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DYNAMIC STATE OF CONCANAVALIN A RECEPTOR INTERACTIONS ON FIBROBLAST SURFACES

CH. HUET* and A. BERNADAC

Institut de Recherches Scientifiques sur le Cancer, P.O. Box 8, 94800-Villejuif (France)

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SUMMARY

Cultured normal and transformed fibroblasts were treated "in situ" by the concanavalin A-peroxidase labelling technique. It is known that peroxidase recognizes only a fraction of the bound lectin depending on the cell type. Kinetics studies revealed that 80 to 95 % of the peroxidase and only 10 % of the lectin are released from the cell surface when the labelled cells were reincubated at 37 °C. It is shown that it is mostly the concanavalin traced by peroxidase that is released and also that the lectin and the enzyme are shed as a complex or concomitantly. Consequently, the shedding pattern of the enzyme is used to demonstrate heterogeneity in the lectin binding sites: there are two main components labelled by concanavalin and peroxidase, one which has a short period (from 6 to 16 min) and another one with a much longer one (1.3 to 3 h).

It is shown that when cells are incubated at 37 °C after a lectin treatment, secondary binding forces occur between the lectin and cell surface components which render the lectin unavailable for inhibiting sugars. Under the same conditions, some peroxidase can still be bound and a slight agglutination can still occur.

INTRODUCTION

The phenomenon of glycoprotein turnover has been extensively studied by Warren and Glick [1] with isolated plasma membranes from L cells. With non-dividing cells they found that there is a constant replacement of membrane components while for dividing cells, although synthesis occurs, there is a great reduction in turnover. The same group [2] showed that after these cells have been treated with trypsin, there is no special repair mechanism to deal with the damage. The surface membrane is replaced in the normal course of turnover.

Hughes et al. [3] working with non-growing TA 3 cells found that the incorporation of tritiated glucosamine was very similar whether or not the cells had been treated with neuraminidase. This indicates that there is a "de novo" synthesis of the

* To whom correspondence should be sent.

carbohydrate chains and that the repair is not effected by the transfer of sialic acid to the oligosaccharide chains which have been enzymatically depleted of their sialosyl residues. Thus, the cell does not have a specific repair mechanism, but relies on the normal membrane turnover to maintain the integrity of its cell surface.

Using an ellipsometric method, Poste [4] measured the rate of synthesis and the thickness of the cell material. The rate of synthesis was found greater in transformed cells than in the normal parent cells.

Surface changes have also been demonstrated using lectins (see reviews of Burger [5] and Nicolson [6]). With concanavalin A, Karsenti and Avrameas [7] studied the glycans metabolism on the lymphocytes. They detected the lectin by virtue of its binding spontaneously to peroxidase and showed a release of peroxidase in the incubation media. Other studies have been carried out by Jones [8] who measured the release of bound lectins (concanavalin A and phytohaemagglutinin) from red blood cells and from lymphocytes. This author demonstrated that several components with different half lives are involved.

In previous papers, we reported differences in the internalization of concanavalin A receptors between transformed cells and their normal controls [9] and analyzed the relationship between the amount of lectin bound and the quantity of the marker enzyme fixed on the cell surface [10]. The ultrastructural study was carried out using the concanavalin A-peroxidase method [11] and it demonstrated the penetration of peroxidase-labelled material as the cell surface membrane became unlabelled.

In the present work, we investigate quantitatively how the lectin and the peroxidase molecules behave after their binding to the cell surface when the cells are further incubated at 37 °C. This had led us to study whether or not there is synthesis or turnover of the receptor sites on the surface membrane.

MATERIALS AND METHODS

The basic techniques have been described in detail elsewhere [9]. Additional methods are described below.

Cell cultures

Fibroblasts in secondary culture were obtained by trypsinization of Syrian Hamster embryos (EH cells). As transformed cells we used Cl_2TSV_5 cells kindly supplied by Professor P. Tournier in our institute.

Reagents

Concanavalin A was prepared by affinity chromatography and was obtained from Miles Yeda (Israel). The lectin was labelled according to the method of Kalb and Levitzki [12] with ^{63}nickel obtained from I. R. E. (Belgium). This method was used because we obtained high specific activities ($15–16 \cdot 10^6$ cpm/mg). The transition metal ion binds to a specific site which is different from the sugar binding site; this method modifies neither the external charge of the protein molecule nor its molecular configuration (it has been shown that acetylation or succinylation can induce dimerization [13]). The horse radish peroxidase was RZ 3 grade and was found homogenous by analytical centrifugation analysis (Boehringer, Germany). *O* - Dianisidine was ob-

tained from Merck (G. F. R.) and the inhibiting sugars (α-methylmannoside, mannose, glucose, cellobiose) from Sigma Co. (U.S.A.).

