is responsible for the substantial decrease in the rate of uptake of asialo-fetuin at 37°C in the presence of concanavalin A.

The observed decrease in the association constant does not, however, indicate a drastic modification of the receptor or its immediate environment by concanavalin A. A 4-fold change in the association constant implies a change in the free energy of binding of 3 kJ/mol (at physiological temperatures), whereas the change in free energy on binding of methyl α-D-mannoside to concanavalin A is about 25 kJ/mol, and the change in free energy on binding of asialo-fetuin to the hepatocyte receptor is 51 kJ/mol. A possible explanation for the effect of concanavalin A on the association constant of the receptor

for asialo-fetuin is simply steric hindrance as a consequence of binding of concanavalin A to the receptor, which is itself a glycoprotein containing mannose residues (Kawasaki & Ashwell, 1976).

The effect on concanavalin A on the degradation of asalio-fetuin is very marked. The result that concanavalin A has to be present during the process of endocytosis in order to influence the fusion of endocytic vesicles with lysosomes is in agreement with previous work in macrophages (Edelson & Cohn, 1974). In the digestion of asialo-fetuin by the isolated hepatocyte, the rate of fusion of endocytic vesicles with lysosomes seem to be slow (Tolleshaug *et al.*, 1979). The rate has been shown to be sensitive to several familiar drugs (Kolset *et al.*, 1979; Berg & Tolleshaug, 1980). It appears from our results that the process of formation of endocytic vesicles proceeds normally in the presence of concanavalin A. As concanavalin A cannot cross the membrane, it must be found on the inside of membrane of the endocytic vesicle. Accordingly, it appears that concanavalin A affects fusion by influencing the distribution of those membrane components that are responsible for the fusion between endocytic vesicles and lysosomes.

References

Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol.* **41**, 99–128

Beeck, H., Ullrich, K. & von Figura, K. (1979) *Biochim. Biophys. Acta* **583**, 179–188

Berg, T. & Tolleshaug, H. (1980) *Biochem. Pharmacol.* **29**, 917–925

Berry, M. N. & Friend, D. S. (1969) *J. Cell Biol.* **43**, 506–520

Carpentier, J. L., Gorden, P. & Freychet, P. (1979) *J. Clin. Invest.* **63**, 1249–1261

DiDonato, S., Wiesmann, U. N. & Herschkowitz, R. N. (1977) *Biochem. Pharmacol.* **26**, 7–10

Duncan, R. & Lloyd, J. B. (1978) *Biochim. Biophys. Acta* **544**, 647–655

Edelson, P. J. & Cohn, Z. A. (1974) *J. Exp. Med.* **140**, 1364–1385

Frantz, W. L. & Turkington, R. W. (1972) *Endocrinology* **91**, 1545–1552

Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279**, 679–685

Gonzalez-Noriega, A. & Sly, W. S. (1978) *Biochem. Biophys. Res. Commun.* **85**, 174–182

Gordon, L. M., Sauerheber, R. D. & Esgate, J. A. (1978) *J. Supramol. Struct.* **9**, 299–326

Guillouzo, A. & Feldmann, G. (1977) *J. Histochem. Cytochem.* **25**, 1303–1310

Juliano, R. L., Moore, M. R., Callahan, J. W. & Lowden, J. A. (1978) *Biochim. Biophys. Acta* **513**, 285–291

Kawasaki, T. & Ashwell, G. (1976) *J. Biol. Chem.* **251**, 5292–5299

Kolset, S. O., Tolleshaug, H. & Berg, T. (1979) *Exp. Cell Res.* **122**, 159–167

LaBadie, J. H., Chapman, K. P. & Aronson, N. N. (1975) *Biochem. J.* **152**, 271–279

Langley, J. N. (1905) *J. Physiol. (London)* **33**, 374–413

Nilsson, M. & Berg, T. (1977) *Biochim. Biophys. Acta* **497**, 171–182

Noonan, K. D. & Burger, M. M. (1973) *J. Biol. Chem.* **248**, 4286–4292

Redshaw, M. R. & Lynch, S. S. (1974) *J. Endocrinol.* **60**, 527–528

Roth, J. (1974) *Int. J. Cancer* **14**, 762–770

Sarkar, M., Liao, J., Kabat, E. A., Tanabe, T. & Ashwell, G. (1979) *J. Biol. Chem.* **254**, 3170–3174

Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672

Seglen, P. O. (1976) *Methods Cell Biol.* **13**, 29–59

Sharon, N. & Lis, H. (1972) *Science* **177**, 949–959

Shechter, Y., Hernaez, L. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5788–5791

So, L. L. & Goldstein, I. J. (1968) *Biochim. Biophys. Acta* **165**, 398–404

Stahl, P. D., Rodman, J. S. , Miller, M. J. & Schlesinger, P. H. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 1399–1403

Tolleshaug, H. & Berg, T. (1979) *Biochem. Pharmacol.* **28**, 2919–2922

Tolleshaug, H., Nilsson, M., Berg, T. & Norum, K. R. (1977) *Biochim. Biophys Acta* **499**, 73–84

Tolleshaug, H., Berg, T., Frölich, W. & Norum, K. R. (1979) *Biochim. Biophys. Acta* **585**, 71–84

Ullrich, K., Basner, R., Gieselmann, V. & von Figura, K. (1979) *Biochem. J.* **180**, 413–419

Zweig, S. & Singer, S. J. (1979) *J. Cell Biol.* **80**, 487–491