Biochimica et Biophysica Acta, 730 (1983) 359–368
Elsevier Biomedical Press

BBA 71629

TIME-DEPENDENT LECTIN BINDING TO ISOLATED RECEPTORS IN MODEL MEMBRANES

NIKA V. KETIS and CHRIS W.M. GRANT

Department of Biochemistry, University of Western Ontario, London, Ontario, N6A 5C1 (Canada)

(Received October 7th, 1982)

Key words: Glycophorin; Agglutinin; Band 3 protein; Lectin receptor; Concanavalin A; (Model membrane)

Binding of lectins to two integral membrane glycoproteins has been measured in lipid bilayer model membranes and in their cell of origin with an eye to clarifying the basis of time-dependence in such processes. Specific binding was monitored as a function of time using an assay that involved membrane exposure to radiolabelled wheat-germ agglutinin or concanavalin A, and subsequent differential centrifugation to remove unbound material. Qualitatively, the time dependence of lectin binding to the isolated receptors in lipid bilayers was found to be similar to that for the same receptors in the intact cell – hence the phenomenon does not depend for its existence upon receptor interaction with other specific native membrane components. Quantitatively, the time-course was sensitive to structural features of the model membrane involved. The results may be understood by viewing the glycopeptide headgroups as deformable structures which rearrange as a direct result of lectin attachment, and it would appear that rearrangement is essential for high-affinity binding. Model membrane structure was examined by light microscopy and freeze-etch electron microscopy in an attempt to assess the applicability of this type of study to a more detailed analysis of the processes involved in lectin binding. Although the freeze-etch technique is a promising one, it was concluded that heterogeneity in receptor arrangement within the lipid bilayer is the most important limitation to correlation of binding curves with membrane structure.

Introduction

The physical response of membrane receptor glycoproteins to a specific recognition event such as lectin binding is of considerable interest, since it may play a role in initiation and/or modulation of the overall cellular response. In this regard, it is noteworthy that binding of lectins and other specific polypeptides to cell surfaces is time-dependent (see, for example, Refs. 1 and 2). Typically, the amount bound increases over a period of up to 1 h from zero to some limiting value. The most remarkable feature of these time-dependent binding profiles is that they exist: that site saturation is not complete within seconds as in the case of enzyme/substrate interactions. Given the well-known fact that lectin binding may trigger a variety of processes including receptor turnover, patching and capping; and given the complex milieu that is the cell surface; it is tempting to correlate this time-dependence with the functioning of some intricate and meaningful cellular machinery. However, it was very clearly demonstrated as early as 1975 by Schnebli and Bachi that this need not be the case [1]. These authors showed that time-dependent concanavalin A binding occurred in a system of human erythrocytes in which appreciable receptor redistribution and metabolic processes were not possible. We have investigated the phenomenon of lectin binding in a model system of isolated membrane glycoproteins reassembled into lipid bilayers.

Glycophorin (Refs. 3, 4 and references therein) and band 3 (Refs. 5, 6 and references therein) are both transmembrane glycoproteins of the human erythrocyte. Analogous species in more active cells are thought to have the potential for mediating cellular response to specific recognition events.

They occur in approximately equal numbers (some 10^6 copies of each) in the red cell membrane. It has been suggested that band 3 protein exists as dimers [4,7] and that each such dimer associates with one glycophorin, while leftover glycophorin molecules are independent of such association [7]. About 25% of band 3 molecules carry sites appropriate to high affinity concanavalin A attachment [5] – and these comprise the only substantial population of concanavalin A binding sites on the erythrocyte membrane. Similarly, glycophorin is the human red cell binding site for wheat-germ agglutinin. Hence, this system has some unusual advantages for comparisons between model and native membranes – most cell types have more than one receptor glycoprotein for a given lectin.

Glycophorin and band 3 protein are structurally quite different species. The former has a small polypeptide backbone (M_r 12000) which traverses the membrane only once (hydrophobic portion 23 amino acid residues) [3]; and bears 16 short carbohydrate chains of which 15 are 0-linked (Ref. 8 and references therein). In contrast, band 3 protein has a M_r of almost 100000, a complex, folded hydrophobic section, and a single large N-linked carbohydrate chain [5,6,9]. We have previously described lectin-binding profiles and scatchard plot analyses for these receptor glycoproteins in large liposomes [10–12]. The results are strikingly similar to those from analogous experiments with receptors in intact cells.

