Biochimica et Biophysica Acta, 628 (1980) 303–313

BBA 29187

TOXIC EFFECT OF *RICINUS* LECTIN ON HEPATOMA CELLS IN RELATION TO ENZYME MODIFICATION OF THE CELL SURFACE

M. DODEUR, M. AUBERY and R. BOURRILLON

Laboratoire de Biologie et Pathologie Moléculaires des Glycoprotéines, INSERM U.180, C.N.R.S., L.A. 293, Faculté de Médecine, 45, rue des Saints-Pères, 75006 Paris (France)

(Received July 30th, 1979)

Key words: Toxic effect; Ricinus lectin; Enzyme modification; Cell surface; (Hepatoma cell)

Summary

With regard to the toxic effects of *Ricinus* lectin, neuraminidase-treated hepatoma cells have been found to be the most sensitive, and untreated hepatoma cells the least. Cells treated with neuraminidase and galactose oxidase exhibited an intermediate sensitivity.

At 37°C, the number of *Ricinus* lectin molecules bound to untreated, neuraminidase-treated and neuraminidase and galactose oxidase-treated cells required to bring about 30% toxicity within 2 h was $15 \cdot 10^5$, $7.5 \cdot 10^5$ and $11.5 \cdot 10^5$ molecules/cell, respectively. This difference was rather small and suggests that the additional binding sites exposed following enzyme treatment were as efficient in mediating lectin toxicity as those present before enzyme treatment. Positive cooperativity was observed during *Ricinus* lectin binding to enzyme-treated cells at 37°C and the apparent association constant increased with the increase of binding site occupancy.

The binding sites on enzyme-treated cells appeared to be homogeneous since under different physical conditions (4°C) the shape of the Scatchard plot could be altered in such a way as to produce a single line of slope. In contrast to enzyme-treated cells, untreated cells did not exhibit a positive cooperative process either at 37°C or at 4°C.

We found that the toxicity of *Ricinus* lectin paralleled the irreversible specific binding of lectin, suggesting that only this was able to mediate the toxic effect.

Our results are discussed in terms of the possible entry into the cells of *Ricinus* lectin and this occurs more rapidly in enzyme-treated than in untreated cells. This difference agrees with the sequence of events proposed: (i) Binding of *Ricinus* lectin; (ii) Clustering of lectin binding sites; and (iii) Endocytosis.

Introduction

Certain lectins are extremely toxic to animals and can kill animal cells grown in vitro [1—3]. One of the more toxic lectins is ricin from *Ricinus communis* seeds (M_r 60 000), which is more potent on tumour than on normal cells and could thus be a strong anti-tumour agent [4]. It has also been found to provide some therapeutic protection against Ehrlich ascites tumour [5]. The anti-tumour effect of ricin is attributable to its powerful inhibitory action on protein synthesis [6—10]. *R. communis* seeds contain another lectin (M_r 120 000), which, although to a lesser extent than Ricin, also exhibits a toxic effect, mainly on tumour cells [11]. Saltvedt [12] has reported that the toxicity of *Ricinus* lectin is not related to any contamination by ricin. Although few studies have concentrated on the toxicity of *Ricinus* lectin, it has been observed that, like ricin and abrin [10], *Ricinus* lectin is involved in an interaction with ribosomes in acellular systems and inhibits cell protein synthesis.

In the present paper, we report our investigations into the sensitivity of tumour cells (Zajdela ascites hepatoma cells) to the toxicity of *Ricinus* lectin as related by the interactions of *Ricinus* lectin before and after modification of cell surfaces by specific enzymes. This was done in order to establish whether the binding sites, either unmasked or modified, could be involved in mediating the toxic effect.

Materials and Methods

Cells. Ascites hepatoma, initially induced by dimethylaminobenzene in Wistar rats, was obtained from Dr. Zajdela (Faculté des Sciences, Orsay). These hepatoma cells were obtained 7 days after intraperitoneal injection of 7-day-old tumour cells (0.25 ml containing $2.5 \cdot 10^7$ cells) into 250 g rats (Charles Rivers, France). The tumour-bearing animals were killed by cervical dislocation and the tumour cell suspension was harvested and washed four times with Eagle's culture medium.

Lectin. R. communis was purified by the procedure of Nicolson and Blaustein [13] and solutions of *Ricinus* lectin (M_r 120 000) were prepared at concentrations of 100 μg/ml in 0.15 M NaCl. *Ricinus* lectin was homogeneous and devoid of contamination by ricin as determined by polyacrylamide gel electrophoresis, analytical centrifugation and by the constancy of the specific toxicity during its purification by gel filtration.

