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STIMULATION OF THE BIOSYNTHESIS OF MEMBRANE GLYCOPROTEINS FROM ZAJDELA ASCITES HEPATOMA CELLS BY *ROBINIA* LECTIN

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SUMMARY

Membrane glycoprotein biosynthesis of ascites hepatoma cells is followed by [^{14}C]glucosamine and [^{3}H]leucine incorporation into cells in culture. The rate of incorporation is strongly increased by the addition of *Robinia* lectin in culture medium. Labeled glycoproteins are released from lectin stimulated and non-stimulated cells by trypsin digestion. Studies of labeled trypsinates on sodium dodecyl sulfate gel electrophoresis and Sephadex G-200 filtration exhibit two fractions both labeled with [^{14}C]glucosamine and [^{3}H]leucine and having different molecular weights, one over 200 000 and the other about 2000. Identical results are obtained when external membrane glycoproteins are solubilized by sodium deoxycholate. Comparison of surface glycoproteins isolated by trypsinization from control cells labeled with [^{3}H]-glucosamine and from lectin stimulated cells labeled with [^{14}C]glucosamine displays no significant qualitative differences between glycoprotein fractions released from both cell groups.

INTRODUCTION

The glycoprotein coat which appears to be on the outer surface of all cells [1–4] has been shown to be implicated in such biological cell characteristics as cellular growth control, adhesivity and cell recognition [5–8]. Alterations of membrane glycoproteins have been assumed to represent key-steps in cell transformation [9–12].

Mitogenic lectins have been shown to stimulate the growth of normal cells [13–15] and to reduce that of transformed cells [15–18]. Although uncertainties subsist concerning the mechanism of lectin action on cells, the initial event appears to be its binding to a glycoprotein receptor site on the cell surface [13, 19–22].

It seemed of interest to know if lectin, which has such opposite actions on the growth of normal and transformed cells, could change the cellular metabolism of glycoproteins, mainly that of membrane glycoproteins. To date only normal human lymphocytes have been investigated for lectin action on cell growth and glycoprotein metabolism [23].

As part of our studies on membrane glycoproteins of normal and transformed cells, investigation is being made of both structure and biosynthesis of surface glycoproteins in rat ascites hepatoma cells. The present report is concerned with the problem of biosynthesis in cells, whether or not they are stimulated by mitogenic lectin.

MATERIALS AND METHODS

Reagents

Chemicals. L-[4(n)-^{3}H]leucine (specific activity 40 Ci/mM), D-[1-^{14}C]-glucosamine (specific activity 57 Ci/mM) and D-[1-^{3}H]glucosamine (specific activity 11 Ci/mM) were purchased from C.E.A. (Saclay, France).

Eagle's minimum essential medium was obtained from Institut Pasteur (Paris) and supplemented with a 2-fold concentration of vitamins and amino acids, 10% foetal calf serum, penicillin (200 μg/ml Specia) and streptomycin (1 mg/ml Specia).

Other chemical products were the highest purity commercially available.

Lectin. Preparation and characterization of *Robinia pseudoacacia* lectin have been previously reported [24]. Its mitogenic properties have been described [25].

Cells. Cells of Zajdela ascites hepatoma were obtained 7 days after transplantation of 0.3 ml of a 7-day old tumor without removing the ascitic fluid. The tumor-bearing animal was killed by cervical dislocation and the tumor cell suspension was taken off and washed 3-4 times with culture medium.

Labeling of cell cultures

The cells were counted with a hemocytometer and then resuspended at a concentration of about $2.5 \cdot 10^5$ cells per ml in Eagle's minimum essential medium. Ascites cells (2.5 ml) were cultured on 50 mm plastic dishes (Falcon) for 8 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. For labeling times of one cell cycle or more, labeled precursors were added directly to the culture medium of each culture dish, namely 7.5 μCi of L-[4(n)-^{3}H]leucine to radiolabel proteins and 2μ Ci of D-[1-^{14}C]glucosamine to radiolabel glycoproteins.

To study the lectin effect on the precursors incorporation time course, *Robinia* lectin, leucine and glucosamine were simultaneously added to each culture dish. Lectin optimum concentration was determined by measuring the amounts of glucosamine and leucine incorporated as a function of the amount of lectin added, namely between 1 and 20 μg of lectin per ml of culture medium; it was found to be 6.7 μg per ml of culture medium and lectin at that concentration was introduced into each culture dish before incubation.

At various times of culture (1–8 h), the reaction was stopped by cooling in ice; cells were centrifuged (600 × *g* for 5 min) and washed three times with iced 0.15 M sodium chloride. Cold 20% trichloroacetic acid (2 ml) was added directly to the cell pellet which was kept at 4 °C for 1 h. After four washings with ice cold 5% trichloroacetic acid, the precipitate was solubilized in 10 ml of instagel (Packard) and the radioactivity counted in an Intertechnique liquid scintillation spectrometer.

When cell labeling was necessary for investigating cell-coat components, culture was proceeded for 8 h at a concentration of $2.5 \cdot 10^5$ cells/ml in culture flasks containing 25 ml of culture medium. All the radioactive precursors were initially

added: either 20 μCi of D-[1-^{14}C]glucosamine and 75 μCi of L-[4(n)-^{3}H]leucine, or 20 μCi of D-[1-^{14}C]glucosamine, or 15 μCi of D-[1-^{3}H]glucosamine. Lectin at a final concentration of 6.7 μg/ml was added directly into the culture flasks where cells were incubated.

