ISOLATION OF A LECTIN FROM LIVER PLASMAMEMBRANE AND ITS BINDING TO CELLULAR MEMBRANE RECEPTORS IN VITRO

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1. Introduction

Recently, much interest has been stimulated by the fact that the surface membrane of mammalian cells possess receptor sites for the binding of lectins from plant seeds [1,2]. Such data has led to completely new perspectives regarding cell surface architecture and membrane fluidity of normal and virus transformed cells [3,4]. In addition, the binding of plant lectins to various types of animal cells has been shown to induce changes in many diverse physiological phenomena, such as mitosis [5], phagocytosis [6,7], protein synthesis [8] and cell-to-cell contact [9]. Understanding of a common biochemical basis of these apparently non-related properties of lectins has not yet been attained.

We have previously demonstrated that a general cellular location of plant lectins is within membranes [10–12]. These lectins most probably are composed of two distinct classes, those constituting secretory glycoproteins present in isolated membrane vesicles during transport to the cell wall [11] and those lectins, for example ricin, which appear to be actual components of membranes [12].

We thought therefore that it was highly probable that some analogous form of carbohydrate-binding proteins could be present within animal cell membranes and we decided to investigate such a possibility using plasmamembranes isolated from bovine liver.

In this report, we demonstrate that protein solubilized from plasmamembranes (PM) can bind to carbohydrate groups present at the surface of rabbit erythrocytes. This protein can therefore be classified as a lectin. With the aid of hemagglutination as an auxiliary system for the quantitative determination of the binding capacity we were able to demonstrate that the PM-lectin can bind in vitro to receptors present on the surface of PM as well as other cellular membranes.

2. Experimental

Membrane fractions of PM, Golgi apparatus (GA), rough microsomes (RM) and smooth microsomes (SM) were prepared from bovine liver cells essentially as described by Fleischer [13,14]. In this system and fractionation scheme, it has been demonstrated that cross contamination between fractions is minimal.

We extracted isolated membranes using two extractants previously shown to be efficient in the solubilisation of active lectins from plant cell membranes [10]. Fractions were sonicated in 0.5 M potassium phosphate, pH 7.1, centrifuged at 100 000 *g* (SW 50 L rotor, Beckman Spinco ultracentrifuge) for 15 min at 4°C to repellet membrane material, which was further sonicated in 0.1 M EDTA containing 0.06% v/v Triton X-100, and recentrifuged as before. The two extracts: 1 (phosphate extract) and 2 (EDTA-Triton extract) were dialysed extensively against 0.05 M sodium phosphate pH 7.3, 0.9% w/v NaCl (PBS). Non-diffusible material from all extracts and membranes was made up to constant volume (6.0 ml) with PBS and tested for the presence of lectins by the agglutination assay using trypsinised

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rabbit erythrocytes, as described previously [10]. The effect of sugars and glycosides on the agglutination activity of the lectin was investigated using the titer plate method [10]. Protein was determined using the method of Lowry [15], using bovine serum albumin as the standard.

In order to investigate whether isolated lectin could bind to liver cell membranes in vitro, isolated vesicles were suspended in PBS, and incubated with aliquots of extract 2. After incubation at 37°C for 15 min the suspension was centrifuged and the supernatant was tested for agglutination activity against rabbit erythrocytes.

3. Results and discussion

Lectin activity was recovered only in extract 2 of the PM fraction (see control in fig.1), in which 0.12 μg protein/50 μl was sufficient to just agglutinate all erythrocytes. No lectin activity was found in any other fraction, although protein was present (concentrations of protein in extract 2 as $\mu g \cdot ml^{-1}$: PM 155, GA 465, RM 175, SM 970). This result is partly in contrast with results obtained for plant cell membranes, where lectin activity was recovered in extracts of all membranes isolated (PM, GA, ER and mitochondrial) [10–12], and therefore may indicate that other lectins are present in cellular membranes of liver cells, but are non-active towards surface carbohydrates on rabbit erythrocytes. It is of interest that preliminary studies have indicated that a high titer against trypsinized rabbit erythrocytes could be obtained using extracts of microsomal and mitochondrial fractions from bovine pancreas.

In order to investigate whether the isolated PM lectin can bind to liver cell membranes in vitro, isolated vesicles were suspended in PBS and incubated with aliquots of lectin. We were able to demonstrate that receptors for the PM lectin are present on the surface of isolated vesicles, and that the relative amounts of vesicle material required to achieve saturation of available lectin binding capacity was different for the various membrane fractions (fig.1). Since the amount of vesicles was measured by protein determination, and the vesicles may contain varying proportions of proteins non-involved in complex formation, it is difficult to attach quantitative