All solutions were made in a phosphate buffer 0.01 M, pH 7.4, containing NaCl, KCl, $MgCl_2$ and $CaCl_2$ (saline).

Cell surface labelling

In all experiments, lectin and enzyme were used at a concentration of 50 μg/ml and all incubations with these reagents were made at room temperature, for 15 min.

The incubation schedule of each experiment is summarized in Table I. The variations of time which are given below refer to the duration of the saline treatment at 37 °C.

Experiment a: evaluation of concanavalin and peroxidase in the reincubation medium

Cells were labelled with the radioactive lectin, washed with saline and immediately incubated with peroxidase. The cultures were then washed 6 times to eliminate unbound and non-specifically trapped enzyme. (It was controlled so that no peroxidase could bind directly to the cell surface in the absence of lectin). Then the cultures were reincubated with saline for 0.5, 1, 2 and 4 h at 37 °C. At each time point, radioactivity and peroxidase activity were measured both on cells and in supernatants. To measure the amount of peroxidase fixed on cells the enzyme was removed from the cell surface by a 15-min incubation in a 0.1 M mannoside solution. It was ascertained that there was no more detectable peroxidase activity on the surface after this treatment. The enzyme activity was determined with *O*-dianisidine [14], 30 s after mixing the reagents in the spectrophotometer cuvette; absorbance was automatically recorded. The peroxidase values reported are the differences between the activities in the incubation media and the corresponding sixth washing solutions. Binding to the serum proteins which cover the bottom of the culture flasks was found negligible as compared to the amount bound to the cells [15].

Since the experiments were done at room temperature, the molecular weight of the concanavalin tetramer (108 000) was used for the determination of the number of lectin molecules bound to the cell surface. The amount of dimer is negligible under such conditions [15].

The proteins were measured according to the method of Lowry et al. [16].

Experiment b: measurements of variations in the number of receptor sites on the cell surface. In these experiments, the cell monolayer was submitted to 4 successive incubations: (1) ^{63}Ni - concanavalin for 15 minutes at room temperature (2) Saline at 37 °C for 0.5, 1, 2, and 4 hours, respectively (3) ^{63}Ni - concanavalin at room temperature for 15 minutes again. (4) Peroxidase for 15 minutes at room temperature to reveal the available lectin. In another set of experiments, the first incubation with lectin was carried out using unlabelled lectin.

Experiment c: determination of lectin availability for different inhibiting sugars. After labelling for 15 min at room temperature with radioactive concanavalin and post-incubating in saline at 37 °C, the cells were further incubated for 15 min at room temperature in different sugar solutions (mannoside, mannose, glucose, and cellobiose in each case the sugar solution was 0.1 M).

Experiment d: estimation of the lectin-induced agglutinability. To test the agglutinability we measured the lectin-induced adhesion of single cells in suspension to a cell

layer fixed on the bottom of the culture flask [17, 18]. Briefly, a batch of cells was allowed to grow in a medium containing [^{3}H]thymidine. These radioactive cells were removed from the layer by treatment with 0.02 % EDTA (30 min at 37 °C) and the radioactive cell suspension adjusted to $1.5 \cdot 10^6$ cells per ml. Two ml of this cell suspension were then poured over a cell layer ($4 \cdot 10^6$ cells/25 cm^2) which was previously treated with lectin and incubated in saline at 37 °C as in the other experiments. After 20 min of gentle swirling, the supernatant was poured off; the monolayer with the attached cells was then washed three times. The radioactivity of the monolayer served as an estimate of the specific agglutinability enhanced by the non-specific cell adhesion. This non-specific cell adhesion was measured by the amount of single cells remaining on a monolayer which was untreated by the lectin. The specific concanavalin induced agglutination was given by the difference of the 2 values.

Concanavalin-induced fixation of the radioactive cell suspension on serum coated flasks (i.e. flasks filled with culture medium only and incubated at 37 °C for 48 h) is also reported.

RESULTS

(*a*) *Release of concanavalin and peroxidase in the incubation medium*

With the transformed cells the amount of concanavalin tetramer bound is $56 \cdot 10^{12}$ molecules/mg cell proteins. The normal cells fixed about 4 times more, i.e. $200 \cdot 10^{12}$ molecules/mg cell proteins. Differences between transformed and normal cells were also detected when the lectin was traced by peroxidase: the respective values were 7.25 and $60 \cdot 10^{12}$ molecules/mg cell proteins (i.e. about 8 times more peroxidase on the normal cells than on the transformed ones) [10].