Release of glycoprotein coat from cells

Trypsin treatment. Labelec cells were harvested and washed three times with 0.15 M sodium chloride/0.01 M sodium bicarbonate at pH 7.5. Then cells were resuspended at a concentration of 10^6 cells per ml in the same buffer containing L-(tosylamido-2-phenyl)ethyl chloromethylketone-treated trypsin 0.004% (Worthington). A mild shaking was maintained for 20 min at 37 °C and reaction was stopped by the addition of soybean trypsin inhibitor 0.008% (Sigma). Cells were centrifuged for 5 min at 600×*g* and the radioactive supernatant solution that was designated "trypsinate" was isolated after a last centrifugation for 15 min at 15 000×*g*.

Solubilization by sodium deoxycholate. Labeled cells were washed as above and resuspended at a concentration of 2×10^6 cells per ml in 0.15 M sodium chloride solution at pH 6.8. To this cellular suspension was added an equal volume of a solution of 0.1% sodium deoxycholate in 0.15 M sodium chloride solution (pH 6.8). After 2 min at room temperature, cells were centrifuged for 5 min at 600×*g*. The pellet was then discarded and the supernatant was centrifuged again for 15 min at 15000×*g*. The resulting deoxycholate extract was kept at −20 °C.

Phase-contrast microscopy study enabled us to observe that the cells showed no gross morphological alterations after sodium deoxycholate treatment.

Polyacrylamide gel electrophoresis

Whole cells (about $2\cdot10^6$) and trypsinates were first dissolved in 100 μl of a solution containing 1% sodium dodecyl sulfate/1% 2-mercaptoethanol and immediately placed into a boiling-water bath for 2 min. 20 μl glycerol was added to the cooled sample to increase its density and then 25 μl of bromophenol blue. 7 and 10% polyacrylamide gels were prepared following the Davis method [26] partially modified according to Hunt and Brown [27]. Samples were applied on gels (90×3 mm) which were run at pH 8.3 with 1 mA/gel until bromophenol blue was 20 mm from the bottom end of the gel. Gels were calibrated for molecular weights with bovine serum albumin: dimer, 136 000 and monomer, 68 000; ovalbumin, 43 000; trypsin, 25 000 and cytochrome *c*, 12 500. After electrophoresis, gels were frozen and sliced into 1.5 mm thick disks with a gel slicer. Each slice was dissolved by 0.5 ml of hydrogen peroxide in a counting vial for 12 h at 37 °C; 10 ml of Instagel scintillation fluid were added and radioactivity was counted in an Intertechnique liquid scintillation spectrometer.

All procedures were done in the same way for cell coat material removed from lectin-stimulated and non-stimulated cells.

Gel filtration

Sephadex G-200 columns (90×2 cm) were equilibrated either in 0.1 M pyridin acetate pH 6.5 (trypsinate) or in 0.05% sodium deoxycholate solution (extract). Elution was carried out with a corresponding buffer at a constant flow rate of 5 ml/h. The void volume and the total volume of the column were determined by being run

through blue dextran and valine DNP. It was calibrated with proteins of known molecular weights: Immunoglobulin G, 150 000; ovalbumin, 43 000 and cytochrome *c*, 12 500. Fractions were collected and aliquots of 200 μl were counted with 10 ml of Instagel scintillation fluid (Packard) for the determination of the $^3H/^{14}C$ ratio of each fraction. In all cases, the recovery from the columns was about 90% of the applied counts.