Labelling of Ricinus lectin. Radioactive labelling of the lectin was carried out according to the method of Miller and Great [14] using [^{3}H]acetic anhydride (2 Ci/mmol, C.E.A., Saclay, France). Labelled lectin was purified by gel filtration on Sephadex G-25, the eluting buffer being 0.005 M $NaHCO_3$/0.15 M NaCl, pH 7 (buffer A).

The specific activity of *Ricinus* lectin was $4 \cdot 10^6$ dpm/mg. The labelled lectin behaved exactly like native lectin in the erythro-agglutination test. Labelled *Ricinus* lectin solution was prepared at a final concentration of 500 μg/ml in buffer A.

Binding studies. The binding experiments were performed as previously described [15]. The specific binding of the lectin was established as the differ-

ence between the quantities of lectin bound in the presence and in the absence of 0.1 M D-galactose. Experimental values were plotted according to the method of Scatchard [16], taking into account the molecular weight of the lectin (120 000).

Enzyme-treatments of cells. Neuraminidase treatment and galactose-oxidase treatment of hepatoma cells were performed as previously described [15]. The sialic acid content of the supernatant was determined by Warren's method [17]. This treatment released into the supernatant 75 μg of sialic acid/10^8 cells. The cell pellet was washed twice with buffer A and finally resuspended either in buffer A for the binding assay or in Eagle's medium for the toxicity experiments. *Vibrio cholerae* neuraminidase (Behringwerke, 500 units/ml) and *Polyporus circinatus* galactose oxidase (Sigma Chemical Co., U.S.A., 425 units/8 mg) were devoid of protease activity as determined by the azocoll test [18]. 95% of the enzyme-treated cells were viable as estimated by the trypan blue exclusion test.

Sodium borohydride treatment of cells. Neuraminidase and galactose oxidase-treated cells were incubated with sodium borohydride as previously described [19]. The cells were then washed and resuspended as described above. 90—95% of the cells were viable.

Cultures. The hepatoma cells were cultured as previously described [15]: *Ricinus* lectin was added to cell suspensions at final concentrations ranging from 0.4 to 40 μg/10^6 cells. A sample of the cell suspension was counted in a hemocytometer. Cell viability was tested by trypan blue dye exclusion. Cytolysis was investigated using inverted microscopic examination.

Assay method for the toxic effect of Ricinus lectin. The toxic effect of *Ricinus* lectin on protein synthesis was estimated by the incorporation of [^{3}H]-leucine [6—10].

The cells were pulsed for 1 h with 3 μCi/dish of [^{3}H]leucine (specific activity 58 Ci/mmol, Radiochemical Centre, Amersham). The incoporation of radioactivity into the cells was assayed after cell lectin incubation of various time intervals.

The cells were washed twice with 0.15 M NaCl and left overnight suspended in 2 ml 10% trichloroacetic acid. The precipitate was washed twice with 5% trichloroacetic acid and counted for radioactivity.

In order to test the specificity of the lectin toxicity by using its specific inhibitor, *Ricinus* lectin (3 μg/ml) was incubated with D-galactose (0.1 M final concentration) for 15 min at 20°C before being added to the cells.

Results

Quantitative interaction of Ricinus lectin with untreated and enzyme-treated hepatoma cells

At 37°C, the untreated and enzyme-treated cells rapidly bound the lectin to plateau values within 15 min.

Two types of binding curves could be distinguished for untreated and enzyme-treated cells; a hyperbola, which characterized the binding of lectin to untreated cells, and a sigmoid curve which characterized the binding of *Ricinus* lectin to enzyme-treated cells (Fig. 1). Neuraminidase-treated and neuramini-

