Vol. 95, No. 3, 1980 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 14, 1980 Pages 1299-1305

GANGLIOSIDE HEADGROUP DISORDER AS A SEQUEL TO LECTIN BINDING

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Received June 24,1980

SUMMARY: Evidence is presented that gangliosides show a measurable tendency to exist in clusters in lipid bilayer systems; and that these clusters are subject to disruption by a lectin binding event.

INTRODUCTION

The events that surround lectin binding to eucaryotic cells have been made the subject of considerable investigation in recent years because of their presumed significance to physiological recognition processes. It is well known that lectins bind with high selectivity to sugar residues of glycolipids and glycoproteins; and that by so doing they can sometimes initiate a chain of events leading to a metabolic response on the part of the cell. We have employed headgroup spin labeled gangliosides and glycoproteins in an attempt to focus with minimal ambiguity on the possible role of early structural changes in such phenomena. Gangliosides were chosen as a suitable family of glycolipids for study because of their documented involvement in a variety of specific recognition events (reviewed in 1 - 5). For our glycoprotein work we have employed the human erythrocyte species, glycophorin, which is widely accepted as an example of a transmembrane glycoprotein (6 and ref. therein). Both gangliosides and glycophorin are able to function as receptors for WGA, a lectin with high affinity for NANA residues (6 - 8).

Abbreviations: BSA, bovine serum albumin; WGA, wheat germ agglutinin; NANA, N-Acetylneuraminic acid.

0006-291X/80/151299-07$01.00/0

Given that binding of a lectin involves headgroup oligosaccharide interaction with, and perhaps crosslinking by, a multivalent macromolecule; one might expect oligosaccharide chain immobilization to be a basic initial result. Indeed in our hands this is the *rule* at high lectin concentrations. However, we demonstrate here that at *low* lectin concentrations, at least for gangliosides in lipid bilayers, the headgroups can actually become more mobile - presumably reflecting a disruption of pre-existing, non-covalent interactions. The concept that a binding event might lead to disruption of a cell surface receptor aggregate is of potential interest in understanding the role of gangliosides as recognition sites.

MATERIALS AND METHODS

L-α-dimyristoyl and L-α-dipalmitoyl phosphatidylcholine, cholesterol and egg phosphatidylcholine were obtained from Sigma. The latter (Type III-E) was further purified by column chromatography on silicic acid. Dioleoyl phosphatidylcholine was obtained from Serdary Res., London, Canada. All phospholipids were pure, as judged by thin layer chromatography on silica gel G (Stahl). Gangliosides were isolated from beef brain by a modification of the method of Kanfer (9) in which gangliosides obtained from the initial Folch extraction were purified by chromatography on silicic acid (Bio Rad 200-400 mesh eluting with $CHCl_3$/MeOH) and checked for purity by thin layer chromatography. Ca^{2+} and Mg^{2+} were added as their chlorides, and EDTA as the disodium salt. HEPES, WGA and globulin free BSA were obtained from Sigma. Spin labeled derivatives of gangliosides and glycophorin illustrated in Figure 1 were prepared as described by us previously (10-13).

Lipid mixtures were made by dissolving appropriate amounts of each in chloroform/methanol and pumping extensively under vacuum to remove traces of solvent. Dried lipid mixtures were suspended in buffer by vigorous vortexing.

EPR spectra were recorded on a Varian E12 spectrometer equipped with a TM_{110} cavity. Probe temperature was measured with a copper constantan thermocouple and was 21°C unless otherwise specified.

RESULTS AND DISCUSSION

The characteristics of headgroup spin labeled gangliosides and glycophorin have been described by us previously (e.g. 10-13). The derivatives used in the work described here have nitroxide rings covalently attached to headgroup sugar residues: either randomly distributed amongst the various sugars (COOH-linked, Figure 1a), or restricted to NANA residues (NH_2-linked, Figure 1b). The number of labels per ganglioside or glycoprotein is on the order of 1. Note that in the case of glycophorin these labels are presumably distributed randomly amongst the 16 available oligosaccharide chains (12). We have