Materials and Methods

Membrane glycoproteins were isolated from outdated bank blood: glycophorin by the method of Marchesi and Andrews [13], and the concanavalin A receptor by a modification of the method of Findlay [14] as described earlier [15]. The details of their incorporation into lipid bilayer structures have been described by us previously [10–12,15,16]. Synthetic lipids were obtained from Sigma and were pure as judged by thin-layer chromatography on silica gel G (Stahl). Total erythrocyte lipid was prepared by extraction of freeze-dried ghosts with 2 : 1 chloroform/methanol and then repeated drying and extraction of the soluble fraction. 2-Chloroethanol was from Eastmann-Kodak and unlabelled lectins were from Sigma. ^{125}I-labelled wheat-germ agglutinin was prepared according to the general method of Markwell [17]: it was typically 71% trichloroacetic acid-precipitable and had a specific activity of $4 \cdot 10^5$ cpm/μg of protein. [^{3}H]Concanavalin A was from New England Nuclear and was 75% trichloroacetic acid-precipitable with a specific activity of $1.8 \cdot 10^6$ cpm/μg. ^{125}I was from Amersham. Serum albumin was either 96–99% fraction V from Sigma or ultrapure globin-free – both of which gave the same results. Dextran T 500 was from Pharmacia. Peroxidase-linked wheat-germ agglutinin was Type VI from Sigma; ferritin-linked concanavalin A was from Sigma or CalBiochem-Behring Corp. Rhodamine-labelled concanavalin A was from Vector Laboratories.

The lectin-binding assays employed in this work were based on incubation of radiolabelled lectins with erythrocytes or model membranes and subsequent differential centrifugation to remove unbound material. The details have been described elsewhere [10,11]. With the exception of samples for freeze-etch electron microscopy, all liposomes used in these studies were very large, multilamellar structures produced by hydration of dried films [10,11]. Samples for electron microscopy were either small liposomes from the latter preparations (separated by differential centrifugation) or were prepared by dialysis of detergent solutions containing lipid plus glycoprotein [15,16]. Both preparations had the same appearance by electron microscopy. All binding experiments were done with 3 mg/ml serum albumin or Dextran T 500 in the buffers (pH 7.4 phosphate-buffered saline containing Ca^{2+} and Mg^{2+}).

Samples for electron microscopy were quenched in Freon 22 cooled in liquid nitrogen. They were freeze-etched at -100°C (2 min etching) using a Balzers BA501 high-vacuum coating device. Replicas were cleaned in commercial bleach and then acetone prior to examination with a Philips EM 200. If liposomes were to be labelled with wheat-germ agglutinin-peroxidase, they were incubated with this material for 30 min at 0°C. The samples were then washed twice in saline, fixed with glutaraldehyde (Polysciences EM grade) for 2 h at 0°C, washed once more and allowed to dialyze overnight. They were then exposed to diaminobenzidene [18] (Sigma) at 0°C for 10 min and dialyzed against 5 mM buffer for 2–5 h.

Results

Binding curves

Figs. 1 and 2 illustrate the time-dependence of high-affinity lectin binding to intact human erythrocytes. In our hands, such curves can vary marginally, depending upon the history of the red blood cells (e.g., freshly drawn vs. outdated bank blood) – the data shown are for fresh blood. The curves obtained are similar to lectin-binding time-courses reported by other workers [1,2] – rising logarithmically from zero to a plateau representing site saturation at the concentrations of lectins employed. Schnebli and Bachi have previously reported a time-course for concanavalin A binding to human erythrocytes [1]; and our data are in good agreement with theirs. It is well known that in the mature human erythrocyte neither the receptors for concanavalin A nor those for wheat-germ agglutinin show gross redistribution in response to lectin binding: both remain essentially randomly dispersed (Refs. 1, 19 and references therein). Yet clearly, high-affinity attachment of concanavalin A and wheat-germ agglutinin are processes requiring a finite time (40 min and 15 min, respectively, to reach site saturation). If receptor rearrangement is a controlling factor in this system, it occurs over distances of no more than a few hundred ångstroms.