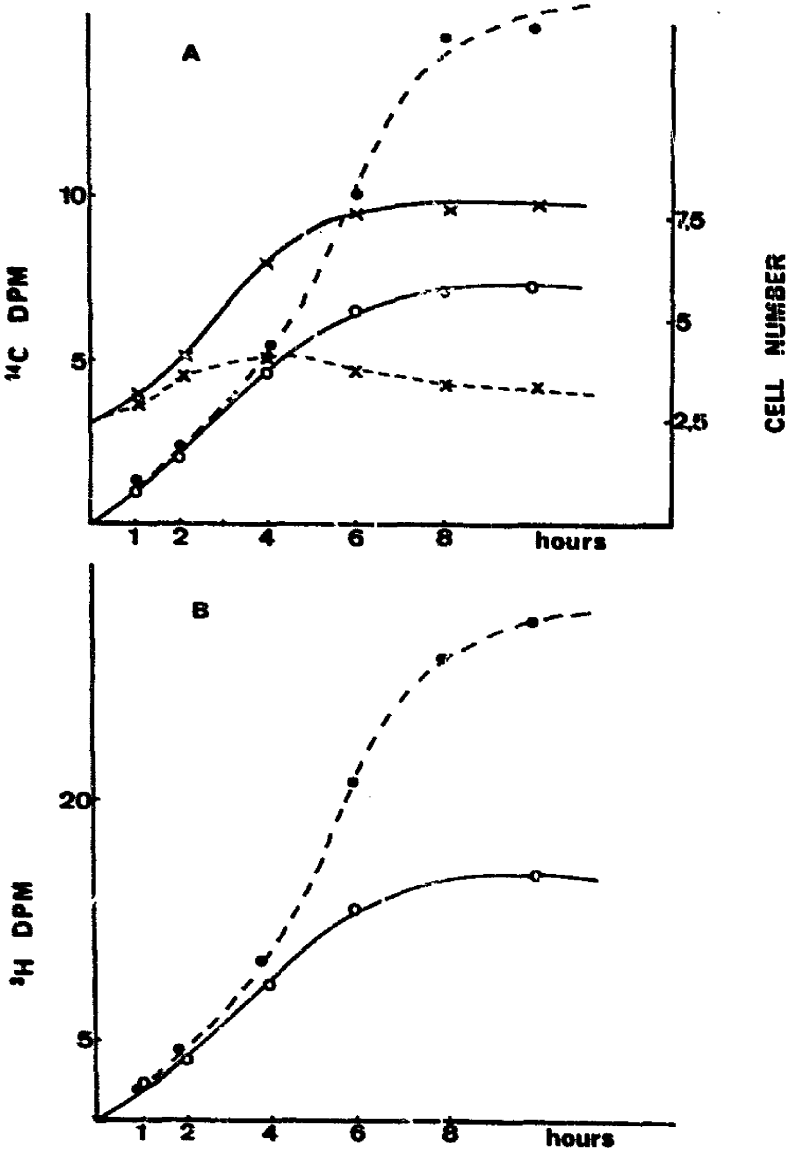

Fig. 1. ***Robinia*** **lectin effect on labeling kinetics of ascites cell cultures. Ascites cells ($2.5 \cdot 10^5$/ml) were grown in 2.5 ml suspension cultures in the presence of (A) 3 μCi/ml [^{3}H]leucine or (B) in the presence of 0.8 μCi/ml [^{14}C]glucosamine. At the times indicated reaction was stopped and radioactivity (dpm/10^5 cells) was counted in trichloroacetic acid insoluble fraction of control cells, (○—○); of lectin stimulated cells, (●– – –●). Control cell growth, (×—×); lectin stimulated cell growth, (×– – –×) are represented in (A).**

RESULTS AND DISCUSSION

1. *Incorporation of [^{14}C]glucosamine and [^{3}H]leucine into lectin stimulated and non stimulated cells*

Hepatoma ascites cells in culture incorporate [^{14}C]glucosamine and [^{3}H]-leucine at a low and constant rate which is stimulated when *Robinia* lectin is added.

The incorporation rate of two radioactive precursors into the trichloro-acetic acid precipitable proteins of control cells is first linear and then becomes

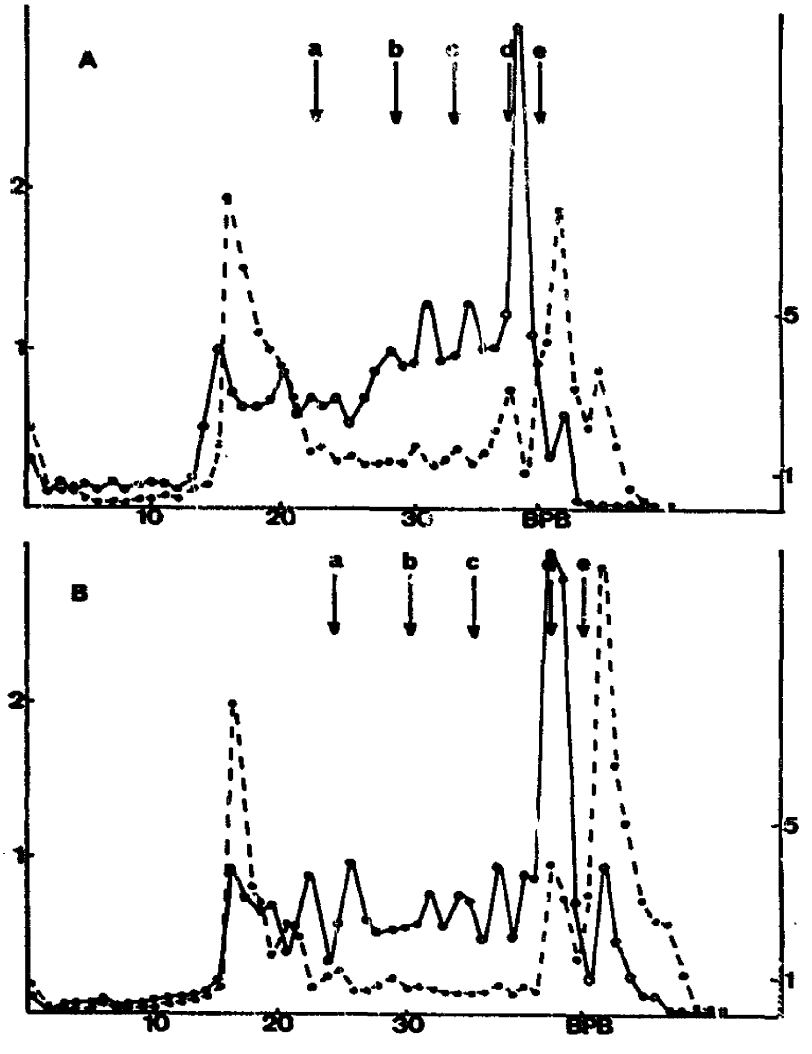

Fig. 2. Polyacrylamide gel electrophoresis of proteins and glycoproteins from whole control cells (A) and lectin stimulated cells (B). Cells were grown in the presence of [^{3}H]leucine and [^{14}C]glucosamine. Aliquots (10^{6} cells) were solubilized with sodium dodecyl sulfate 1 % containing 1 % 2-mercapto-ethanol and subjected to electrophoresis on 7 % polyacrylamide gels calibrated with bovine serum albumin: a, dimer; b, monomer; c, ovalbumin; d, trypsin; e, cytochrome *c*. (○—○), [^{3}H]dpm · 10^{-4}, (●– – –●), ^{14}C dpm · 10^{-4}. BPB, bromophenol blue.

constant after 6–7 h of culture. Fig. 1 shows that cell response to lectin is not immediate but requires a 4-h lag. Then, the incorporation into lectin-stimulated cells shows a point of inflexion between the fourth and sixth hour of culture, resulting in a 2-fold stimulation of the rate by the eight hour of culture. The same delay is necessary to observe the lectin effect on cell growth [15].