When we measured the lectin/enzyme ratio in the reincubation medium, significant differences were obtained between the transformed cells and the normal cells: after 30 min and 4 h at 37 °C in saline the respective values were 0.87 and 0.97 for the transformed cells and 0.27 and 0.34 for the normal cells (Table II). These values indicate that under our experimental conditions, where all the receptors may not be involved, one molecule of concanavalin is released concomitantly either with 1 or with 3 molecules of peroxidase.

TABLE I

INCUBATION SCHEDULES

Concanavalin A and peroxidase incubations were made at room temperature, at 50 μg/ml and for 15 min. The cells were incubated in saline for 30, 60, 120 and 240 min at 37 °C. ⊣⊢, indicates washes with saline; ▲, indicates time = 0 in the experiments.

Experiment	Incubation schedules				
a	^{63}Ni-concanavalin A	⊣⊢	Peroxidase	▲	Saline
b	^{63}Ni or unlabelled concanavalin A	⊣⊢	▲saline	⊣⊢	^{63}Ni-concanavalin ⊣⊢ peroxidase
c	^{63}Ni-concanavalin A	⊣⊢	▲saline	⊣⊢	Sugar inhibitor
d	Unlabelled concanavalin A	⊣⊢	▲saline	⊣⊢	^{3}H-labelled cells

TABLE II

EXPERIMENT a: CONCANAVALIN AND PEROXIDASE RELEASE IN THE REINCUBATION MEDIA

Measurements and calculations were carried out as described in the text. Values are given for 30 min and 4 h of reincubation in saline, at 37 C.

		Concanavalin A		Peroxidase		Concanavalin A/Peroxidase
		μg/mg Cell protein	Molecules/mg cell protein	μg/mg cell protein	Molecules/mg cell protein	
Cl_2TSV_5 cells	30 min	0.48	$2.7 \cdot 10^{12}$	0.23	$3.1 \cdot 10^{12}$	0.87
	4 h	0.94	$5.2 \cdot 10^{12}$	0.41	$5.6 \cdot 10^{12}$	0.97
EH cells	30 min	1.8	$10 \cdot 10^{12}$	2.7	$37 \cdot 10^{12}$	0.27
	4 h	3.5	$19.5 \cdot 10^{12}$	4.2	$57.5 \cdot 10^{12}$	0.34

The amount of peroxidase remaining cell-associated can also be expressed as a percentage of the initial amount bound and plotted against time. It becomes then evident that the relative amounts of remaining bound concanavalin are very similar for both cultures, though more peroxidase is observed on the transformed cell surface (Fig. 1). If the kinetics of peroxidase retention are plotted on a log scale against time, it is clearly seem that this phenomenon consists of at least two major components, a slow one and a fast one; the slow component is defined by drawing the tangent to the curve at time $t = 4$ h. Its intercept with the ordinate determines the percentage of this slow component at time $t = 0$. Subtraction of this slow component from the experimental curve gives the fast component. For the transformed cells, the fast component (representing 45 % of the total bound peroxidase) was found to have a half life of about 16 min whereas the other (55 %) has a half life of about 3 h. (Fig. 2a). The results

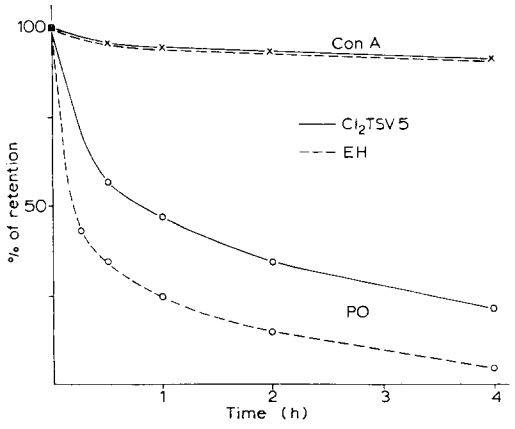

Fig. 1. Experiment a: percentage of concanavalin and peroxidase retention on cells. Cells were labelled with ^{63}Ni-concanavalin and peroxidase, and postincubated in saline at 37 °C. Most of the enzyme is shed from the cells whereas most of the lectin remains cell associated. ×, concanavalin; ○, peroxidase; —, transformed cells; - - -, normal cells.