Figs. 3A, 4A illustrate time-courses for lectin binding to glycophorin and concanavalin A receptors, respectively, in lipid bilayers. It would appear

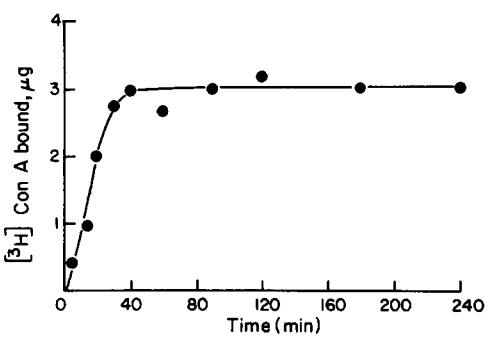

Fig. 1. Time-dependence of [^{3}H]concanavalin A binding to intact human erythrocytes (in (Ca^{2+}, Mg^{2+})-containing phosphate-buffered saline (pH 7.4) with 3 mg/ml bovine serum albumin). Each sample comprised $1.9 \cdot 10^8$ cells and 200 μg lectin in a total volume of 300 μl. Temperature, 22°C.

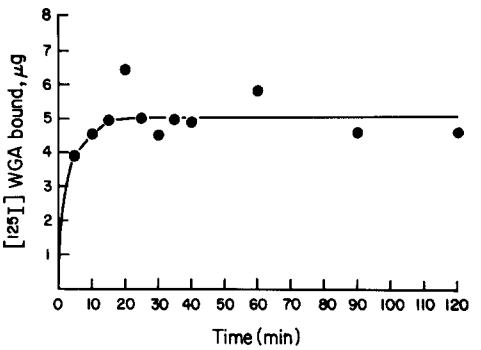

Fig. 2. Time-dependence of ^{125}I-labelled wheat-germ agglutinin binding to intact human erythrocytes (in (Ca^{2+}, Mg^{2+})-containing phosphate-buffered saline (pH 7.4) with 3 mg/ml bovine serum albumin). Each sample comprised $1.9 \cdot 10^8$ cells and 200 μg lectin in a total volume of 300 μl. Temperature, 22°C.

that time-dependent high-affinity lectin binding to glycoprotein receptors can take place in the complete absence of any other component specific to

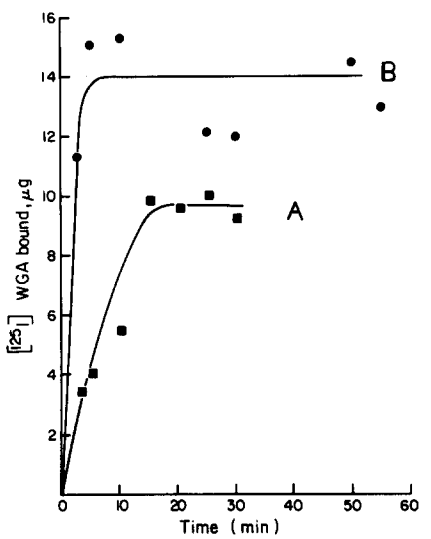

Fig. 3. A. Time-dependence of ^{125}I-labelled wheat-germ agglutinin binding to large, sealed liposomes of dimyristoylphosphatidylcholine-cholesterol (4 : 1.5 mole ratio) bearing glycophorin (2 : 1 lipid/protein weight ratio). Assayed in pH 7.4 phosphate-buffered saline containing Ca^{2+} and Mg^{2+}, and 3 mg/ml bovine serum albumin. Each sample comprised $0.4 \cdot 10^7$ liposomes and 200 μg lectin in a total volume of 300 μl. Temperature, 22°C. B. As A, except that the liposomes contained both glycophorin and concanavalin A receptor (1 : 1 mole ratio) and the lipid/protein weight ratio was 2 : 1. Each sample comprised $5.2 \cdot 10^7$ liposomes and 200 μg lectin in a total volume of 300 μl.