Since the major incorporation of glucosamine and leucine is expected to happen in glycoproteins and proteins, it seems that *Robinia* lectin stimulates the biosynthesis of these cell components whereas it inhibits cell proliferation. These results can be compared with those obtained by Hayden et al. [23] who have carried out the only studies available concerning the action of a mitogenic lectin on glycoprotein metabolism. The Phaseolus lectin (PHA) induces a stimulation of glycoprotein biosynthesis in lymphocytes whereas it stimulates the growth and blast transformation of these cells. Thus, it appears that the stimulation of glycoprotein biosynthesis by lectin in both types of cells does not occur by the same mechanism, or that glycoprotein biosynthesis is not correlated to cell proliferation.

2. *Polyacrylamide gel electrophoresis of proteins and glycoproteins from whole cells*

Whole cell proteins and glycoproteins were studied by electrophoresis on 0.1 % sodium dodecyl sulfate/7 % polyacrylamide gels in order to compare glycoproteins of stimulated and non-stimulated cells. Two sets of cells are grown for 8 h in the presence of [^{14}C]glucosamine and [^{3}H]leucine: the first one with *Robinia* lectin, the second without lectin. Cells are then harvested and washed and aliquots are dissolved in 1 % sodium dodecyl sulfate/1 % 2-mercaptoethanol and subjected to electrophoresis. Labeled proteins and glycoproteins are localized on the gel by radioactivity measurements. Electrophoresis profiles of non-stimulated and stimulated cell-glycoproteins are shown in Figs. 2A and B respectively. Nearest the top of the gel, an important band of [^{3}H]leucine and [^{14}C]glucosamine labeled components is found in the molecular weight range, 150 000–200 000. Other peaks of glycopeptides are found which run with the bromophenol blue or just ahead of the dye and have very low molecular weights (lower than 10 000). However, the actual molecular weights may be somewhat different due to the fact that glycoproteins migrate in an anomalous fashion as compared with globular proteins on sodium dodecyl sulfate polyacrylamide gels.

The comparison of the two profiles suggests that no new glycoproteins formed after lectin treatment can be detected by this method.

Thus, glycoproteins of ascites cells can be separated into two major classes by electrophoresis on sodium dodecyl sulfate polyacrylamide gels. However, these two classes which have very different molecular weights are detected by [^{3}H]leucine and [^{14}C]glucosamine radioactivity and it is possible that other classes of glycoproteins exist which are not labeled in this way.

3. *Release of surface glycoproteins*

In order to determine which class of these glycoproteins is present on cell surface two different methods are used to release surface glycoproteins.

(*a*) *Proteolytic digestion.* Trypsin treatment on intact cells: the trypsin mild treatment just releases outer glycoproteins from cells without lysing them, as demonstrated by phase-contrast microscopy. The two sets of double-labeled cells are trypsin

TABLE I

DISTRIBUTION OF [^{14}C]GLUCOSAMINE AND [^{3}H]LEUCINE IN WHOLE CELLS, IN TRYPSINATED CELLS AND IN CORRESPONDING TRYPSINATES AND IN DEOXYCHOLATE EXTRACTS

Control cells and lectin stimulated cells were labeled for 8 h with [^{14}C]glucosamine and [^{3}H]leucine, harvested and washed three times with 0.15 M sodium chloride, 0.01 M sodium bicarbonate (pH 7.5). Radioactivity in dpm was measured in aliquot of whole cells and trypsinated cells (10^6 cells). The amount of cell coat glycoproteins released from whole cells by trypsinization (0.4 μg trypsin/10^6 cells) or by sodium deoxycholate solubilization (0.1 %) were determined by radioactivity measurements in all supernatants.

Fraction	Control cells		Lectin stimulated cells	
	Radioactivity dpm/10^6 cells	(%)	Radioactivity dpm/10^6 cells	(%)
[^{3}H]leucine				
Cell pellet	20 972	100	27 110	100
Trypsinated cell pellet	18 451	88	23 857	88
Trypsinate	1 887	9	2 720	10
Deoxycholate extract	5 093	25	6 700	25
[^{14}C]glucosamine				
Cell pellet	21 765	100	29 870	100
Trypsinated cell pellet	17 847	82	24 722	83
Trypsinate	3 482	16	5 070	17
Deoxycholate extract	8 310	38	11 928	40

treated and the distribution of [^{14}C]glucosamine and [^{3}H]leucine is studied in trypsinated cell pellets and in each corresponding trypsinate as compared with non-trypsinated cell pellets. Results are given in Table I.

Trypsin treatment appears to remove the same proportion of labeled components from normal- as from lectin-stimulated cells, namely 17 % of [^{14}C]glucosamine labeled compounds and 10 % of [^{3}H]leucine labeled compounds. On the other hand lectin stimulates incorporation of [^{3}H]leucine and [^{14}C]glucosamine into trypsinate: incorporation rates are increased by a factor of 30 % for both amino acid and osamine. These results therefore agree with the assumption that lectin would act on cellular metabolism by a global stimulation of glycoprotein biosynthesis.