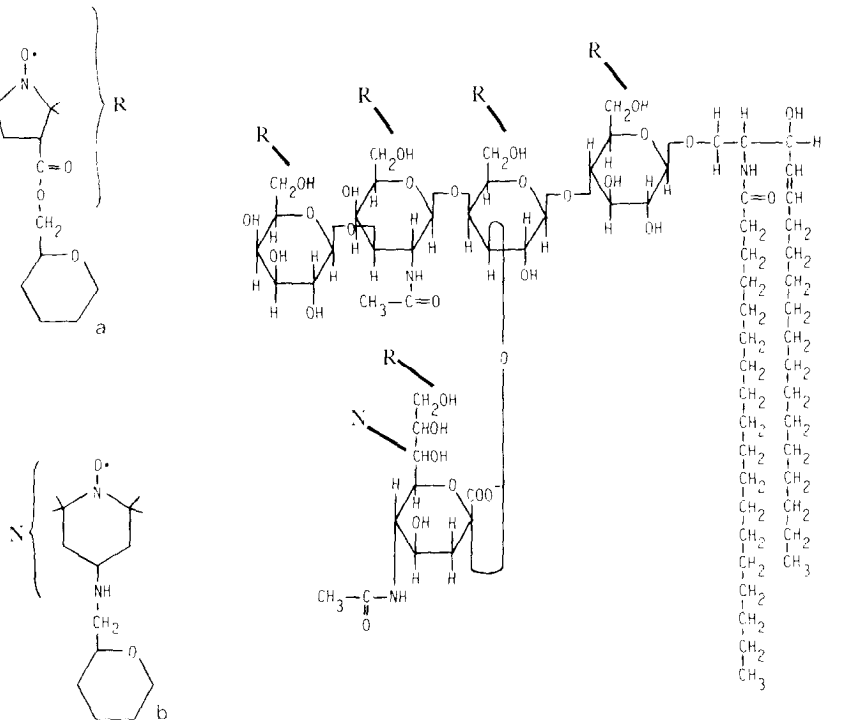

Figure 1. Mode of attachment of headgroup spin labels to glycolipid and glycoprotein

(a) The COOH-coupled label which has a prediliction for primary alcohol functions and is scattered throughout the oligosaccharide chains

(b) The NH_2-coupled label which is specific for NANA residues

A typical ganglioside is shown for illustration purposes. Only a fraction of the possible attachment points are actually occupied by spin labels, especially when using the type in Ia.

extensively studied such labeled carbohydrate bearing components in systems ranging from organic solvent to intact cells (10-13), and have recently begun to investigate their response to lectin binding. Figure 2a typifies the effect of WGA binding on the oligosaccharide headgroup of glycophorin in lipid bilayers. It is very similar to plots reported previously for WGA binding to glycophorin free in solution (12,13). The parameter plotted on the Y-axis is the spin label correlation time, τ_c , measured by the method of Keith et al (14) as described previously (15); and is *inversely* related to headgroup freedom of motion. The result shown in Figure 2a is essentially what one might predict: increased headgroup immobilization with increased lectin binding - the only

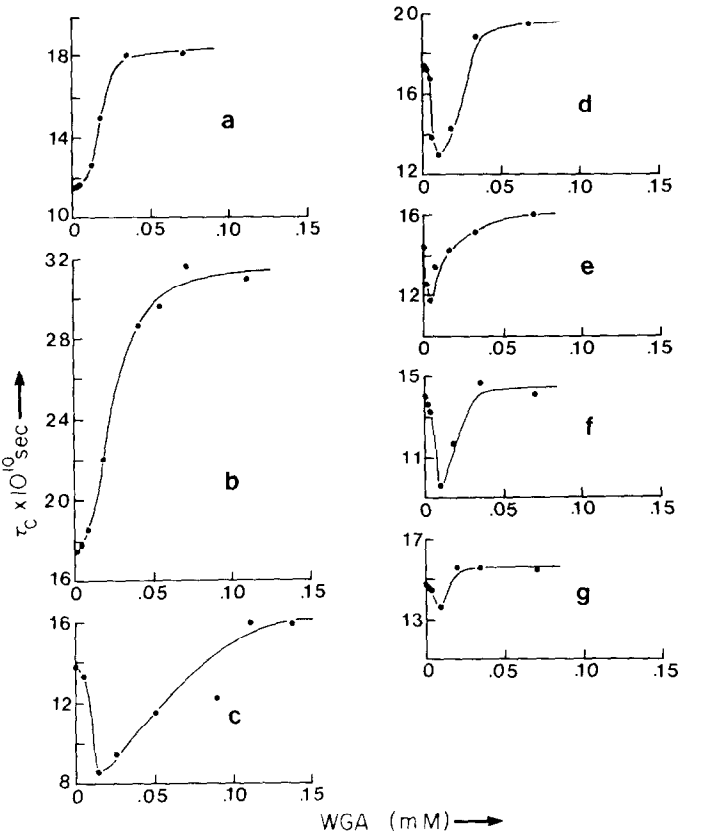

Figure 2. Effect of lectin binding on headgroup oligosaccharide dynamics in a variety of different systems

The Y-axis parameter (spin label correlation time, τ_c,) is *inversely* related to headgroup motional freedom. EPR samples were made by adding 20 µl of various stock WGA solutions to equal volumes of the glycolipid or glycoprotein specimens. Lipid concentrations were 40 µmol/ml for 2a, 10 µmol/ml for 2d, and 28 µmol/ml for 2c,e,f,g (all samples unsonicated). Spectra were recorded 3 min after mixing

(a) Typical data obtained using the spin labeled *glycoprotein*, glycophorin, for comparison. Glycophorin was assembled at a lipid/protein molar ratio of 180:1 into 1:1 egg phosphatidylcholine/dipalmitoyl phosphatidylcholine bilayers by dialysis from detergent solution (dodecyltrimethylammonium bromide) as described elsewhere (12,16). The bilayer structures were harvested by centrifugation. Glycophorin was spin labeled on random sugar residues (Figure 1a). Medium 0.1 N KCl containing 2 mM Ca^{2+} and Mg^{2+}, 0.3% BSA, and buffered with 5 mM HEPES pH 7.3