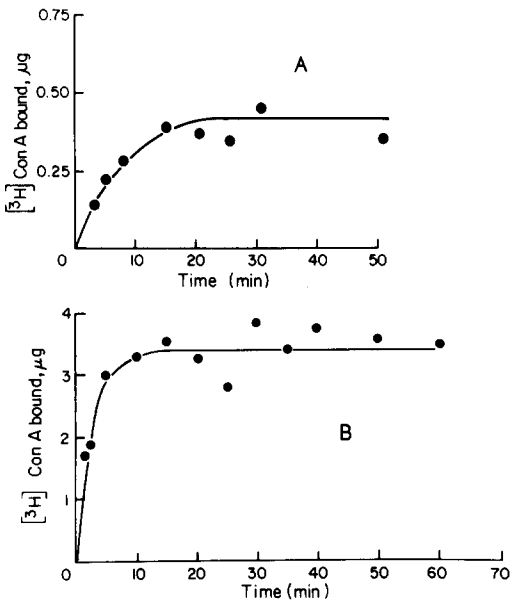

Fig. 4. A. Time-dependence of [^{3}H]concanavalin A binding to large, sealed liposomes of dimyristoylphosphatidylcholine-cholesterol (4 : 1.5 mole ratio) bearing concanavalin A receptor glycoprotein (1 : 1 lipid/protein weight ratio). Assayed in pH 7.4 phosphate-buffered saline containing Ca^{2+} and Mg^{2+}, and 3 mg/ml bovine serum albumin. Each sample comprised $1.2 \cdot 10^7$ liposomes and 200 μg lectin in a total volume of 300 μl. Temperature, 22°C, B. As A, except that the liposomes contained both glycophorin and concanavalin A receptor (1 : 1 mole ratio) and the lipid-to-protein weight ratio was 2 : 1. Each sample comprised $5.2 \cdot 10^7$ liposomes and 200 μg lectin in a total volume of 300 μl.

the cell of origin. Hence, the phenomenon certainly does not demand the participation of a complex membrane machinery, or even interactions with other specific components. It may be worth noting that wheat-germ agglutinin/glycophorin binding in this particular bilayer system (4 : 1.5 mole ratio dimyristoylphosphatidylcholine/cholesterol) occurs at about the same rate as it does in intact cells (complete in 15–20 min), while the time-course of lectin binding to the concanavalin A receptor has dropped from 40 min to 10 min. We feel that this difference is correlated with a tendency on the part of the concanavalin A receptor to exist in dense clusters in our bilayer systems (see following sections). This would permit rapid multivalent lectin binding with only minimal headgroup reorientation.

Since glycophorin and band 3 proteins are thought to interact in the native membrane [4,7], we have recorded lectin-binding curves for liposomes containing both receptors at once. Typical time-courses are given in Figs. 3B, 4B. The time taken to reach site saturation is shortened by 50% or more in each case. The latter observation is consistent with previous claims that the two families of receptors can also interact with one another in model membranes [11,16]. However, it is clear from the following sections that a more detailed interpretation of such experiments with more than one receptor would require a better knowledge and control of receptor arrangement than is possible in our model system.

The question arises as to whether one might be able to manipulate model membrane lipid composition to sort out the relative contributions of receptor lateral diffusion and simple headgroup reorientation to the time-course of lectin binding. For instance the bilayers upon which Figs. 3, 4 are based permit gross receptor redistribution over the time-course of the binding experiment [15,19]. Switching to a rigid lipid membrane such as dipalmitoylphosphatidylcholine-cholesterol at 22°C should prevent long-range receptor lateral redistribution and greatly slow even short-range lateral diffusion [20]. Fig. 5 shows that this approach does result in a measurable prolongation of the time course for lectin binding (compare to Fig. 3A), but not the drastic change one would expect

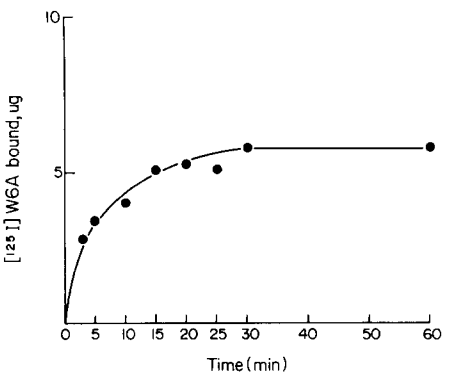

Fig. 5. Time-dependence of ^{125}I-labelled wheat-germ agglutinin binding to (rigid) liposomes of dipalmitoylphosphatidylcholine-cholesterol (4 : 1.5 mole ratio) in phosphate-buffered saline containing Ca^{2+} and Mg^{2+}, and 3 mg/ml bovine serum albumin. Temperature, 22°C. Each sample comprised $0.8 \cdot 10^7$ liposomes and 200 μg lectin in a total volume of 300 μl.

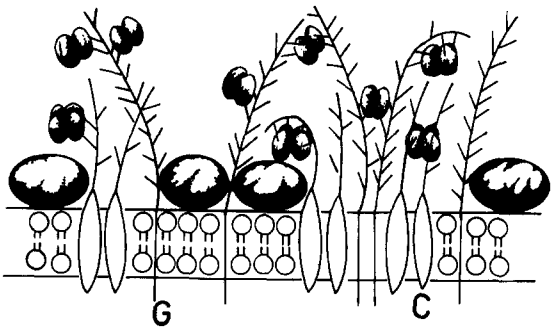

Fig. 6. Schematic illustration of the possible modes of multidentate liganding for wheat-germ agglutinin (dimer) binding to glycophorin (G) and concanavalin A (tetramer) binding to concanavalin A receptor (C). Inter- and intramolecular contributions have been indicated. Interactions with sites other than oligosaccharide have been ignored, although they may be significant (for example, Ref. 22). Adsorbed serum albumin is shown as shaded spheroids at the lipid/water interface.