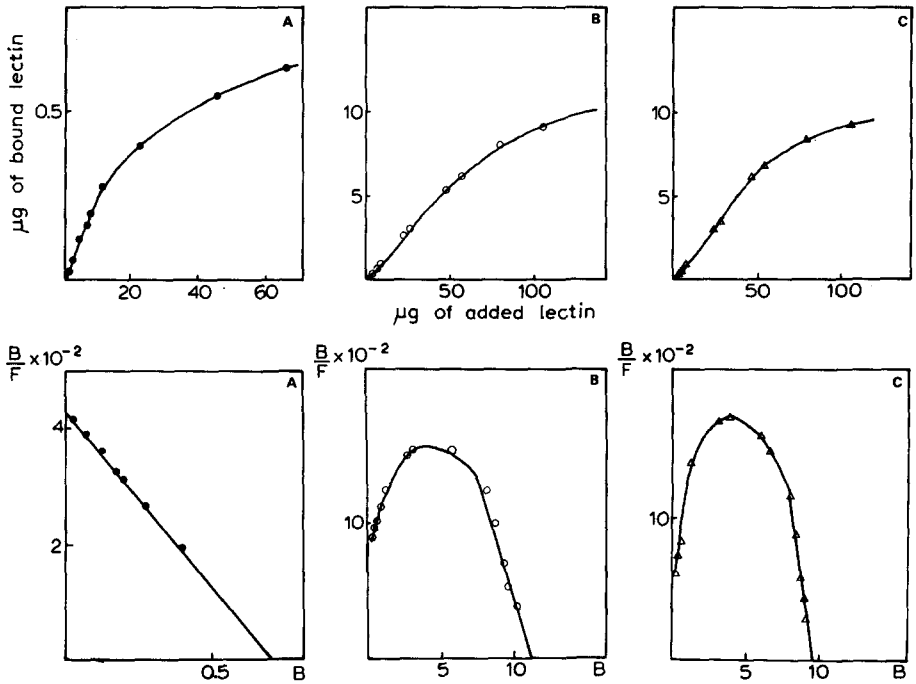

Fig. 1. Binding at 37°C of *Ricinus* lectin on untreated and enzyme-treated cells. The data were plotted according to the method of Scatchard: top, saturation curves for *Ricinus* lectin binding to hepatoma cells. Each point gives the range of values obtained from four separate experiments. Abcissa, μg of added *Ricinus* lectin, ordinate, μg of bound *Ricinus* lectin/10^6 cells; bottom, Scatchard plots. *B* represents the amount (μg/10^6 cells) of lectin bound and *F* represents the amount (μg/10^6 cells) of free lectin. Abcissa, *B*, ordinate, *B/F*. A, untreated cells (●——●); B, neuraminidase-treated cells (○——○); and C, neuraminidase and galactose oxidase-treated cells (△——△).

dase and galactose oxidase-treated cells bound more labelled *Ricinus* lectin than did the untreated cells (Table I). The untreated cells gave linear Scatchard plots, whilst the enzyme-treated cells gave a bell-shaped curvilinear line. Positive cooperativity (convex downward) was seen at low concentrations of lectin

TABLE I

ASSOCIATION CONSTANT AND BINDING SITE NUMBER OF *RICINUS* LECTIN AT 4°C AND 37°C IN UNTREATED AND ENZYME-TREATED CELLS

Each point gives the range of values obtained from four separate experiments and the standard error does not exceed 10%. n.t., not tested.

Treatment	Amount of bound lectin at saturation (μg X 10^6 cells)		Number of binding sites (N X 10^7/cells)		Association constant (K_a) (M^{-1} X 10^6)	
	4°C	37°C	4°C	37°C	4°C	37°C
No enzyme used	2	0.7	1	0.35	0.56	1.44
Neuraminidase	7.9	11.5	4	5.8	0.56	2.4
Neuraminidase + galactose oxidase	3.8	9.8	1.9	4.9	0.56	4.5
Neuraminidase and galactose oxidase + sodium borohydride	7.9	n.t.	4	n.t.	0.56	n.t.

(Fig. 1). Neuraminidase-treated and neuraminidase and galactose oxidase-treated cells had 16 and 14 times more binding sites than untreated cells. The apparent association constant estimated at saturation was 1.7 and 3 times higher in neuraminidase-treated and neuraminidase and galactose oxidase-treated cells, respectively, than in untreated cells (Table I).

We used the direct kinetic method according to De Meyts and Roth [20] to demonstrate the site-site interactions of the positive cooperative process during the *Ricinus* lectin binding to enzyme-treated cells. When all sites were empty, the $\overline{K}_a$ was $0.5 \cdot 10^6\ M^{-1}$ in both neuraminidase-treated and neuraminidase and galactose oxidase-treated cells. When all sites were occupied, the $\overline{K}_a$ was 2.6 and $5 \cdot 10^6\ M^{-1}$ in neuraminidase treated and neuraminidase and galactose oxidase-treated cells, respectively. This showed that the affinity increased 5.2- and 10-fold, respectively, with the increased binding site occupancy.

