CONCANAVALIN A BINDING BY ISOLATED PLASMA MEMBRANES AND ENDOMEMBRANES FROM LIVER AND MAMMARY GLAND

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Plasma membrane and other endomembrane components from rat liver and bovine mammary gland were assayed for their ability to bind the jack bean lectin concanavalin A (Con A). Rat liver plasma membrane and the apical plasma membrane-derived milk fat globule membrane (MFGM) had the greatest capacity to bind Con A. Rough microsomes and nuclear membranes bound only small amounts of Con A and Golgi apparatus bound intermediate levels of the lectin. Binding sites were assymetrically distributed on the membranes.

1. Introduction

Con A and other plant lectins have been used extensively to study the nature and distribution of binding sites on the surfaces of normal and transformed cells. Different lectins have different specificity; Con A binds to terminal α-D-mannopyranoside or α-D-glucopyranoside residues [1]. These studies have followed three forms: utilization of lectins to determine agglutinability of cells (e.g. [2–4]), quantitative binding studies with radioactive lectins (e.g. [3–8]), and studies of the distribution of surface saccharides using ferritin-conjugated agglutinins (e.g. [9,10]) or agglutinin–peroxidase conjugates [11–13]. Using these techniques it was shown that surface membrane carbohydrates are externally disposed [9,10] and transformed cells were reported to differ from untransformed controls in number [14] and distribution [15,16] of binding sites and in agglutinability [4]. However, comparison of different results is complicated by the fact that differences in incubation conditions or the degree of confluency of cells can greatly affect the magnitude of binding [3,4].

Since it is apparent that problems such as endocytosis [3,10,17] could be overcome by using isolated membranes rather than intact cells, it is surprising that lectin binding to isolated plasma membranes has not yet been studied. Mitochondria appear to be the only purified cellular component studied in this regard [18,19] although Hirano et al. [10] observed that ferritin-conjugated lectins were bound by plasma membranes and also internal surfaces of endomembrane vesicles when added to myeloma cell homogenates.

2. Experimental procedures

Nuclei and nuclear membranes [20] rough endoplasmic reticulum [21–23], smooth microsomes [21,23], Golgi apparatus [21–24], plasma membrane [21,22] and MFGM [25] fractions were isolated from rat livers or from mammary tissue or milk of lactating Holstein cows. Morphological and biochemical characterization has shown these fractions to be contaminated by no more than 10% with other membrane fractions. The MFGM is known to be derived directly from the apical plasma membrane of mammary secretory cells [23,25,26]. Membrane fractions were washed twice by centrifugation after suspension in PBS (0.15 M NaCl, 1 mM KH_2PO_4, 10 mM Na_2HPO_4, 1 mM $MgCl_2$, pH 7.2). Unless otherwise specified, all fractions were frozen and thawed at least three times prior to assay.

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Con A (Serva, Heidelberg) was acetylated [27] with ^{3}H-labelled acetic anhydride (400 mCi/mMole, New England Nuclear) to a specific activity of 1.54×10^{7} cpm/mg as determined under the conditions used for binding assay.

Membrane fractions were incubated with the desired amount of Con A in PBS in a final volume of 1.0 ml. After incubation with agitation, the assay mixture was transferred to a Whatman GF/C glass fiber filter under vacuum [13]. The filter was washed with 4×5 ml portions of PBS (further washing caused no decrease in radioactivity) and was then transferred to a scintillation vial containing 1 ml of Nuclear Chicago solubilizer. After one hour at 37°C, 10 ml of a toluene-based scintillation fluid containing PPO and POPOP were added and radioactivity was determined. Parallel incubation mixtures contained 2 mM α-methyl mannoside in addition to the above constituents. Specific binding was measured as the difference between the values obtained in the absence and presence of α-methyl mannoside. This in effect corrects for nonspecific binding, entrainment, adsorption or aggregation of Con A [5].

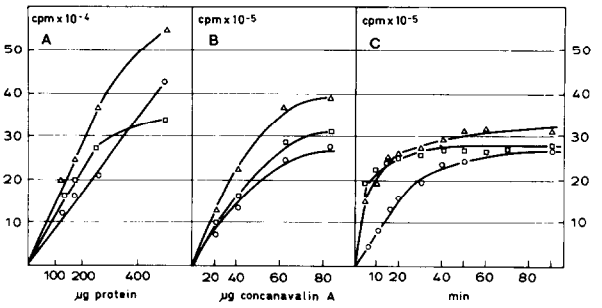

Fig. 1. Concanavalin A binding by bovine milk fat globule membrane. Binding was measured at three incubation temperature, 0°C (○), 19°C (△) and 37°C (□). In A and C. Con A was present at a level of 84 μg/assay. In A and B, incubation times were 40 min. In B and C the concentration of membrane protein was 170 μg/assay. Values are specific binding determined as the difference between binding in the presence and absence of α-methyl mannoside. Other conditions are given in the text. In B and C data are expressed as cpm/2 mg protein.