if lateral diffusion alone were the determinant of the time-course for lectin binding. Fig. 6 has been included to aid visualization of the various primary processes discussed above that might be expected to confer time-dependence upon lectin binding to glycophorin or band 3. It attempts to illustrate the fact that considerable potential exists for complex headgroup rearrangement to optimize highly directional multidentate liganding. The degree of headgroup mobility invoked in this figure is, if anything, conservative. Note that when receptors are already tightly clustered, multidentate liganding should be more rapidly achieved.

Electron microscopy

Theoretically, far more subtle investigations of membrane-receptor function could be performed with a well controlled and uniform model system. Hence, membrane characterization is a key issue. Freeze-etch electron microscopy is a technique which offers high-magnification views of extensive regions of membrane surfaces. It has found considerable application in studies of receptor distribution on cell membranes: the approach being typically to use ferritin-conjugated antibodies or lectins to localize a particular receptor. To our knowledge, the same technique has not previously been applied to model membranes. The role of the attached ferritin (a large, stable structure) is to cast a more substantial shadow than would lectin or antibody alone during subsequent platinum shadowing of the specimen. Ferritin appears in the micrographs as a precise sphere marking the location of the lectin-receptor complex. Alternatively, one may employ lectins or antibodies covalently coupled to peroxidase, an enzyme which precipitates about itself (in the presence of diaminobenzidene) a deposit of insoluble material. This deposit is irregular in size and shape. Fig. 7 illustrates the appearance of liposomes exposed to ferritin- or peroxidase-labelled lectins. Samples were handled at 0°C (and fixed with glutaraldehyde in some cases) to prevent lectin-induced structural alterations. Very large (cell size) liposomes were unsuitable for freeze-etch work, since the combination of their extreme size and multilamellar nature makes oblique views of the outermost lamellae statistically unlikely.

We have reported previously that glycophorin disperses fairly diffusely in lipid bilayer membranes and shows little tendency for bilayer disruption, while the concanavalin A receptor in our hands aggregates into areas of apparently disrupted bilayer [16]. These general observations seem to be born out by the patterns of lectin binding seen in Fig. 7. Fig. 7A illustrates the surface appearance of a liposome bearing glycophorin and incubated alternately with peroxidase-linked wheat-germ agglutinin and diaminobenzidene. Figs. 7A–E are electron micrographs of liposomes made using extracted erythrocyte lipid enriched in phosphatidylethanolamine, which should permit optimal glutaraldehyde fixation (see Materials and Methods). Figs. 7B and C simply show that high-affinity lectin binding in this system is dependent upon a surface coat of adsorbed material [10], and is reversed by inhibitory surgar. Figs 7D,E illustrate the distribution of bound wheat-germ agglutinin on the same liposomes coated with Dextran T-500. Fig. 7F shows the same experiment performed with liposomes of dimyristoylphosphatidylcholine-cholesterol – the lipid mixture used in deriving the binding curves in Figs. 3 and 4. Figs 7G,H illustrate the appearance of liposomes bearing concanavalin A receptor and labelled with ferritin-linked concanavalin A. The latter receptor is typically densely packed, and where it covers large areas of the liposome (Fig. 7G) the bilayer is buckled and