Gel electrophoresis of trypsinates: each proteolysate is studied by electrophoresis on 10 % polyacrylamide gels containing 1 % sodium dodecyl sulfate and 1 % 2-mercaptoethanol. Profiles of radioactivity electrophoretic migration are displayed in Fig. 3.

The overall distribution of the glycoproteins in the gels appears to be similar in the two diagrams, both displaying two classes of ^{3}H and ^{14}C labeled glycoproteins: the heavy one with an apparent molecular weight of about 150 000–200 000 and the other, which runs with bromophenol blue, with a molecular weight of less than 10 000. These two classes of glycoproteins have also been observed on diagrams of solubilized whole cells (Fig. 2). Besides, there is no evidence for any new species of glycoprotein material issuing from intact glycoprotein proteolysis during trypsinization. However, more subtle differences between the trypsinates from stimulated and non-stimulated cells cannot be ruled out. The low molecular weight compounds are resolved into

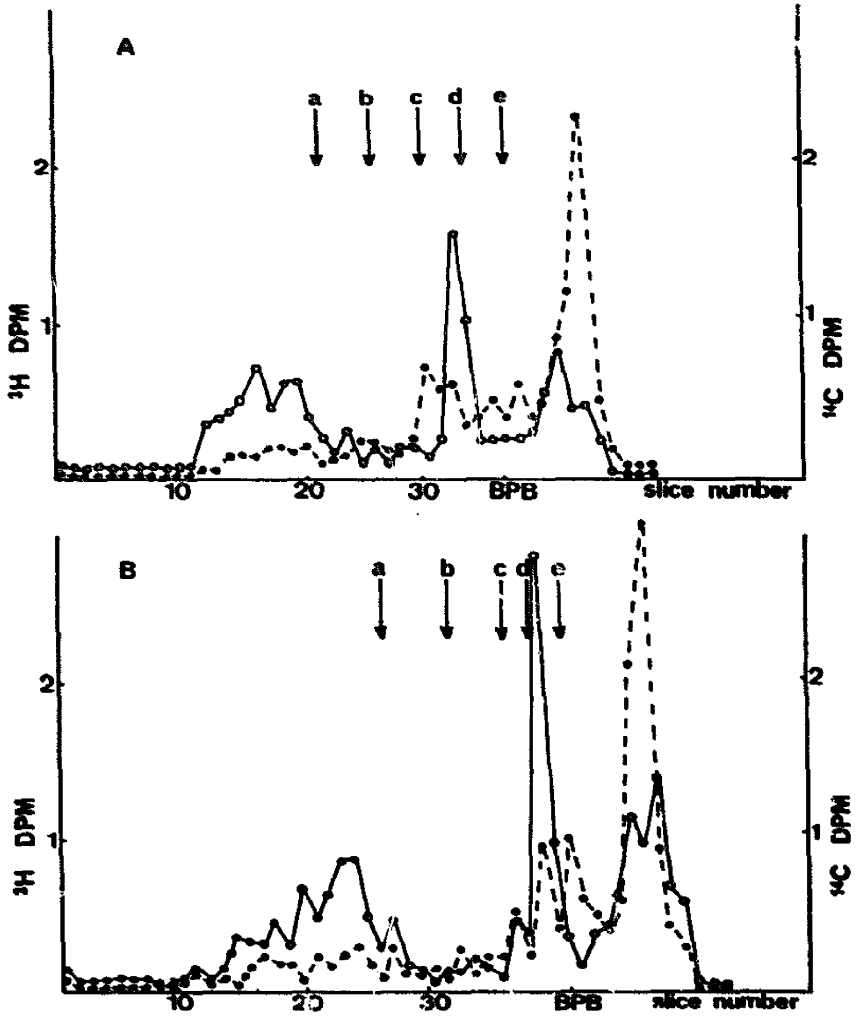

Fig. 3. Polyacrylamide gel electrophoresis of control trypsinate (A) and trypsinate from lectin stimulated cells (B). [^{3}H]leucine and [^{14}C]glucosamine labeled cells were treated for 20 min with 0.004 % trypsin at 37 °C. After centrifugation, the supernatant designated trypsinate was solubilized in 1 % sodium dodecyl sulfate 1 % 2-mercaptoethanol and subjected to electrophoresis on 10 % polyacrylamide gels calibrated with bovine serum albumin: a, dimer; b, monomer; c, ovalbumin; d, trypsin and e, cytochrome c. (○—○), ^{3}H dpm · 10^{-3}; (●- - -●), ^{14}C dpm · 10^{-3}. BPB, bromophenol blue.

two peaks: one with a molecular weight of 5000–7000 and the other which migrates ahead of bromophenol blue.