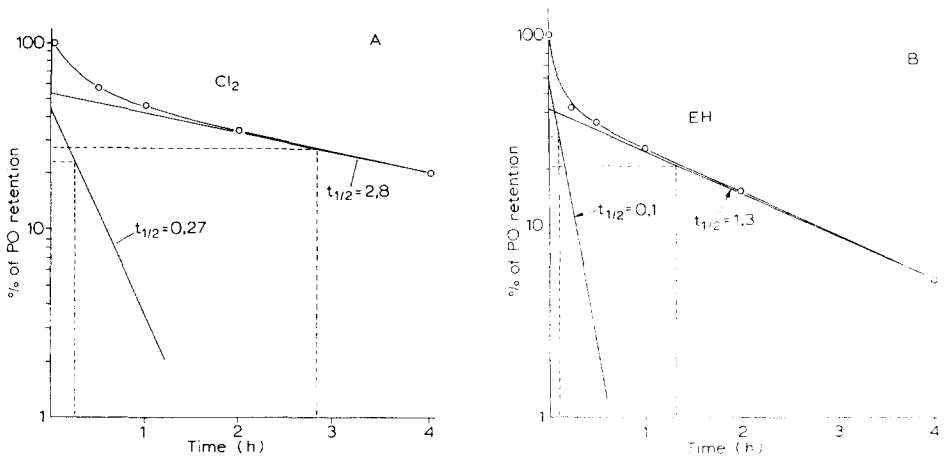

Fig. 2. Experiment a: percentage of peroxidase retention on transformed cells (A) and on normal cells (B). Data are plotted on a logarithmic scale as a function of time. The two components are determined graphically (see text).

obtained for the normal cells are slightly different; 59 % of the total amount of the bound peroxidase has a half life of about 6 min whereas 41 % has a half life of about 1.3 h (Fig. 2b).

A control experiment was also undertaken where the incubations were done in the following sequence; lectin, saline at 37 °C and only then peroxidase. In this type of experiment we found that the same amount of peroxidase could be bound on the cell surface after 4 h in saline as that which was found remaining after the same incubation period, on the cell membrane in the "lectin - peroxidase - saline" experiments described above. These results are developed later in Fig. 7.

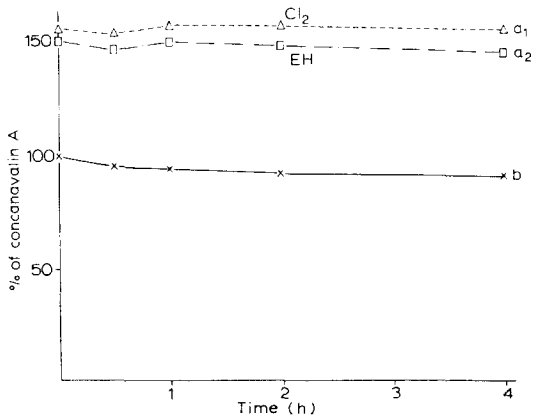

Fig. 3. Experiment b: variation in the number of receptor sites. Cells were submitted to two successive radioactive lectin treatments with an intermediate saline incubation at 37 °C. The 100 % were determined as the amount fixed after one lectin labelling at time $t = 0$. a_1, lectin bound on the transformed cells; a_2, lectin bound on the normal cells; b, same as lectin retention on Fig. 1.

TABLE III

EXPERIMENT b: CONCANAVALIN AND PEROXIDASE BINDING AFTER TWO TREATMENTS WITH RADIOACTIVE LECTIN

Cultures are treated as described in Table I. The results are expressed in μg/mg cell proteins. PO, peroxidase.

	Incubation time (h)									
	0		0.5		1		2		4	
	Concanavalin A	PO	Concanavalin A	PO	Concanavalin A	PO	Concanavalin A	PO	Concanavalin A	PO
Cl_2TSV_5 cells	15.6	0.58	15.4	0.43	15.7	0.49	15.7	0.48	15.5	0.44
EH cells	54.4	9.7	53	7.2	54	6.0	53.3	6.0	52.2	6.5

(*b*) *Variation in the number of concanavalin receptor sites.* With two incubations with the radioactive lectin, similar lectin bindings were measured on cells, whether or not these 2 incubations had been separated by a 37 °C incubation. Results are shown in Table III and in Fig. 3. However, a slight increase of about 10 % was obtained after 4 h of reincubation in saline when only the second lectin binding procedure was performed with radioactive lectin. This increase in the second binding is due to the availability of concanavalin sites which have been freed by the lectin release during reincubation in saline. The same increase could also be calculated graphically in Fig. 3 by subtracting Curve b from Curve a_1 or a_2.