(b) Ganglioside labeled on random sugars (Figure 1a) but *free in solution* at 0.7 mg/ml. Medium 0.145 N NaCl buffered with 5 mM HEPES pH 7.3, and containing 5 mM EDTA

(c) Ganglioside labeled on NANA residues (Figure 1b), at 4.8 mol % in bilayers of egg phosphatidylcholine. Medium 0.145 N NaCl containing 5 mM EDTA and 0.3% BSA, buffered with 5 mM HEPES

(d) Ganglioside labeled on random sugars, at 5 mol % in egg phosphatidylcholine. Medium 0.145 N NaCl containing 5 mM EDTA, 0.3% BSA, and buffered with 5 mM HEPES pH 7.3

noteworthy aspect being that the curve obtained is (+) cooperative. A similar phenomenon is seen for WGA binding to gangliosides in solution (Figure 2b). However the effect of WGA binding to gangliosides in lipid bilayers shows a remarkable difference: at *low* lectin concentrations, the headgroup mobility may actually increase (Figures 2c - g). In all cases, as the concentration of lectin is increased to high levels, headgroup mobility decreases - presumably in response to crosslinking by the tetravalent lectin. The point of maximal lectin-induced oligosaccharide mobilization (the dips in curves (c-g)) occur at lectin concentrations such that the majority of the ganglioside is not bound. For instance in Figure 2c the ratio of *total available* WGA to available ganglioside is 1 : 35. Hence it would appear that binding to a small fraction of the gangliosides can result in a *general* increase in motional freedom. This observation can be readily understood if one assumes that the gangliosides exist in clusters which are perturbed by an initial binding event. However, although we have claimed previously that there should be a certain tendency toward attractive forces amongst oligosaccharide chains (10,15), we have until now assumed that small quantitites of gangliosides would disperse quite extensively in simple lipid bilayers.

There are various mechanisms which might be expected to contribute to ganglioside clustering. Attractive forces amongst oligosaccharide chains have been mentioned above. Probably more important though is the well known semi-crystal nature of hydrated membrane lipids with resultant phase transition and phase separation phenomena (e.g. 17-19). On the basis of chain length differences, headgroup differences, or glycerol vs. ceramide backbone differences, gangliosides may fail to fit optimally into a phosphatidylcholine

(e) Ganglioside labeled on NANA residues, at 5 mol % in dioleoyl phosphatidylcholine. Medium 0.145 N NaCl containing 5 mM EDTA, buffered with 5 mM HEPES pH 7.3

(f) Ganglioside labeled on NANA residues, at 0.5 mol % plus 4.5 mol % *unlabeled* ganglioside in egg phosphatidylcholine. Medium 0.145 N NaCl containing 5 mM EDTA and buffered with 5 mM HEPES pH 7.3

(g) Same as (f) but in bilayers of egg phosphatidylcholine containing 10 mol % cholesterol as well

lattice. Probably chain length differences are not a key factor in the observation described here since beef brain gangliosides are largely C_{18}/C_{20} sphingosine with C_{18} fatty acids and we see the phenomenon in egg phosphatidylcholine and dioleoyl phosphatidylcholine (Figure 2).

Bunow and Bunow have shown by scanning calorimetry that, below 30 mol % ganglioside, the melting behaviour of ganglioside/stearoyl oleoyl phosphatidylcholine mixtures has a measurable concentration dependence (20). In other words, some mixing must occur. Combining this observation with our own evidence of ganglioside-ganglioside proximity, it would seem likely that the ganglioside 'clusters' are in equilibrium with a sizable pool of laterally dispersed ganglioside. This is probably to be expected from the theory of bilayer phase separation behaviour proposed originally by Chapman and McConnell and coworkers (17,21).

We have recently invoked the same phenomenon of ganglioside clustering to explain our observation that the host bilayer phase transition temperature correlates poorly with temperature effects on headgroup mobility of incorporated gangliosides (11). Briefly, ganglioside headgroups are more mobile in fluid than in rigid bilayers; however, the temperature-induced increase in ganglioside headgroup mobility occurs some 5 - 6°C below the melting point of the surrounding host matrix (and can be abolished by the presence of Ca^{2+}, an ion which should lead to closer ganglioside packing (10)). If gangliosides are segregated into patches the above observation can be readily understood on the basis of high local concentrations.

CONCLUSIONS

The observations presented here have two implications:

(i) that gangliosides show a striking tendency to cluster spontaneously in phospholipid bilayers

(ii) if, by whatever mechanism, gangliosides occur in aggregates in real cells (10,22), the possibility exists that a specific binding event directed against these gangliosides can disrupt the aggregate, thereby freeing the original receptor for subsequent redistribution.

ACKNOWLEDGEMENTS

This research was supported by grants from the National Cancer Institute and the Medical Research Council of Canada.

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