It is currently assumed that this last peak is glycolipid material [28, 29]. In order to check this possibility, lipid soluble radioactivity is extracted from the whole trypsinate with chloroform/methanol (2 : 1). 80 % of the radioactivity is recovered in the aqueous layer and electrophoretic diagrams of this fraction are exactly identical to those obtained before extraction of lipids. Therefore, this result is consistent with the glycopeptide nature of very low molecular weight fractions. Gel electrophoresis

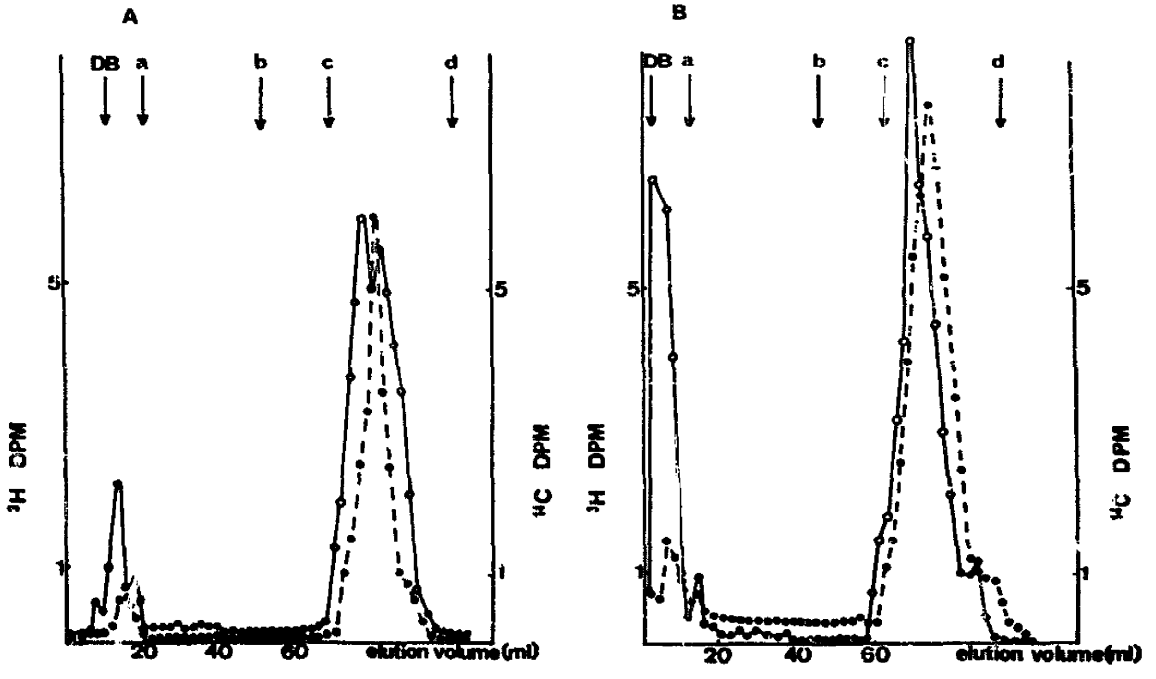

Fig. 4. Gel filtration on Sephadex G-200 column of trypsinates released from control cells (A) and lectin stimulated cells (B). Trypsinates obtained from [^{3}H]leucine and [^{14}C]glucosamine labeled cells were fractionated on Sephadex G-200 column (90 × 2 cm) in 0.1 M pyridin acetate buffer (pH 6.5) calibrated with dextran blue (DB); a, immunoglobulin G; b, ovalbumin; c, cytochrome *c*; d, valine DNP. (○—○), ^{3}H dpm · 10^{-2}; (●- - -●), ^{14}C dpm · 10^{-2}.

diagrams show that the radioactivity bands corresponding to the two classes of glycoproteins are broad and have an irregular shape which is different from that observed with single globular protein species. So it is likely that each class consists of more than a single species of glycoproteins.

Gel filtration of trypsinates: each trypsinate is chromatographed by gel filtration on a column of Sephadex G-200. Elution patterns of trypsinate isolated from control and lectin stimulated cells are similar and exhibit two glycoprotein fractions both labeled with [^{14}C]glucosamine and [^{3}H]leucine (Fig. 4A and B): one which is excluded on Sephadex G-200 and has a molecular weight above 200 000, the other which has a very low molecular weight. It is noteworthy that no fraction only labeled with leucine can be observed.

The size of the second fraction is precised by chromatography on a calibrated column of Sephadex G-50. The elution patterns of these fractions appear quite similar, each having one peak in the 2000–3000 molecular weight range.

Double label of trypsinates with glucosamine: a sharper comparison between trypsinates from stimulated and non-stimulated cells can be obtained by labeling these cells with [^{14}C]glucosamine and [^{3}H]glucosamine respectively.

The two cell sets are then mixed and trypsin treated. The trypsinate is examined by polyacrylamide gel electrophoresis as previously described. From Fig. 5 it appears that electrophoretic profiles of ^{3}H labeled and ^{14}C labeled components are quite