Similar results were obtained when cells were incubated at 37 °C in a serum-free medium with galactose instead of glucose as the source of energy.

When the second incubation with the lectin was followed by peroxidase treatment, we found that for the transformed cells (Fig. 4a) the amount of peroxidase

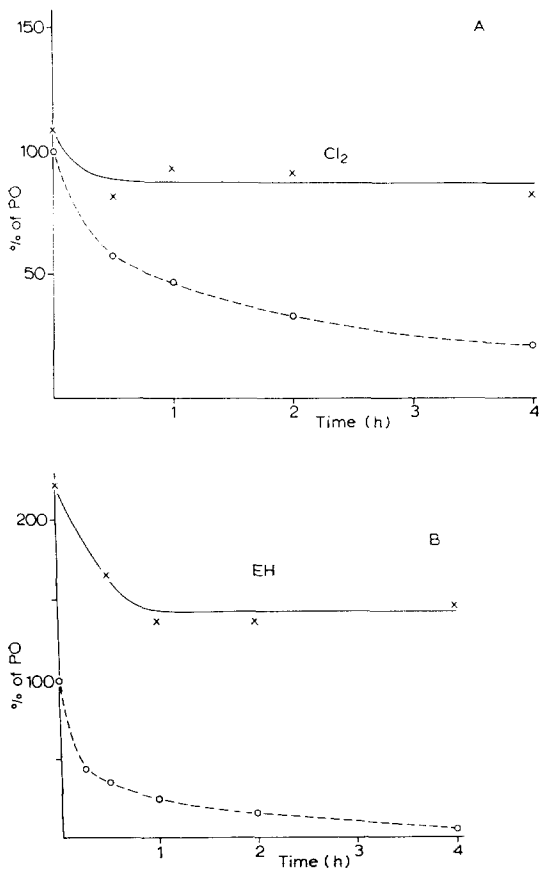

Fig. 4. Experiment b: variation in the number of receptor sites. Cells were submitted to two successive radioactive lectin treatments with an intermediate saline incubation at 37 °C. The lectin was then detected by peroxidase. The 100 % fixation of peroxidase was determined as the amount of enzyme released by mannoside, at time $t = 0$, after one concanavalin labelling. A, % of peroxidase on the transformed cells; B, % of peroxidase on the normal cells; the dotted lines are the same as peroxidase retention on Fig. 1.

TABLE IV

EXPERIMENT c: EFFECT OF INHIBITING SUGARS ON THE CONCANAVALIN LABELLING

The cells were labelled with ^{63}Ni-concanavalin and post-incubated in saline at 37 C. Then the lectin release due to a sugar incubation was measured. Results are given in μg/mg cell protein of Cl_2TSV_5. The same experiment with saline alone has no measurable effect.

Incubation time (h)	methylmannoside	mannose	glucose	cellobiose
0	5.9	4.2	2.7	0.4
0.5	3.9	2.5	0.8	0.2
1	3.0	2.4	0.3	0.15
2	2.9	1.2	0.6	0.2
4	2.6	1.3	0.5	0.2

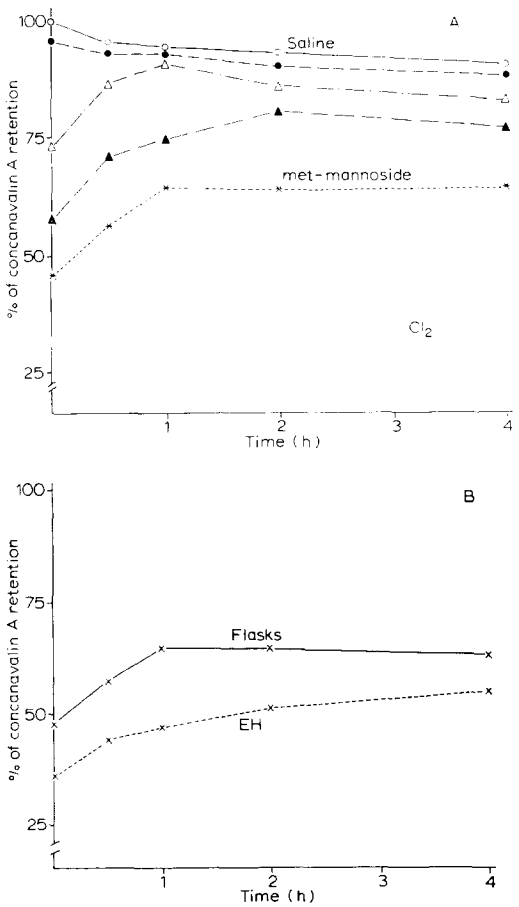

Fig. 5. Experiment c: lectin availability for different inhibiting sugars. Sugars were applied after concanavalin and saline incubations. A, retention on transformed cells; B, retention on normal cells (—) and on serum-coated flasks (- - -). ○ ○, saline (as in Fig. 1); ● ●, cellobiose; △ △, glucose; ▲ ▲, mannose; * *, methylmannoside. The percentage of retention is defined as (amount of lectin remaining after sugar)/(amount of lectin on cells at time 0) × 100.