When the *Ricinus* lectin binding to untreated and enzyme-treated cells was investigated at 4°C, the temperature which is generally used for this type of study, enzyme-treated and untreated cells gave hyperbolic curves of saturation and linear Scatchard plots. After galactose oxidase treatment, the subsequent reduction of galactosyl residues with sodium borohydride induced an increase of *Ricinus* lectin binding sites to the level noted in neuraminidase-treated cells (Table I).

Effect of Ricinus lectin on [^{3}H]leucine incorporation in untreated and enzyme-treated hepatoma cells

1. Dose response (Fig. 2). After 2 h, the lectin at a final concentration of 0.5 μg/ml, caused a marked inhibition (36%) of the [^{3}H]leucine incorporation

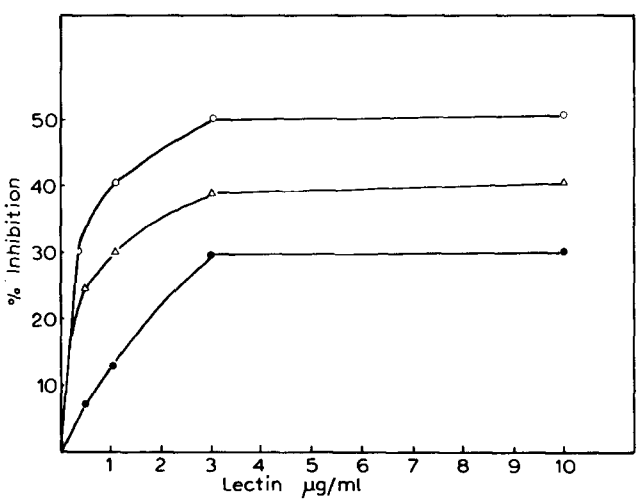

Fig. 2. Dose-response of *Ricinus* lectin effect as estimated by the inhibition of [^{3}H]leucine incorporation at 37°C in untreated and enzyme-treated cells after a 2 h culture period. Each point gives the range of values obtained from four separate experiments. Abcissa, μg of *Ricinus* lectin/ml; ordinate, % inhibition as compared to the control cultured in the absence of *Ricinus* lectin. ●———●, untreated cells; ○———○, neuraminidase-treated cells; △———△, neuraminidase and galactose oxidase-treated cells. The incorporation of [^{3}H]leucine in cpm/10^6 cells was 2689 ± 217, 2415 ± 253 and 2013 ± 14 for untreated, neuraminidase-treated and neuraminidase and galactose oxidase-treated cells, respectively.

TABLE II

EFFECTS OF *RICINUS* LECTIN (12 $\mu g/10^6$ CELLS) ON THE INCORPORATION OF [^{3}H]LEUCINE IN ENZYME-TREATED AND UNTREATED CELLS IN TERMS OF TIME

The hepatoma cells were incubated with *Ricinus* lectin (3 $\mu g/ml$) at 37°C for various lengths of time from 1 to 4 h, and [^{3}H]leucine was added for a 1 h pulse at 0, 1, 2 and 3 h. Each value is the average obtained from four separate experiments. The values given in parentheses represent the effect of *Ricinus* lectin expressed as a percentage of the control.

	cpm/10^6 cells at various times (h) under the influence of *Ricinus* lectin.			
	1	2	3	4
Untreated cells	942 ± 74	2689 ± 217	2817 ± 205	5510 ± 420
+ *Ricinus* lectin	1035 ± 184 (+9)	1698 ± 108 (—37)	967 ± 95 (—66)	1024 ± 88 (—82)
Neuraminidase-treated cells	1353 ± 172	2415 ± 253	2285 ± 19	6632 ± 147
+ *Ricinus* lectin	767 ± 81 (—44)	911 ± 75 (—62)	191 ± 11 (—92)	250 ± 15 (—96)
Neuraminidase and galactose oxidase-treated cells	997 ± 88	2103 ± 14	2191 ± 117	8645 ± 558
+ *Ricinus* lectin	774 ± 64 (—23)	1482 ± 54 (—44)	196 ± 21 (—92)	373 ± 22 (—96)

in neuraminidase-treated cells, whilst in untreated cells this inhibition was slight (7%). In cells treated with galactose oxidase following neuraminidase treatment, *Ricinus* lectin had an intermediate toxic effect (25%). An increase in the lectin concentration brought about an increase of the *Ricinus* lectin toxic effect to plateau levels of 50%, 40% and 30% in neuraminidase-treated, neuraminidase and galactose oxidase-treated and untreated cells, respectively, for a final lectin concentration of 3 μg/ml.