3. Results

Since there have been no previous studies of Con A binding to isolated membranes it was necessary to determine the optimum conditions for assay. For this purpose we used the apical plasma membrane-derived MFGM [23,25,27]. MFGM specifically bound large amounts of Con A. At the three incubation temperatures studied, 0,19 and 37°C, binding was linear up to at least 300 μg of MFGM protein (fig. 1). Within the linear range binding was highest at 19°C and lowest at 0°C. At a constant membrane protein concentration of 170 μg, binding increased in a nearly linear fashion as the Con A concentration was increased up to about 80 μg. At higher Con A concentrations there was little further increase in binding, indicating that binding sites became saturated (fig. 1). Results from time course assays showed that saturation of binding sites was attained much more rapidly at 19 or 37°C than at 0°C (fig. 1). At the higher temperatures nearly maximum amounts of Con A were bound within the first 20 min of incubation; continuing incubation for periods up to 90 min resulted in little additional increase in the amount of Con A bound. At 0°C binding did not reach maximum values until about 60 min. Nevertheless, at saturation the concentrations of Con A bound were nearly the same at all temperatures. This indicates that there is little difference in accessibility of Con A to binding sites over this temperature range. In contrast, with intact cells it has been shown that as much as double the amount of Con A is bound at temperatures above about 12–15°C [13]. The present results suggest that this increased binding with cultured cells may be due to the increased endocytosis of Con A at the higher temperature.

Morphological investigations of milk fat globule secretion have shown that the globule is enveloped in such a way so that the externally disposed surface of the membrane surrounding the globule is the same surface as that which was externally disposed on the plasma membrane [25,26]. Thus this system can be used, to study assymetry of distribution of constituents of the surface membrane, particularly with large molecules such as Con A (the molecular weight of the active form is 120 000; [28]) which would not be expected to penetrate the membrane. Such studies are facilitated by the fact that the MFGM, as isolated, resists vesiculation and mostly exists in the form of nonvesicular membrane sheets [25,29]. Over the range of membrane protein concentrations from 30 to 200 μg, both the intact

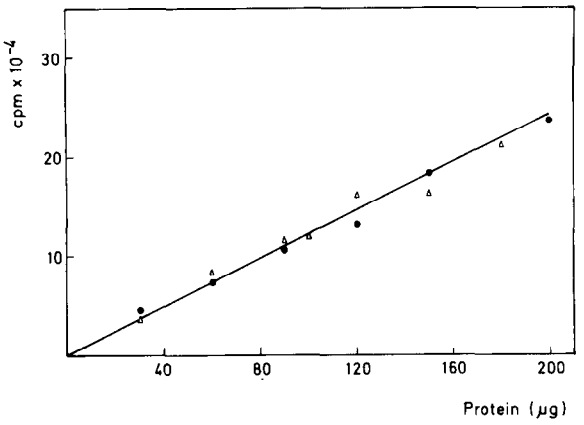

Fig. 2. Concanavalin A binding by intact milk fat globules (•) and isolated milk fat globule membrane (△). Assay mixtures contained 84 μg Con A and the indicated amount of membrane protein. Incubation was at 37°C for 40 min. Values are specific binding determined as the difference between binding in the presence and absence of α-methyl mannoside. Other conditions are given in the text.

fat globule and the isolated MFGM bound nearly identical amounts of Con A (fig. 2). Binding was linear over this membrane protein concentration range. Since there is thus no difference in accessibility of Con A to binding sites in the intact globule and isolated membrane, it follows that all of the binding sites are externally disposed in the membrane surrounding the globule. A similar external disposition of the lectin binding sites on cultured cells has been shown by Nicolson and Singer [9] with ferritin-conjugated lectins. In addition to Con A-binding residues, protein-bound sialic acid is also externally disposed on the MFGM (T. W. Keenan, unpublished observations).

If the carbohydrates are externally disposed on the surface membrane it is logical to assume that they are internally disposed in intracellular vesicles and cisternae and thus may not be accessible to Con A in 'right side out' isolated membrane vesicles (see Discussion). To test this, liver Golgi apparatus fractions were assayed for their ability to specifically bind Con A. Freshly prepared Golgi apparatus fractions had limited ability to bind this lectin. This may have been due in part to residual sucrose, a weak inhibitor of Con A binding, in the fraction. Washing the fraction twice with distilled water increased the binding capacity to a considerable extent; this increase was only partially reversed by addition of sucrose to the assay mixture. Freezing and thawing and/or sonication of the washed fraction, treatments known to cause perforation and rupturing of vesiculated membranes, yielded a further increase in binding capacity. Maximum binding was achieved after three cycles of freezing and thawing; further freezing cycles or sonication did not affect binding capacity. Thus this treatment appeared to yield maximum accessibility of binding sites to Con A and all fractions were subjected to three cycles of freezing and thawing, with interposed homogenization and centrifugation steps, before assay. These treatments, freezing or sonication, had no effect on Con A binding by the MFGM.