TABLE V

EXPERIMENT d: LECTIN-INDUCED AGGLUTINABILITY

A cell monolayer (or a serum-coated flask) was treated with concanavalin. The specific lectin-induced adhesion is measured by means of the addition of a suspension of a [^{3}H]thymidine-labelled cells. Results (specific adhesion) are given in cpm · 10^5 per flask.

	Incubation time (h)					Controls	
	0	0.5	1	2	4	Saline*	Cells**
Cl_2 + [^{3}H]Cl_2	12.8	10.8	8.2	7.2	5.2	3.3	19.3
Empty flask + [^{3}H]Cl_2	14.4	—	—	—	14.1	2.7	19.3
EH + [^{3}H]EH	2.9	1.5	0.9	0.7	0.3	0.4	4.6
Empty flask + [^{3}H]EH	3.6	—	—	—	3.5	0.5	4.6

* The monolayer was incubated in saline without lectin. This measures the non-specific adhesion.
** Radioactivity of the cell suspension added.

bound had not been modified. However, in the case of the normal cultures (Fig. 4b) we obtained an increase in the peroxidase binding after the second concanavalin incubation, compared to the 100 %.

(*c*) *Lectin availability for different inhibiting sugars.* Immediately after concanavalin binding, i.e. without post-incubation in saline, it was possible to remove only 60 % of the lectin by treatment with the most effective inhibiting sugar, α-methyl-mannoside. The amount of removable lectin diminished as sugars with lower affinities were used (Table IV). No significant difference in the amount of lectin removed was noted for cells treated either with cellobiose or with saline alone without sugar. These experiments were performed on the two cell types and on serum-coated flasks with no cells. In all cases, and for both substrates used here, i.e. cells or flasks, the inhibitor

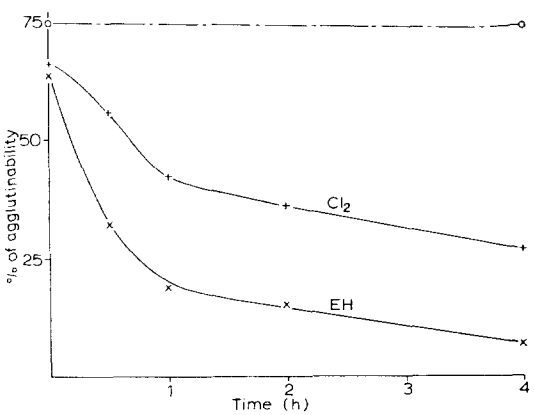

Fig. 6. Experiment d: lectin induced agglutinability. As the cells are incubated at 37 °C, the agglutinability is decreased. The serum-coated flasks exhibit a constant rate of lectin-induced fixation. + +, transformed cells; × ×, normal cells. The dotted lines represent the lectin-induced fixation on serum-coated flasks. The percentage of agglutinability is defined as (lectin-induced fixation of cells)/(total-amount of cells added) × 100.

effectiveness was always diminished when the concanavalin-labelled substrate was incubated at 37 °C before the sugar treatment (Fig. 5).

(*d*) *Lectin-induced agglutinability*. The variations in the fixation of suspended cells to the monolayer are reported in Table V and expressed in % of cellular-induced agglutinability in Fig. 6. When using the previously described method [19], the normal cells always presented a very low agglutinability as compared to the transformed ones. But in the present experiments, when only the monolayer was directly treated with concanavalin and a lectin-free cell suspension was added, the normal cells and the transformed cells showed very similar rates of fixation induced by the lectin. For both cell types, agglutinability decreased when cells were reincubated at 37 °C. We, like Rottmann et al. [18], were unable to dissociate the agglutinated cells by a further treatment with methyl-mannoside.

On the other hand, serum-coated flasks, i.e. incubated for 48 h with culture medium only, exhibited a constant lectin-induced adhesion of 75 % (Fig. 6).