2. Time-course (Table II). After 1 h of culture, [^{3}H]leucine incorporation was not altered by *Ricinus* lectin (3 μg/ml) in untreated cells, whereas in neuraminidase-treated cells a significant and marked inhibition of 44% was noted. This inhibition was only 23% in neuraminidase and galactose oxidase-treated cells.

After 2 h of culture, the inhibition of [^{3}H]leucine incorporation reached 62%, 44% and 37% in neuraminidase-treated, neuraminidase and galactose oxidase-treated and untreated cells, respectively.

After 3 h of culture, *Ricinus* lectin almost totally inhibited the [^{3}H]leucine incorporation (92%) in enzyme-treated cells, but inhibition was only 66% in untreated cells.

Finally, after 4 h of culture, no significant difference was noted in untreated and enzyme-treated cells since *Ricinus* lectin almost totally inhibited (82—96%) the [^{3}H]leucine incorporation.

No cytolysis was observed in the presence of *Ricinus* lectin in either the untreated or the enzyme-treated cells at any time during the experiment. In addition, *Ricinus* lectin had no effect on the number of cells stained with trypan blue as compared with controls incubated without lectin. In all cases, cell death was approximately 5—10%. Thus, *Ricinus* lectin did not kill the hepatoma cells. It is important to note that in the absence of lectin, enzyme treatment did not in itself alter the [^{3}H]leucine incorporation as compared to untreated cells.

In order to test the specificity of its toxic effect, *Ricinus* lectin was pre-incubated with 0.1 M D-galactose before being added to the cell cultures. These experiments were run after 2 h of culture, since it was only after 2 h that untreated cells began to be sensitive to the Ricinus lectin toxicity. Under these conditions, the lectin effect was reduced by 96, 80 and 88% in untreated, neuraminidase-treated and neuraminidase and galactose oxidase-treated cells, respectively. These results showed that the *Ricinus* lectin toxicity on hepatoma cells was specific.

Release of bound labelled Ricinus lectin and consequences of its toxic effect

In an attempt to investigate the minimum time of lectin-binding to obtain a toxic effect, the hepatoma cells were incubated in the presence of labelled *Ricinus* lectin (3 μg/ml) at 37°C for various lengths of time. At the end of each incubation time, the cells were washed with Eagle's medium containing D-galactose (0.1 M final concentration) for 15 min at 20°C. The amount of *Ricinus* lectin remaining bound to the cells and its toxic effect were investigated at various times (Fig. 3).

1. Amount of remaining bound labelled Ricinus lectin. For specifically-bound lectin, 100% was released from untreated cells after 15 and 30 min of