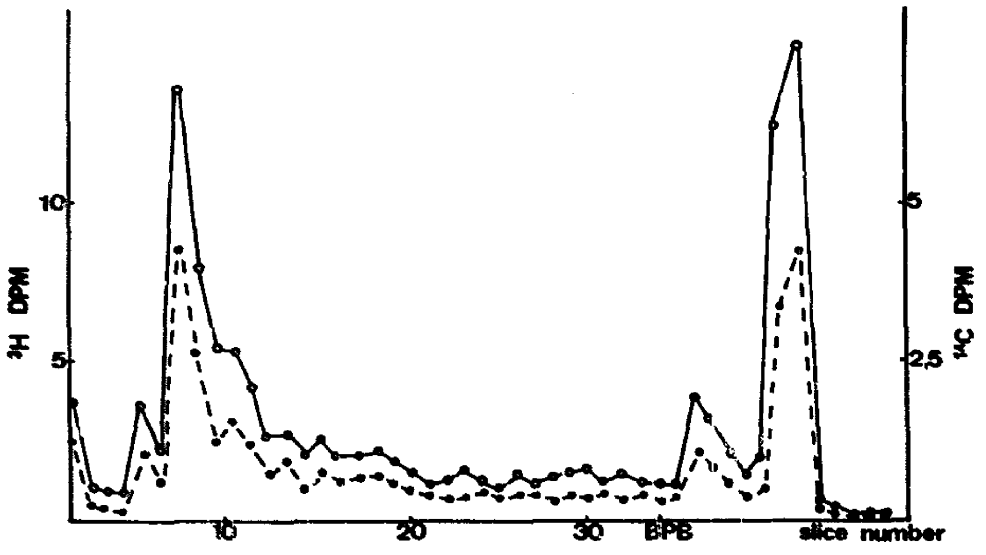

Fig. 5. Polyacrylamide gel electrophoresis of trypsinates double labeled with [^{3}H]- or [^{14}C]glucosamine. Control cells and lectin stimulated cells were grown in the presence of [^{3}H]glucosamine and [^{14}C]glucosamine respectively. The two cells sets were mixed and treated by trypsin as described previously. Trypsinate was solubilized in 1 % sodium dodecyl sulfate 1 % 2-mercaptoethanol and subjected to electrophoresis on 10 % polyacrylamide gels: (○—○), ^{3}H dpm · 10^{-3}; (●- - -●), ^{14}C dpm · 10^{-3}.

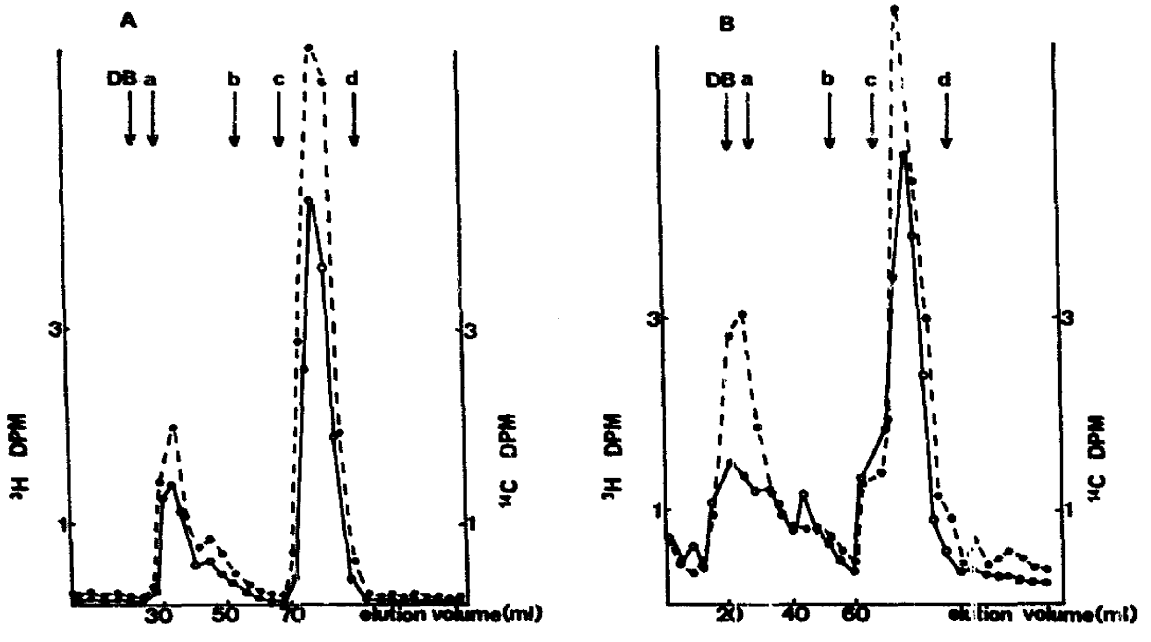

Fig. 6. Elution profiles on Sephadex G-200 column of deoxycholate extracts from control cells (A) and lectin stimulated cells (B). [^{3}H]leucine and [^{14}C]glucosamine labeled cells were solubilized by sodium deoxycholate (0.05 %) for 2 min at 20 °C. After centrifugation the supernatant designated deoxycholate extract was fractionated on Sephadex G-200 column (90 × 2 cm) equilibrated in 0.05 % sodium deoxycholate and calibrated with dextran blue (DB): a, immunoglobulin G; b, ovalbumin; c, cytochrome *c* and d, valine DNP. (○—○), ^{3}H dpm · 10^{-3}; (●- - -●), ^{14}C dpm · 10^{-3}.

similar. These results confirm those obtained by gel electrophoresis and gel filtration of trypsinates from two cell sets, as described above.

Therefore, trypsin digestion of intact ascites cells releases from cell surfaces two distinct size classes of glycoproteins, namely a macroglycoprotein fraction of molecular weight 200 000 or more, and a low molecular weight fraction. It is not yet possible to determine whether the latter fraction arises from degradation of the higher molecular weight fraction, or whether the two fractions are unrelated. The presence of lectin in the culture medium does not induce apparent qualitative modifications in the cell coat glycoproteins but only a stimulation of their biosynthesis.

(*b*) *Solubilization of cell membrane by sodium deoxycholate.* After 8 h culture, [^{3}H]leucine and [^{14}C]glucosamine labeled cells are treated by 0.05 % sodium deoxycholate which solubilizes membrane glycoproteins from intact cells and radioactivity distribution is measured in the cell pellet and extract. It is clear from Table I that the proportion of labeled material released by solubilization is larger than that released by trypsinization. These results are in good agreement with the assumption that detergent solubilizes components which are deeply rooted in the membrane and are not reached by proteolysis.