DISCUSSION

The analysis of the effects of reincubation in saline at 37 °C on concanavalin-peroxidase labelled cells brought out several points: (1) No marked difference was observed between normal and transformed cells when considering the shedding percentage of initially bound amount and not the absolute quantities: in both cases, most of the enzyme (80–95 %) was found in the supernatant after reincubation in spite of the fact that 90 % of the lectin remained cell-associated (controls have been made to ascertain that peroxidase did not induce any lectin shedding). (2) A marked difference between the two cell types was evidenced when the ratios lectin/enzyme were measured in the reincubation media; the ratio was 1 for the transformed cells and 0.3 for the normal parent cells. These ratios were not found to be time dependent since no significant differences were obtained between 30 min and 4 h of reincubation. (3) In both cases the kinetics of peroxidase shedding from the cell surface showed that there is a heterogeneity of binding sites. Two main components with different releasing rates were detectable: one has a short half life (6 min for the normal cells and 16 min for the transformed cells) whereas the second has a much longer half life (1.3 and 3 h for the normal and the transformed cells, respectively). These results could not be demonstrated by radioactive lectin binding alone because of the sensitivity of the method being too low.

Two possible explanations could be suggested: (a) The concanavalin and the peroxidase are shed from the cell surface as a complex or concomitantly and not as two independant molecules. If this is correct then at least two different kinds of complexes must exist; one which consists of 1 peroxidase molecule bound to 1 concanavalin molecule on the transformed cell surface; the other made of 3 molecules of peroxidase bound to one molecule of concanavalin on the normal cells. According to this hypothesis, the kinetics of peroxidase retention reflect the behaviour of the lectin molecules themselves. It is then likely that the binding forces between the concanavalin and its membrane receptors are different in the two cell types studied. This difference could be explained by the nature of the receptor sites, by interactions between the bound lectin and surrounding molecules from the cell surface and/or by the fact that one or three peroxidase molecules had been bound to the lectin. The active

site of the concanavalin involved in the binding to the cell surface could then have been modified and the affinity of its stereospecific site toward its corresponding ligand (i.e. the cell surface receptor) is altered. (b) Concanavalin and peroxidase may also be shed independently from the cell surface. As in the first hypothesis, it is possible to implicate the same kinds of interactions on the "lectin-peroxidase-receptor" system. Then the binding between the lectin and the enzyme must be taken into consideration, instead of the binding between the lectin and the cell receptor. The interactions on the concanavalin molecules do not induce the shedding of the lectin but of the peroxidase.

We have represented in Fig. 7 tentative models for these two hypotheses. At first it has to be pointed out that there are two types of lectin molecules on the cell surface: those which are recognized by peroxidase and those which are not [10]. Hence, if the shed concanavalin is not a peroxidase positive lectin molecule, then in the "lectin-saline-peroxidase" procedure the amount of peroxidase that binds after the saline incubation (model A') should be the same as that bound without any reincubation in saline (model C). In fact, the amount of peroxidase that was measured on the cell surface was smaller (model B') and similar to that measured on the cells after

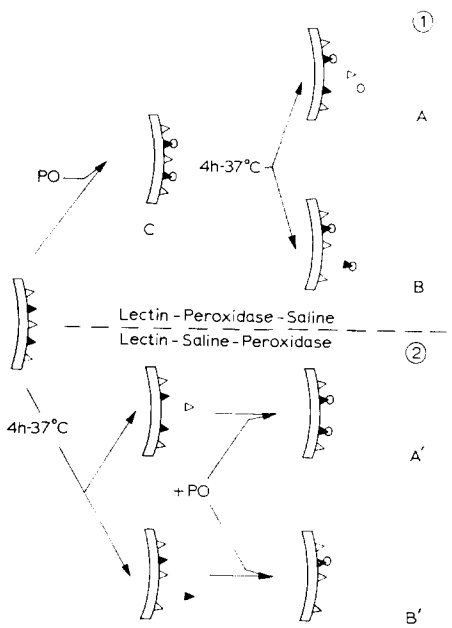

Fig. 7. Tentative models for the lectin-peroxidase shedding. 1. (top) surface labelled by lectin, treated by peroxidase, and reincubated 4 h at 37 °C. A, lectin and enzyme are shed independently i.e. enzyme negative lectine molecules are shed. B, lectin and enzyme are shed concomitantly i.e. enzyme positive lectine molecules are shed. 2. (bottom), surface labelled by lectin, reincubated 4 h at 37 °C and only then traced with enzyme. A', the enzyme positive lectin molecules are not released, therefore the same amount of peroxidase should be found whether or not cells have been incubated at 37 °C in saline. (Compare to C which represents the cell surface labelling without any incubation in saline). B', The enzyme positive lectin molecules are shed, hence less peroxidase is measured on the cell surface, compared to C, but as in model A or B. Because less peroxidase was measured after the reincubations in saline, models B and B' supporting the concomitant shedding are valid. ▲, peroxidase positive lectin; △, peroxidase negative lectin ○, peroxidase.