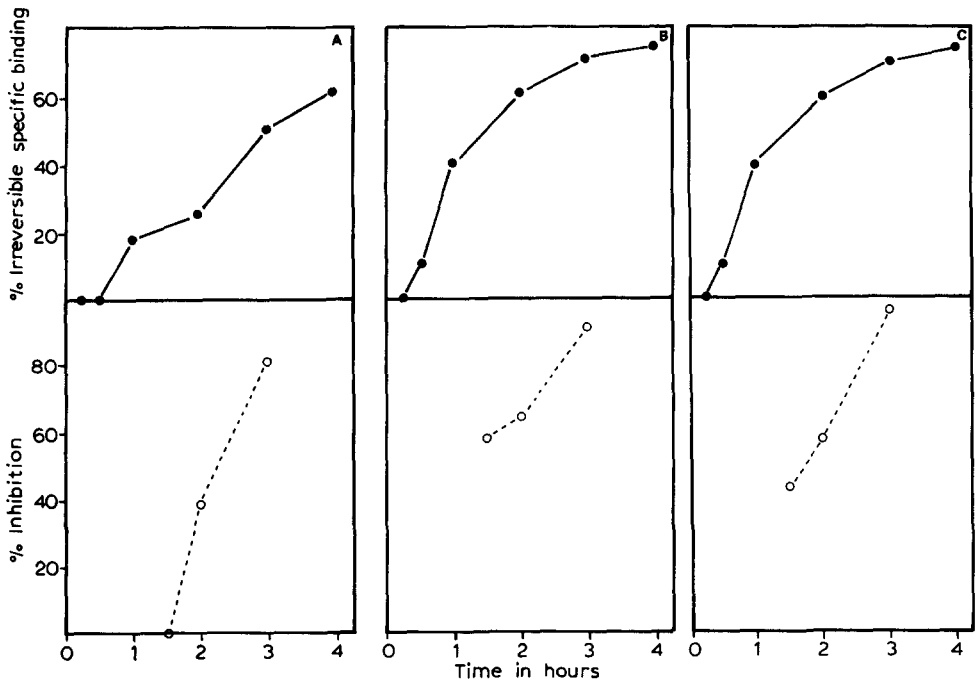

Fig. 3. Kinetics of the irreversible specific binding of *Ricinus* lectin (3 μg/ml) at 37°C from untreated and enzyme-treated cells and subsequent toxic effect. ●——●, specific irreversible binding of *Ricinus* lectin expressed as a percentage;

$$\frac{\mu g \text{ of bound lectin following D-galactose washing} - \mu g \text{ nonspecifically bound}}{\mu g \text{ total bound} - \mu g \text{ nonspecifically bound lectin}} \times 100$$

Amount of lectin remaining bound following D-galactose washing is determined as described in Results. Total binding and nonspecific binding are determined as described in Materials and Methods. ○- - - - -○, inhibition of [^{3}H]leucine incorporation. The cells were incubated with *Ricinus* lectin (3 μg/ml) at 37°C for different periods of time (30, 60 and 120 min). At the end of each incubation, the cells were incubated with D-galactose (0.1 M) for 15 min at 20°C, washed, and then [^{3}H]leucine was added. After a 60 min greater incorporation period, the amount of [^{3}H]leucine incorporation into protein was estimated (90, 120 and 180 min). The values are expressed as percentage of inhibition as compared to the controls without lectin. Each value is the average obtained from four (binding studies) and two separate experiments (toxicity studies). Abcissa, time (h). Ordinate (top), percentage of specific irreversible bound *Ricinus* lectin; (bottom), [^{3}H]leucine incorporation (percent control). (A) untreated cells; (B) neuraminidase-treated cells; (C) neuraminidase and galactose oxidase-treated cells.

binding time, whereas, after 15 min, only 90% was released from enzyme-treated cells. Again, after longer binding times, progressively less *Ricinus* lectin was removable by D-galactose.

2. Toxic effect of remaining bound Ricinus lectin. After 30 min contact with cells, *Ricinus* lectin caused no inhibition of [^{3}H]leucine incorporation in untreated cells. However, in neuraminidase-treated and neuraminidase and galactose oxidase-treated cells, there was marked inhibition (58 and 43%, respectively). After 60 min contact, *Ricinus* lectin brought about 39% inhibition in untreated cells and 64 and 57% in neuraminidase-treated and neuraminidase and galactose oxidase-treated cells. After 120 min contact, the reaction was similar in untreated (81%) and enzyme-treated cells (91—95%).

Discussion

The toxicity of *Ricinus* lectin to hepatoma cells was found to vary with the cell-surface alterations. Neuraminidase-treated cells appeared to be the most sensitive to the toxic effect of *Ricinus* lectin, as estimated by [^{3}H]leucine incorporation, and untreated cells the last sensitive. Neuraminidase and galactose oxidase-treated cells exhibited an intermediate sensitivity to *Ricinus* lectin toxicity.

