AGGLUTINATION BY LECTINS OF LIPOSOMES PREPARED FROM TOTAL LIPIDS OF ERYTHROCYTES

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SUMMARY: Liposomes prepared from total lipid extracts of human and rabbit erythrocyte ghosts were tested for agglutinability with lectins. Soya bean lectin aggregated liposomes prepared with human or rabbit lipids in keeping with the fact that galactose is the predominant sugar in glycolipids. Wheat germ lectin agglutinates liposomes prepared from rabbit lipids but not human lipids. The internal position of acetylglucosamine in the major globoside of rabbit erythrocytes would indicate that wheat germ lectin can interact with a non-terminal sugar. Concanavalin A did not agglutinate either liposomes from human or rabbit lipids. We conclude that glycolipids form an additional class of lectin receptors on membranes.

It is known that most non-transformed tissue culture cells and erythrocytes are rendered more agglutinable by lectins after mild trypsinization of these cells (1). Previous work has shown that proteolytic treatment renders erythrocyte ghost membranes more agglutinable as well (2). Surprisingly, after prolonged proteolysis at high Pronase concentrations, which removed all of the detectable protein, agglutination was actually enhanced with several lectins (2). This suggested that some lectins interacted with glycolipids or small glycopeptides. We therefore prepared total lipid extracts from human and rabbit red blood cell membranes to see whether liposomes prepared from such biological extracts could be shown to be agglutinable. Reconstitution experiments have been reported using purified phospholipids with incorporating glycoproteins, showing the ability of lectins to interact with these proteins. For example, Redwood et al. (3) used purified egg lecithin and purified glycophorin while MacDonald and MacDonald (4) used mostly synthetic diisostearoyllecithin and purified glycophorin. More recently, Surolia et al. (5) employed

synthetic dipalmitoyl-lecithin and purified individual brain gangliosides. We report the use of total lipid extracts from human and rabbit erythrocyte ghosts to make the liposomes. This allows us to determine if liposomes prepared with the glycolipid/lipid proportions similar to that of parental erythrocyte ghosts are able to be agglutinated by lectins. We show that the agglutinability of the liposomes agrees with the different known carbohydrate composition of the glycolipids extracted from rabbit and human erythrocytes.

MATERIALS AND METHODS

Ghosts were prepared from human and rabbit erythrocytes by the method of Dodge et al. (6) and lyophilized. Total lipids were extracted with 10 ml of $CHCl_3$-MeOH (2:1) per 100 mg dry weight of lyophilized material, and reextracted with 5 ml of $CHCl_3$-MeOH and the 2 extracts pooled. To prepare multilamellar liposomes, 0.5 ml of the lipid extract was dialyzed as previously described (7) in the absence of bovine serum albumin. As a control, we prepared purified sphingomyelin containing liposomes. A 1% solution of sphingomyelin in $CHCl_3$-MeOH (2:1) was made and 0.5 ml of this solution was dialyzed as described (7). SBA† was prepared as described (8). WGA was a gift from Dr. J. Sheppard. GlcNAc, galNAc and methyl α-mannopyranoside were obtained from Pfanstiehl Laboratories. Con A was obtained from Miles Laboratories, and sphingomyelin from Sigma Chemical Co.

Agglutination was measured at RT (20-22°C) by following the change in absorbancy (in effect turbidity) in a Zeiss spectrophotometer at 500 mμ (5). The assay mixture contained in 1.0 ml of PBS (140 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, 1 mM KH_2PO_4) enough liposome to bring the absorbancy to 0.1. The lectins were added after the initial reading in 0.02 ml PBS. Reversal of agglutination was assayed by the addition of 0.02 ml of 1 M sugar haptens in PBS.

RESULTS

As shown in Figure 1, addition of SBA (40 μg/ml) to liposomes prepared from human lipids produces an immediate and continuous rise in absorbancy which plateaus in about 60 minutes. After the leveling off of the absorbancy, the addition of galNAc produced an immediate drop in absorbancy indicating reversal of agglutination. Addition of gluNAc, a hapten specific for WGA, or methyl α-mannopyranoside, a sugar specific for con A, produced only minor decreases in optical density. Note that when either con A (200 μg/ml) or WGA (200 μg/ml) was added to the liposomes derived from human erythrocyte

†Abbreviations: SBA, soya bean agglutinin; WGA, wheat germ agglutinin; con A, concanavalin A; glcNAc, N-acetylglucosamine; galNAc, N-acetylgalactosamine; glc, glucose; gal, galactose.

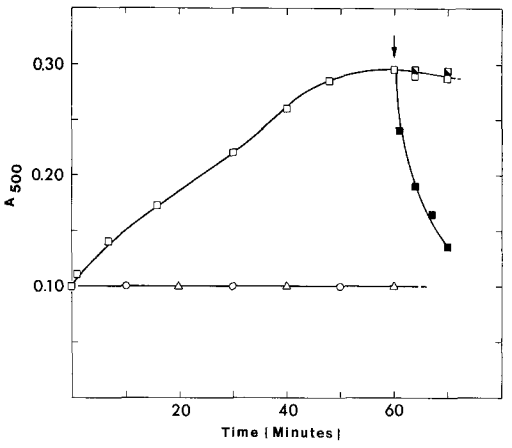

Fig. 1. Time course of aggregation of liposomes prepared from human lipids. Abscissa, time in minutes. Ordinate, absorbancy at 500 mμ. Lectins added at 0 time; □ , soya bean agglutinin, 40 μg; Δ, concanavalin A, 200 μg; O, wheat germ agglutinin, 200 μg. At 60 minutes, 50 μM of ■ , acetylgalactosamine or ◻ , acetylglucosamine or ◪ , mannopyranoside were added.