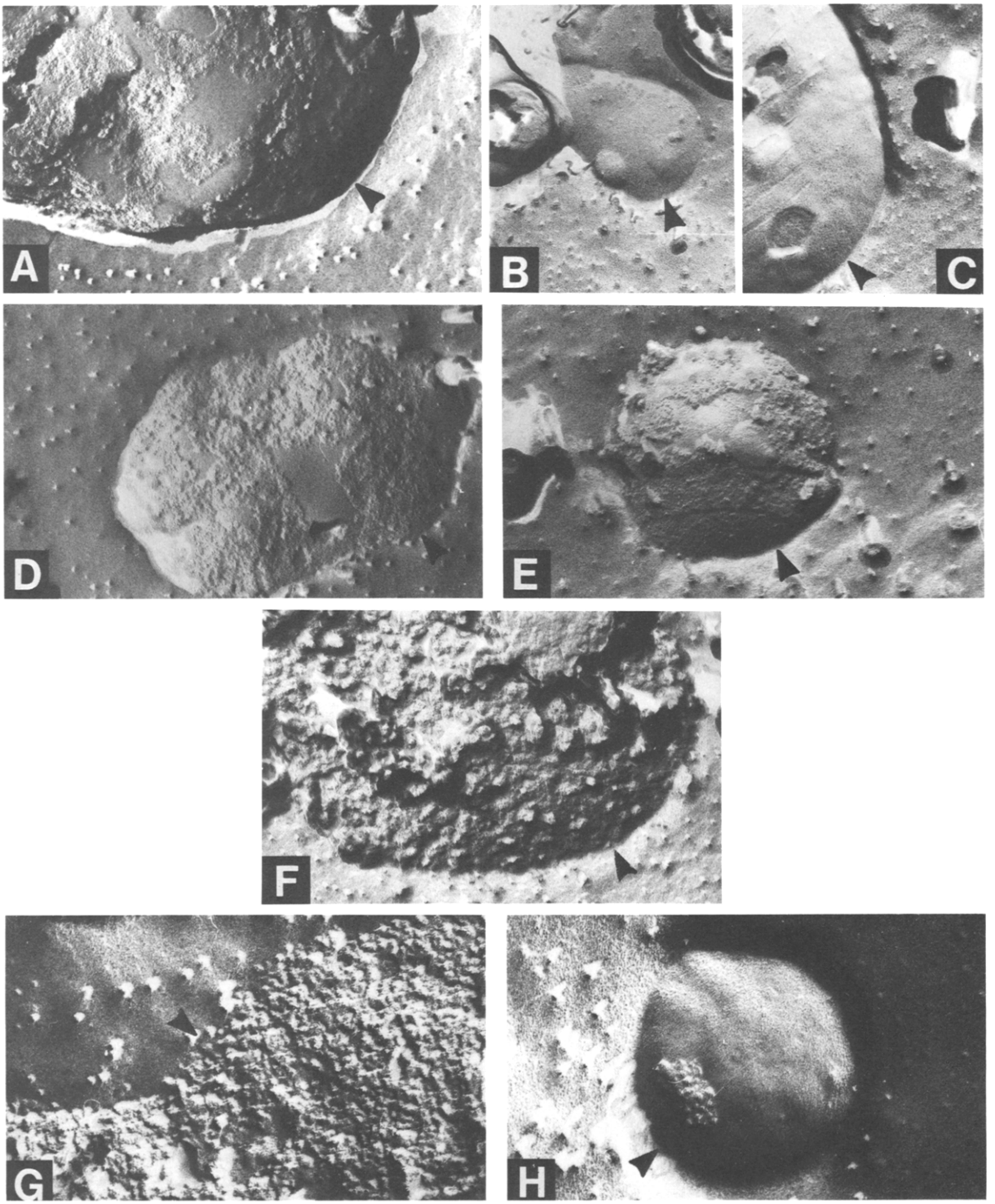

Fig. 7.

deformed. An important point is that the pattern of bound lectin is not simple; it shows wide variability even within a given preparation of liposomes. This basic appearance was the same whether our liposomes were made by hydration of dried lipid films, or by dialysis of detergent solutions. This important limitation makes possible only gross correlation of structure with observed binding behaviour.

Fluorescence microscopy

In some respects, a better overall picture of the liposomes used for binding assays was obtained with the lower magnification technique of fluorescence microscopy. Samples of assay liposomes could be incubated with fluorescent lectin and then washed by differential centrifugation to remove unbound material. We have previously used this approach on liposomes bearing glycophorin and stained with fluorescein-labelled wheat-germ agglutinin [11]. For instance, in fluid lipid bilayers (dimyristoylphosphatidylcholine/cholesterol at 22°C), bound lectin had a grossly patchy distribution; while in rigid lipid bilayers (dipalmitoylphosphatidylcholine/cholesterol at 22°C) the surface fluorescence remained homogeneous after a 15 min incubation [11]. With the concanavalin A receptor, a different picture has been obtained: in general, rhodamine-labelled concanavalin A bound in a patchy distribution, regardless of whether the liposomes were fluid or rigid (Fig. 8). Large areas of most liposomes showed no fluorescence when stained in this way, while others were intensely fluorescent. This is in agreement with the electron microscopy results of the previous section – that the concanavalin A receptor assumes an inherently clustered distribution in our model membranes.