A direct quantitative correlation between the number of lectin-binding sites occupied and the effects of lectin has been proposed [3]. Although the binding between 0 and 4°C is considered a measure of the true number of lectin-binding sites on the cell surface [21], the increase of this number noted at 4°C following enzyme treatment of the cells cannot be directly related to the increased cell sensitivity to *Ricinus* lectin toxicity measured at 37°C. This is because the characteristics of *Ricinus* lectin binding are significantly modified with the increase in temperature up to 37°C. However, it is important to note that at 4°C the differences in the numbers of *Ricinus* lectin binding sites on untreated and enzyme-treated cells coincided with the specificity of *Ricinus* lectin for intact galactosyl residues [13] and with the location of these residues in the cell-surface glycoproteins [22—23]. The change of the binding characteristics of *Ricinus* lectin at 37°C following enzyme treatments can be directly related to the increased level of hepatoma cell sensitivity to *Ricinus* lectin toxicity since the number of bound *Ricinus* lectin molecules required to cause a 30% toxicity within 2 h was not significantly different in untreated and enzyme-treated cells. As can be seen in Fig. 2; 0.5, 1 and 3 μg of *Ricinus* lectin/ml were needed to bring about a 30% toxicity in neuraminidase-treated, neuraminidase and galactose oxidase-treated and untreated cells, respectively. Taking into account the data given by saturation curves (Fig. 1), it was observed that $7.5 \cdot 10^5$ and $11.5 \cdot 10^5$ molecules/cell were bound to neuraminidase-treated and neuraminidase and galactose oxidase-treated cells, respectively, whilst $15 \cdot 10^5$ molecules/cell were bound to untreated cells. It therefore appeared that the additional binding sites exposed following neuraminidase treatment would be as efficient in mediating lectin toxicity as the binding sites present before the neuraminidase treatment. After galactose oxidase treatment of hepatoma cells, the binding sites were also as efficient in mediating lectin toxicity as those present on untreated cells, and those on neuraminidase-treated cells. Similarly, the binding sites for ricin exposed after neuraminidase treatment of BHK 21 and HeLa cells [24—25] and those present before enzyme treatment would appear to be equally efficient since the number of cell-bound ricin molecules required to bring about an equal level of toxicity in these cells was similar before and after neuraminidase treatment.

If there were one or more independent sets of lectin binding sites on the cells, and all sites of each set had the same intrinsic affinity for lectin in the absence of cooperativity, the Scatchard relationship should apply. However, in the presence of the positive cooperativity process in enzyme-treated hepatoma cells, as suggested by the bell-shaped curves, the measurement of the association constant could only be an approximate approach. Analysis of the De Meyts and Roth [20] plots showed that the affinity average would support a positive

cooperative process in the enzyme-treated cells. Cooperativity is not solely a function of the multivalence of the lectin. It is also dependent on the properties of the cells to which it binds [26—28]. In binding phenomena, positive cooperativity implies that the binding constant of the ligand-receptor association increases as the extent of occupancy of receptor sites increases [29]. Our results showed that when the *Ricinus* lectin binding sites were empty, a limiting affinity $\overline{K}_e$ was obtained ($0.5 \cdot 10^6\ M^{-1}$), whereas when the sites were filled, a higher affinity $\overline{K}_f$ was reached ($2.6 \cdot 10^6\ M^{-1}$ for neuraminidase-treated cells and $5 \cdot 10^6\ M^{-1}$ for neuraminidase and galactose oxidase-treated cells). This suggests that, during the positive cooperative process in the enzyme-treated cells, the apparent affinity constant increased with binding site occupancy. Moreover, our results strongly suggest that the binding sites were homogeneous, since under different physical conditions (4°C), the slope of the Scatchard plot could be altered in such a way as to reach a single line of slope for enzyme-treated cells.

The qualitative changes in the lectin binding following enzyme treatment may be attributable to either conformational changes in membrane components, their redistribution within the membrane as facilitated by the fluid character of the latter [30,31], or site-site interaction such as clustering. The clustering process affects the mode of lectin binding in a way which increases the binding constant and results in the observed cooperativity [32]. The crucial step in the formation of the cluster was the first cross-linking of the binding sites which was allowed by the multivalence of *Ricinus* lectin under our experimental conditions (37°C).

Noonan and Burger [21] have proposed that the lectin binding at higher temperatures than 0—4°C was the result of nonspecific interaction and endocytosis. We demonstrated that at 37°C, the nonspecific binding of *Ricinus* lectin to hepatoma cells was not in itself able to cause the toxic effect. The nonspecific binding was observed without the subsequent toxic effect and remained unchanged from 15 min to 4 h. In contrast, we observed that the toxic effect of *Ricinus* lectin paralleled the specific binding of the lectin, the toxic effect increasing with the increase in the amount of specific binding. Nicolson [32] noted a similar parallel using ricin and 3T3 cells.

Endocytosis of the lectin therefore seemed to be the more probable explanation, as has been found for ricin and abrin [10,25,32—33]. Our results suggest that the amount of *Ricinus* lectin needed to cause toxicity could enter enzyme-treated cells more rapidly than untreated cells. This difference agrees with the notion of possible clustering of the *Ricinus* lectin binding sites which would occur during the positive cooperative process of lectin binding to enzyme-treated cells, since it has been shown that lectins such as concanavalin A [34] and ricin [32] enter the cells by endocytotic vesicles only after clustering or patching of the binding sites.