The distribution of Con A-binding sites among endomembrane components from rat liver and bovine mammary gland is given in table 1. Relative to total membrane fractions from liver, plasma membrane and Golgi apparatus bound higher levels of Con A and nuclear and endoplasmic reticulum membranes bound much lower levels. Rough endoplasmic reticulum and nuclear membranes bound nearly the same amounts of the lectin. Binding was slightly higher in our smooth microsomes than in the rough endoplasmic reticulum. Compared to endoplasmic reticulum, Golgi apparatus bound more than twice as much Con A. By far the

Table 1
Concanavalin A binding by membrane fractions from rat liver and bovine mammary gland

Fraction	Specific binding*	
	Liver	Mammary gland
Total particulate	18.7	15.6
Nuclei	5.7	
Nuclear membranes	15.4	
Rough endoplasmic reticulum	14.6	19.5
Smooth endoplasmic reticulum	17.8	20.3
Golgi apparatus	63.9	55.6
Plasma membrane	146.0	
Milk fat globule membrane		139.0

* Specific binding is cpm Con A bound/mg protein × 10^{-4}. Incubation was at 37°C for 40 min. Con A was present in a concentration of 84 μg/assay and the amount of membrane protein was from 100 to 200 μg/assay. Other conditions are described in the text. Total particulate refers to the material of total homogenates sedimented by centrifugation at 150 000 *g* for 1 hr.

largest amounts of Con A were bound by the plasma membrane, which was more than twice as active as was Golgi apparatus. Essentially similar results were obtained with membrane fractions from mammary gland. Binding by endoplasmic reticulum was slightly below the level bound by total particulate fractions. Golgi apparatus bound nearly three times more Con A than did endoplasmic reticulum. MFGM bound more than twice the amount of Con A bound by Golgi apparatus. The binding data for nuclear and rough endoplasmic reticular membranes are most probably overestimates. The low levels of binding observed could be explained, for example, by a 10% contamination of the fraction by membranes from the surface, Golgi apparatus and secretory vesicles. Since some contamination of the fractions is inevitable, the true specific binding ability of nuclear and rough endoplasmic reticular membranes is probably much lower than our figures would indicate.

4. Discussion

Results obtained show that plasma membranes from liver and mammary gland bind Con A in a manner predictable from results obtained with intact cells. The use of isolated membrane fractions overcomes the difficulties in assaying surface of intact cells that arise from accumulation of lectins by endocytosis. While plasma membrane and MFGM had the greatest ability to bind Con A, binding was also appreciable in Golgi apparatus fractions. This result is predictable from the hypothesis of membrane flow and differentiation which states that new membrane material formed at the level of rough endoplasmic reticulum or nuclear membrane is transferred to Golgi apparatus where it is differentiated from membrane which is endoplasmic reticulum-like to membrane which is plasma membrane-like. The differentiated membrane components are then utilized in formation of secretory vesicles, the membrane of which is plasma membrane-like and capable of fusing with plasma membrane [30]. The extensive morphological and biochemical evidence supporting this hypothesis has been reviewed [23,30–33]. More pertinent to the present study, Golgi apparatus membranes are intermediate between nuclear and endoplasmic reticulum membranes and the plasma membrane in composition. Thus the results with Con A binding are fully consonant with, and serve as additional confirmation for, this hypothesis.

The distribution of Con A-binding sites in endomembranes from liver and mammary gland parallels the known distribution of glycoprotein and glycolipid carbohydrates [23,31,32]. Con A-binding sites are low in nuclear membranes and endoplasmic reticulum, intermediate in Golgi apparatus and highest in plasma membranes. To what extent the low levels of carbohydrates in nuclear and rough endoplasmic reticular membranes is due to contaminants is not known at present. Many of the studies purporting to show the presence of bound carbohydrates in such fractions suffer from incomplete assessment of the degree of contamination. Glycosyltransferases which add carbohydrates to protein or lipid acceptors are concentrated in Golgi apparatus but are also present in endoplasmic reticulum [22,35–37]. It is now apparent that polypeptide or lipid acceptors are incorporated into the membrane at the level of the endoplasmic reticulum and that carbohydrates are added in a step-wise manner as the membrane flows toward and is differentiated into plasma membrane. The distribution of glycosyltransferases is consistent with this [22,35] and the present results with Con A binding serve as additional evidence in favour of this concept.

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