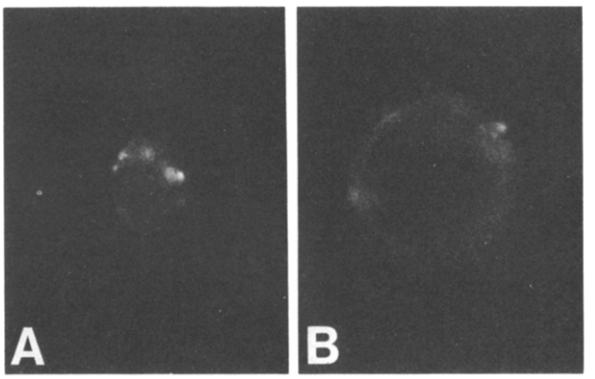

Fig. 8. Rigid lipid liposomes (diapalmitoylphosphatidylcholine-cholesterol in 4:1.5 mole ratio at 22°C) bearing the receptor glycoprotein for concanavalin A and stained with rhodamine-concanavalin A. Lipid/protein ratio 1:1 by weight. Magnification × 1600. Incubated with labelled lectin for 25 min at 22°C.

When liposomes containing both glycophorin and the concanavalin A receptor were stained with a mixture of fluorescein-labelled wheat-germ agglutinin and rhodamine-labelled concanavalin A, the two different lectins could be visualized alternately using coloured filters, or simultaneously without filtering. In greater than 90% of fluorescing liposomes the distribution of both receptors was superimposed – consistent once again with the idea that they interact. But the system has so far proven too complicated to interpret beyond this with any confidence.

Discussion and Conclusions

It seems reasonable to suggest from the original experiments of Schnebli and Bachi [1] that observed time-dependence in lectin-binding to cells

Fig. 7. Freeze-etch electron micrographs of lipid bilayer structures bearing glycophorin (A–F; lipid/protein ratio 2:1 by weight; magnification, × 80000), or concanavalin A receptor (G, H; lipid/protein ratio 1:1; magnification × 103000) and exposed to peroxidase-labelled wheat germ agglutinin or ferritin-labelled concanavalin A, respectively. Samples were incubated with labelled lectins in phosphate buffered saline at 0°C, washed by repeated differential centrifugation, and fixed with glutaraldehyde. Samples were deep-etched for 2 min at −100°C prior to replication with platinum/carbon. The peroxidase-induced precipitate of diaminobenzidene produces an irregular mat of lumpy material wherever large areas of membrane surface have bound wheat-germ agglutinin. Bound concanavalin A is marked by precise spherical ferritin molecules. Arrowheads point from the ice to the membrane edge. A. Lipid mixture erythrocyte lipid/phosphatidylethanolamine (2.4:1, wt. ratio), buffers containing 3 mg/ml bovine serum albumin. B. Same as A but with 0.1 M inhibitory sugar added to buffers. C. Same as A but with no serum albumin in the buffers. D, E. Same as A but with serum albumin replaced by 3 mg/ml Dextran T 500 in all buffers. F. Lipid mixture 4:1.5 mole ratio dimyristoylphosphatidylcholine/cholesterol and with 3 mg/ml Dextran T 500 in buffers. G, H. Lipid mixture 4:1.5 mole ratio dimyristoylphosphatidylcholine/cholesterol, buffers containing 3 mg/ml bovine serum albumin.

may result from passive receptor rearrangements that optimize high-affinity attachment. Our finding of analogous behaviour for receptors in model membranes bears this out. Indeed, it appears that no component of the original membrane is needed other than the receptor itself, so that the time-dependence we see reflects a primary lectin-receptor interaction only. The fact that time-dependent binding occurs in rigid bilayers agrees with the previous workers' conclusion that gross lateral diffusion is not a necessary feature of the time-dependence, and suggests that receptor conformation changes may be important. One might anticipate that further experiments of this sort with various controlled lipid mixtures would be quite informative. Unfortunately, any subtle comparisons between different lipids would demand assurance that the initial arrangement of receptors was not itself sensitive to lipid composition. In fact, it is our opinion that the most serious limitation to model membrane studies of membrane receptors is lack of detailed information about receptor topography. In this regard, fluorescence microscopy and freeze-etch electron microscopy were used to confirm the surface location of lectin-binding sites. Of these two techniques, freeze-etching offers much higher magnification and appears to be a promising technique for relating experimental observations to underlying structure. However, even this technique has not permitted distinction between short-distance lateral diffusion and receptor conformational changes. The main problem here is not the 25 Å limit of resolution in freeze-etching, but rather the variability in receptor arrangement inherent to our liposomes: it is difficult to pick up subtle changes in receptor distribution when distribution is heterogeneous to begin with. Obviously, this problem is compounded by addition of a second family of proteins.