Acknowledgements

We would like to thank Dr. Zajdela for providing the tumour cells, and Miss Font for the *Ricinus communis* lectin. This work was supported by grants from the Délégation générale de la Recherche Scientifique et Technique (75 7 0058),

the Centre National de la Recherche Scientifique (E.R.A. 321), the Ligue Nationale Française contre le Cancer and the Fondation pour la Recherche Médicale Française. M.A. is Chargée de Recherches, INSERM.

References

1 Olsnes, S. and Pihl, A. (1972) FEBS 20, 327—329
2 Olsnes, S. (1972) Naturwissenschaften 59, 497—502
3 Nicolson, G.L. (1974) Int. Rev. Cytol. 39, 90—190
4 Lin, J.Y., Tserng, K.Y., Chi-Ching, C., Lin, L.T. and Tung, T.C. (1970) Nature 227, 292—293
5 Lin, J.Y., Lin, L.T., Chen, C.C., Tserng, K.Y. and Tung, T.C. (1970) T'ai-Wan I Hsueh Hui Tsa Chih 69, 53—57
6 Lin, J.Y., Liu, K., Chen, C.C. and Tung, T.C. (1971) Cancer Res. 31, 921—924
7 Lin, J.Y., Pao, C.C., Ju, S.T. and Tung, T.C. (1972) Cancer Res. 32, 943—947
9 Onozaki, K., Tomita, M., Sakurai, Y. and Ukita, T. (1972) Biochem. Biophys. Res. Commun. 48, 783—788
9 Tomita, M., Kurokawa, T., Onozaki K., Osawa, T., Sakurai, Y. and Ukita, T. (1972) Int. J. Cancer 10, 602—606
10 Nicolson, G.L., Lacorbiere, M. and Hunter, T.R. (1975) Cancer Res. 35, 144—155
11 Kornfeld, S., Eider, W. and Gregory, W. (1974) in Control of proliferation in Animal cells (Clarkson, B. and Baserga, R., eds.), pp. 435—445, Cold Spring Harbor Conference on Cell Proliferation
12 Saltvedt, E. (1976) Biochim. Biophys. Acta 451, 536—548
13 Nicolson, G.L. and Blaustein, J. (1972) Biochim. Biophys. Acta 266, 543—547
14 Miller, J.R. and Great, H. (1972) Biopolymers 11, 2533—2536
15 Aubery, M., Dodeur, M., Roguet, R. and Bourrillon, R. (1978) Exp. Cell Res. 113, 303—310
16 Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660—672
17 Warren, L. (1959) J. Biol. Chem. 234, 1971—1975
18 Moore, G.L. (1969) Anal. Biochem. 32, 122—127
19 Dodeur, M. and Bourrillon, R. (1978) Biochimie 60, 177—181
20 De Meyts, P. and Roth, J. (1975) Biochem. Biophys. Res. Commun. 66, 1118—1126
21 Noonan, K.D. and Burger, M.M. (1973) J. Biol. Chem. 248, 4286—4292
22 Thomas, D.B. and Winzler, R.J. (1969) J. Biol. Chem. 244, 5943—5946
23 Spiro, R.G. (1970) Annu. Rev. Biochem. 39, 599—638
24 Rosen, S.W. and Hughes, R.C. (1977) Biochemistry 16, 4908—4915
25 Sandvig, K., Olsnes, S. and Pihl, A. (1978) Eur. J. Biochem. 82, 13—23
26 Bornens, M., Karsenti, E. and Avrameas, S. (1976) Eur. J. Biochem. 65, 61—69
27 Wright, J.A. and Ceri, H. (1977) Biochim. Biophys. Acta 469, 123—136
28 Prujansky, A., Ravid, A. and Sharon, N. (1978) Biochim. Biophys. Acta 508, 137—146
29 Koshland, D.E. and Neet, K.E. (1968) Annu. Rev. Biochem. 37, 359—420
30 Nicolson, G.L. (1973) Nature (London) New Biol. 243, 218—220
31 Singer, S.J. and Nicolson, G.L. (1972) Science 175, 720—731
32 Nicolson, G.L. (1974) Nature (London) New. Biol. 251, 628—630
33 Olsnes, S. and Refsnes, K. (1978) Eur. J. Biochem. 88, 7—11
34 Willingham, M.C. and Pastan, I. (1978) Cell 13, 501—507