the "lectin-peroxidase-saline" experiment for identical saline reincubations (model A or B). This implies that the shed lectin molecules are traced by the enzyme and also that the lectin and the enzyme are released as a complex or concomitantly: hence only model B is valid (model A is excluded). Another supporting observation is the constant value of the lectin/enzyme ratios measured in the medium all over the reincubation periods.

In previous ultrastructural studies we reported that the surface of the transformed cells became devoid more quickly of concanavalin-peroxidase stain than the normal controls when the cultures were reincubated at 37 °C. At present it is clear that though peroxidase shedding periods were found shorter in the case of the normal cells (Fig. 2) and also though more peroxidase is shed from these same cells (Table II), quantitatively more peroxidase remains on the normal cells than on the transformed ones. This apparent discrepancy is explained by the fact that the normal cells bind 8 times more enzyme than the homologous transformed cells. Consequently, it is not surprising that these cells were seen heavily stained in ultrastructural studies.

The stoechiometric analysis of the concanavalin tracing by peroxidase [9] and its variations led us to ask the following question: Since part of the lectin is recognized by peroxidase and is either endocytosed [10] or released into the medium, do the remaining lectin molecules which are not bound to any peroxidase show a similar behaviour? We would suggest that, in our experimental conditions, most of the lectin remains on the cell surface for the following reasons: (a) We demonstrated that the number of binding sites is constant when measured with radioactive concanavalin after double incubations. Therefore, there is no evidence here for any turnover of the lectin receptors. But this method and the time lapses studied are of course limiting factors; the method might not be sensitive enough to pick up a modified turnover due to the presence of the lectin on receptors, and the time of investigation might have been too short to detect a slow turnover. (b) The experiments with the inhibiting sugars demonstrated that the amount of lectin released by a sugar, after reincubation in saline, is diminished. We believe that pinocytosis alone, although it does occur to a certain degree [9], can only partially explain this result as a similar phenomenon was observed with the serum-coated flasks without cells. Rather, this results from a secondary binding of the lectin to the receptor itself and/or to some components surrounding the receptor sites. These arguments are in agreement with those of Gray [20] and Podder [21] who also suggested that some protein-protein interactions can stabilize the glycoprotein-lectin complex on the cell surface. (c) We have also shown that after reincubations at 37 °C peroxidase binding as well as slight agglutination could still occur.

It is generally admitted that the agglutination between two cells is maintained by a bridge of lectin molecules linking receptors. We have observed that the rate of agglutination for a given cell type could depend on whether there is lectin on only one or both cell surfaces involved in that process. When secondary normal cultures were suspended in a lectin solution, their rate of agglutination was very slight compared to that of transformed cells. But with the method used here in the present work, the rates of agglutination were very similar for both cultures studied, though no trypsin treatment was done on the normal cells (in both methods cells were treated identically by EDTA). It might be that in our experimental conditions a lectin bridge between two cells is formed which would not be possible with these cells if all their receptors were

binding the lectin as in the usual agglutination process. However, this does not allow for the fact that normal cells are agglutinable by high concentrations of lectin. Indeed it is rather puzzling that after lectin treatment, serum-coated flasks without a cell monolayer induced more fixation of the added suspended cells than flasks with a monolayer. However, this phenomenon may be interpreted either in terms of steric interactions which are probably different between the cell surface and the serum-coated flasks or by the quantity of fixed lectin per surface. Configurational changes in the target glycoproteins [22] and pinocytosis might be also partially responsible.

In summary it appears that the lectin binding on the cell surface involves complex mechanisms which may resemble hormone binding [23]. The concanavalin sites exhibit heterogeneity in that some of them allow the fixed concanavalin to react with peroxidase and others do not. Such a heterogeneity was also suggested for concanavalin and other lectins [8, 24, 25].

It seems to us that the kinetics of the peroxidase retention allow us to distinguish several kinds of receptors which differ mainly by their calculated half lives, their agglutination property and which might be classified as follows: those which are traced by peroxidase and have a short half life; those which are traced by peroxidase and have a long half life; those which can induce agglutination and may be traced by the enzyme (agglutination sites are not necessarily peroxidase positive); and those which are not traced by the enzyme and do not induce agglutination.

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