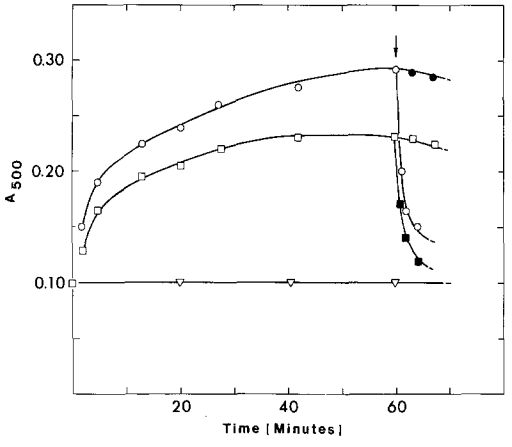

Fig. 2. Time course of aggregation of liposomes prepared from rabbit lipids. Conditions and symbols as in Fig. 1, plus ▽ , without any lectin.

lipid, no significant change in absorbancy was seen. Lower concentrations of these lectins (100, 50 and 25 μg/ml) also had no effect on the aggregation. A control, not shown, was a liposome suspension prepared with sphingomyelin and no absorbancy change was observed when SBA was added.

Figure 2 shows similar experiments using liposomes prepared with total

lipids from rabbit erythrocyte membranes. As in Figure 1, SBA induced vesicle aggregation as indicated by the continuous increase in absorbancy and, upon the addition of the specific hapten inhibitor, galNAc, the optical density returns to the initial value. For WGA there is a gradual increase in absorbancy which can be reversed upon the addition of glcNAc but not galNAc. In the absence of the added lectins there is no significant change in the absorbancy of the liposome suspension during the period of the experiments. When con A (200 to 50 μg/ml) was added (not shown), no increase in optical density was seen.

The maximum size of these liposomes was about one micron when observed under a phase contrast light microscope. This approximates the size found by negative staining electron microscopy of mixed phospholipid liposomes prepared by the dialysis technique used here (9).

DISCUSSION

The simplest explanation for aggregation of liposomes derived from human red blood cell lipids by SBA is the presence of available receptor, i.e., that the major sugars present in glycolipids are gal and galNAc (10). Both of these sugars are known to interact with SBA (1).

Hakomori and Murakami (11) were able to show that neither trihexosylceramide nor human erythrocyte globoside, nor human erythrocyte hematoside inhibit WGA induced agglutination of cells (11). Our liposome preparations containing human lipids were also not agglutinated by WGA. However, there are trace amounts of glycolipids that contain glcNAc residues in human red blood cells and therefore could interact with WGA, such as the glcNAc containing ganglioside, paragloboside and P_1-antigen (12). Some of these glycolipids may have been lost during the preparation of our liposomes (during dialysis), thus accounting for the lack of our liposome preparations agglutinating in the presence of WGA. Another explanation is that the glycolipids have not been lost, but are present in very small concentrations. As Surolia et al. (5) have pointed out, a critical concentration of ganglioside in the liposomes is

necessary to demonstrate agglutinability of their liposomes. Failure of con A to agglutinate our liposomes probably indicates the almost complete absence of glycolipid receptors for con A in intact red blood cells. Proteolysis renders erythrocyte ghosts (2) or enveloped virus particles (13) no longer agglutinable by con A. Klenk and Huang (13) suggest that mannose, the presumed sugar receptor for con A, is present in glycoproteins but not in glycolipids.

The major glycolipid of rabbit erythrocyte membrane (10) has the structure: ceramide-glc-gal-glcNAc-gal-gal, containing both gal, which interacts with SBA, and glcNAc, which interacts with WGA (1). Interestingly, our results indicate that WGA appears to interact with an internal sugar. It is known that con A may interact with internal mannopyranosyl residues of polysaccharides, as demonstrated both by hapten inhibition techniques and by quantitative precipitation methods (14). However, liposomes prepared with purified gangliosides only bound ricinus lectin when the particular ganglioside contained galactose as a terminal residue (5).

Our findings indicate that there are apparently enough receptor glycolipids in total human erythrocyte lipids to make liposomes agglutinable with SBA. Our data also shows that not only brain gangliosides, used by Surolia et al. (5), but also neutral glycolipids, which are the major glycolipids in erythrocyte membranes, can interact with lectins. The amount of total lipid in 10^{10} human red blood cells is about 5 mg, of which 5-10% are glycolipids (15). Since 70% of erythrocyte glycolipid is globoside (15), this type of glycolipid will constitute a minimum of 0.175 mg per 10^{10} cells. From these numbers, one calculates that there are approximately 8×10^6 molecules of globoside (MW 1,300) per human erythrocyte, a number significantly higher than the total binding sites for SBA which has been estimated between $1.0\text{-}1.1 \times 10^6$ (16). If only 5% of the total number of globoside molecules interact with SBA, this would represent 35% of the total binding sites for SBA in the human erythrocyte. Reisner et al. (16) have recently reported that the binding of SBA at low lectin concentrations to human erythrocytes show positive coopera-

tivity. One of their explanations was the unmasking of cryptic sites. Possibly these cryptic sites could be the glycolipids. In any case, since glycoproteins of human red blood cells have already been shown to interact with many lectins (17,18) and probably interact with SBA, two different classes of sites are potentially available to SBA, i.e., glycoproteins and glycolipids. In rabbit red blood cells, glycolipid receptors are available to both SBA and WGA.

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