Deoxycholate extracts from control and lectin-stimulated cells are fractionated on calibrated Sephadex G-200 column and their elution patterns are shown in Fig. 6. The glycoprotein fractions, having the same molecular weight features as the compounds isolated by trypsin treatment, are obtained.

CONCLUSION

From the results obtained in this study, cell coat of Zajdela hepatoma ascites cells appears to be made up of two classes of glycoproteins having different molecular weights, as observed by gel electrophoresis and gel filtration: a high molecular weight group (200 000 or more) and a low molecular weight group (2000–3000). This latter group probably does not arise from trypsin proteolysis of cell surface glycoproteins because it is also present in deoxycholate extract. Although a fraction of these low molecular weight glycoconjugated compounds might possibly arise from the degradation of higher molecular weight compounds, these glycopeptide fractions appear to be structural entities in cell coat rather than degradation products of glycoproteins. Besides, glycopeptide material with a low molecular weight has also been isolated from a variety of membranes [30, 31].

On the other hand, the glycoprotein biosynthesis in hepatoma cells is stimulated by a mitogenic lectin from *Robinia* at the same time as cell growth is inhibited and no morphological changes are induced. This stimulation of glycoprotein synthesis by lectin appears to be a quantitative rather than a qualitative process as shown especially by double label with glucosamine of stimulated and non-stimulated cells. A similar stimulation of glycoprotein biosynthesis has been observed on culture lymphocytes in the presence of another mitogenic lectin. Yet this stimulation is accompanied by proliferation and blast transformation of lymphocytes whereas ascites cells proliferation is reduced by *Robinia* lectin and no increase in cell surface area is evident to warrant glycoproteins biosynthesis stimulation. Anyway, further work will be necessary to state whether these newly synthetised glycoproteins are degraded or excreted as such.

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REFERENCES

1 Morawiecki, A. (1964) Biochim. Biophys. Acta 83, 339–347
2 Benedetti, E. L. and Emmelot, P. (1968) in The Membranes (Dalton, A. S. and Haguenau, F., eds.), pp. 33–120, Academic Press, New York
3 Winzler, R. J. (1970) Intern. Rev. Cytol. 29, 77–125
4 Marchesi, V. T., Tillack, T. W. and Scott, R. E. (1971) in Glycoproteins of Blood Cells and Plasma (Jamieson, G. A. and Greenwalt, T. S., eds.), pp. 94–105, Lippincott and Co. Philadelphia
5 Curtis, A. S. G. (1967) The Cell Surface, Academic Press, New York
6 Roseman, S. (1970) Chem. Phys. Lipids 5, 270–297
7 Moscona, A. A. (1963) Proc. Natl. Acad. Sci. U.S.A. 49, 742–747
8 Waliach, D. F. H. (1968) Proc. Natl. Acad. Sci. U.S.A. 61, 868–874
9 Ohta, N., Pardee, A. B., McAuslan, B. R. and Burger, M. M. (1968) Biochim. Biophys. Acta 158, 98–102
10 Meezan, E., Wu, H. C., Black, P. H. and Robbins, P. W. (1969) Biochemistry, 8, 2518–2524
11 Buck, C. A., Glick, M. C. and Warren, L. (1971) Biochemistry 10, 2176–2180
12 Warren, L., Fuhrer, U. P. and Buck, C. A. (1973) Fed. Proc. 32, 80–85
13 Nowell, P. C. (1960) Cancer Res. 20, 462–466
14 Ling, N. R. (1968) Lymphocyte Stimulation, North Holland Publ. Co. Amsterdam
15 Aubery, M., Font, J. and Bourrillon, R. (1972) Exp. Cell Res. 71, 59–64
16 Shoman, J., Inbar, M. and Sachs, L. (1970) Nature 227, 1244–1246
17 Burger, M. M. and Noonan, K. D. (1970) Nature 228, 512–515
18 Dent, P. B. and Hillcoat, B. L. (1972) J. Nat. Cancer Inst. 49, 373–377
19 Borberg, M., Yesner, J., Gesner, B. and Silber, R. (1968) Blood 31, 747
20 Powell, A. E. and Leon, M. A. (1970) Exp. Cell Res. 62, 315–325
21 Sharon, N. and Lis, H. (1972) Science 177, 949–959
22 Nicolson, G. L. (1974) Int. Rev. Cytol. 39, 89–95
23 Hayden, G. A., Crowley, G. M. and Jamieson, G. A. (1970) J. Biol. Chem. 245, 5827–5832
24 Font, J. and Bourrillon, R. (1968) Biochim. Biophys. Acta 154, 28–39
25 Sharif, A. and Bourrillon, R. (1975) Cell. Immunol. 19, 372–380
26 Davis, B. J. (1964) Ann. N. Y. Acad. Sci. 121, 404–427
27 Hunt, R. C. and Brown, J. C. (1974) Biochemistry 13, 22–28
28 Evans, W. H. and Gurd, J. W. (1971) Biochem. J. 125, 615–624
29 Lenard, J. (1970) Biochemistry 9, 1129–1132
30 Laico, M. T., Ruoslahti, E. I., Papermaster, D. S. and Dreyer, W. J. (1970) Proc. Natl. Acad. Sci. U.S.A. 67, 120–127
31 Glick, M. C., Kimhi, Y. and Littauer, U. Z. (1973) Proc. Natl. Acad. Sci. U.S.A., 70, 1682–1688