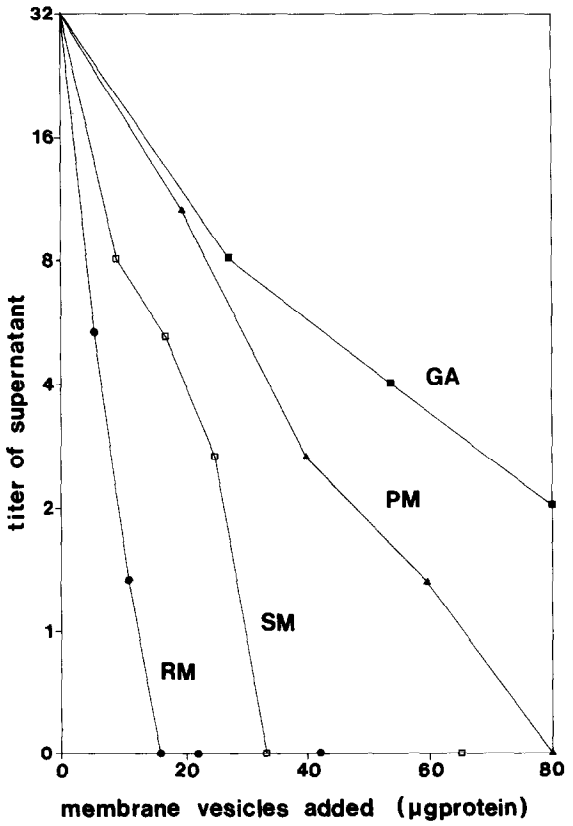

Fig.1. Decrease in the agglutination activity caused by binding of liver PM lectin to different membrane vesicles. 100 μl of PM lectin (15.5 μg protein) was incubated with 100 μl PBS containing different membrane vesicles at the concentrations shown. After 15 min incubation at 37°C and centrifugation at 12 000 *g* at room temp., the agglutination activity of lectin remaining in the supernatant was tested by the hemagglutination assay. Agglutination activity is given as titer, defined as the reciprocal of the greatest dilution at which full agglutination occurred. In cases where the titer of parallel samples differed by one dilution step, the lowest titer multiplied by $\sqrt{2} = 1.41$ is given.

significance to the binding saturation figures. The relative degree of vesiculation of the different disrupted membranes, and the extent to which the different membranes have vesiculated inside-out are also unknown. In this context, it may be of relevance that substantial binding of concanavalin A (Con A) to rat liver cellular membranes (measured as radioactivity recovered in a filter assay after incubation at 37°C for 1 h) could only be obtained after a series of

successive freezing/thawing treatments of the membranes [16]. However, the data presented in this report does demonstrate on a qualitative basis the presence of receptor groups on the plasmamembrane with properties matching the binding sites of the PM lectin.

Preliminary evidence indicates that L-fucose, D-galactose, D-xylose, α-methyl-D-mannoside and N-acetyl-D-galactosamine in final concentrations of 5×10^{-3} M, do not inhibit the agglutination of rabbit erythrocytes by liver PM lectin. *N*-acetyl-D-glucosamine (5×10^{-4} M) reduces the titer from 32 to 6, and di- and tri-β-1-4-oligomers of *N*-acetyl-D-glucosamine (kindly supplied by N. Sharon, Rehovot) were completely inhibitory at the latter concentration. During the course of this work, agglutinating activity of a rabbit hepatic binding protein was described [17]. The inhibition of that agglutination reaction was however caused by sugars different to those reported here for bovine liver PM lectin.

Carbohydrate macromolecules present on the surface of cells have often been implicated in the processes of contact and adhesion between neighbouring cells, providing a basis for morphogenetic cell aggregation [18–21]. This requires the presence of cell surface components with specific recognition properties. The nature of such components has been visualised on a more or less hypothetical basis as antibody-like proteins [18–20] or surface glycosyltransferases [21]. Our results demonstrate the presence of carbohydrate-binding protein in plasmamembrane which can bind to homologous receptors on the same membrane, and therefore allows the suggestion that the processes of contact and adhesion may be mediated by lectin–receptor interaction between adjacent cells.

The application of Con A and other seed lectins have been shown to provide an in vitro stimulus for many varied physiological events [1–9]. A common feature of these diverse events is the requirement that the stimulus is transmitted from the cell surface to the cell interior, and many characteristics of the surface carbohydrate groups defined as lectin receptors are in accordance with their role as stimulus-transmitters [22–28]. We suggest that binding of seed lectins to animal cells may mimic the action normally provided by neighbouring cells. The binding of the PM lectin of one cell to carbohydrate containing receptors at another cell would thus provide the in vivo stimulus for developmental events.

This hypothesis would provide a consistent interpretation of much other experimental data [29–35]. For example, Con A receptors are known to be involved in the spontaneous reaggregation of young chick embryo neural retina cells [9]. Addition of non-agglutinating digests of Con A-t [9], or homotypic PM fractions [29] to these cells, prevents the occurrence of spontaneous reaggregation. This could be explained if the receptor sites on the cell surface membrane were blocked by the binding of the added material and were therefore unavailable for endogenous surface lectin–receptor binding. Similarly, normal low density non-contacted cell lines exhibit a random distribution of intra-membraneous particles, whereas in high density contact inhibited cell cultures, a correlation between degree of cell contact and aggregation of particles has been demonstrated [30,31]. It may be speculated that the clustering of particles represents the formation of endogenous lectin–receptor bonds. It is of interest that the surface receptors of transformed cells remain randomly distributed until clustered by the action of an exogenous lectin, this may imply that a feature of transformed cells is their inability to form in vivo binding of lectin–receptors possibly due to a decreased availability of endogenous plasma membrane lectin.

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Note

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