Is there a plausible molecular mechanism whereby (time dependent) lectin-induced receptor rearrangements could optimize lectin binding? Lectins are proteins of diverse origin, but all have more than one specific sugar-binding site (reviewed in Ref. 21). The affinities of these sites for monosaccharide inhibitors are in the neighbourhood of $1 \cdot 10^3\ M^{-1}$ [22]; and the mechanism of binding is assumed to be similar to that of enzymes for their substrates. A given lectin binds to cell surfaces with a wide range of affinities – although typically, biological researchers concern themselves only with those binding events which are stable to extensive washing procedures (affinity greater than some $4 \cdot 10^6\ M^{-1}$). There are several possible mechanisms whereby lectins with such a modest affinity for monosaccharides might bind with very high affinity to surface structures. Firstly, a given lectin might have a higher affinity for a chain of specific sugars in appropriate linkage. And secondly, a lectin might bind simultaneously to two or more different chains of sugars (whereupon the chelate effect would dictate a greatly increased affinity). In fact, there is evidence in support of both of these mechanisms: for instance, both concanavalin A and wheat-germ agglutinin have been demonstrated to have binding sites which can theoretically recognize linked sugars (Refs. 23–26 and references therein). Bhavanandan and Katlic have demonstrated quite convincingly that in the case of wheat-germ agglutinin binding to glycophorin, it is simultaneous attachment to two or more chains that is important [23]. They showed that, although the latter lectin binds with high affinity to intact glycophorin (with 16 oligosaccharide chains), if the chains are cleaved intact from the polypeptide backbone they are bound only with low affinity, this in spite of the fact that each binding site on wheat-germ agglutinin can accommodate a group of three sugars [23,24]. Since both wheat-germ agglutinin and concanavalin A have four separate sugar-binding sites, it is easy to imagine that multidentate binding could contribute importantly to their affinities for the cell surface. In fact it has been claimed that at a membrane surface all sugar-binding sites of lectins should tend to be occupied where structurally possible [27]. Lectin binding via all four sites may often be sterically feasible, since glycoprotein headgroups are lengthy, close together, and very flexible (see Figs. 5, 6). However, the data of Bornens et al. [28] and that of Wang and Edelman [22] indicate that divalent concanavalin A binds to cell surfaces with an affinity at least roughly comparable to that of tetravalent concanavalin A (data for monovalent concanavalin A affinity does not seem to exist in the literature). Wang and Edelman have suggested the possibility of lectin binding to sites other than

carbohydrate as a source of polyvalent attachment. Whatever the relative importances of these various mechanisms in any given lectin-cell interaction, it seems very likely that inter- and intramolecular headgroup rearrangements will play a key role. This is also a consideration for binding of polypeptide hormones to their receptors [29]. It is our suggestion that, quite apart from any involvement of cytoskeletal components and long-distance receptor translation, local headgroup conformation and reorganization is an important source of time-dependence in lectin binding.

Part of the theoretical framework needed for considerations of this sort already exists. Jennissen [30,31] has demonstrated very clearly through studies of phosphorylase A binding to alkyl-chain derivatives of Sepharose beads that bringing receptors close enough for polyvalent attachment raises binding affinity. Presumably with lectins and oligosaccharide chains, the orientation requirements are stricter and hence degrees of freedom fewer (for instance the extraordinary sensitivity of enzyme-substrate binding to ligand orientation is well known). In the complex meshwork that is the eucaryote glycocalyx, a requirement for optimal orientation and proximity of receptors would be expected to be a likely source of time-dependence. In fact, since lectin binding as a function of time rises from zero, it would appear that receptor rearrangement is an obligate first step in the binding process. Possible degrees of freedom that might account for lectin-induced rearrangement are headgroup deformation/reorientation, headgroup clustering, headgroup uncovering from a sterically unfavourable position to expose new sites and/or make polydentate attachment more feasible, and diffusion in the plane of the membrane. The rapid lectin binding which we observe to densely clustered patches of concanavalin A receptor may be explained on this basis as resulting from statistical ease of finding correctly oriented oligosaccharide chains. A model system with a structure more clearly definable than that employed here would be very useful for further elucidation of the important events associated with lectin-membrane interaction.

Acknowledgements

We would like to acknowledge the technical assistance of M. Hall in preparation of samples for electron microscopy. This work was supported by grants from the Medical Research Council and the National Cancer Institute of Canada.

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