notes on methodology

Binding of phosphatidylcholine-^{14}C to glass

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SUMMARY Lecithin-^{14}C was found to bind to Pyrex glass. The percentage bound varied with different organic solvents, being greatest in dioxane solution. Thorough treatment of the glass with dimethyldichlorosilane prevents the binding, which suggests that charge-charge interactions are involved.

SUPPLEMENTARY KEY WORDS fritted glass · silanized glassware · phospholipid adsorption

IN THE COURSE of studying the binding of lecithin to modified erythrocyte membranes (1) I observed that significant binding of this phospholipid took place to glass from benzene solution. This observation was extended to other organic reagents in an attempt to assess the magnitude and possibly the mechanism of this binding, and methods to overcome it.

Phosphatidylcholine-^{14}C, produced by feeding $^{14}CO_2$ to *Chlorella* algae, was obtained from Applied Science Laboratories Inc. (State College, Pa.) and further purified to a minimum of 95% radiochemical purity (no other labeled peaks) by elution from silicic acid-impregnated glass fiber (ChromAR, Mallinckrodt Chemical Works, St. Louis, Mo.). After dilution with cold carrier bovine or egg lecithin the appropriate band was eluted and studied by chromatography on silver nitrate-impregnated silicic acid. Study of these eluted bands showed that the labeled lecithin contained unsaturated fatty acids.

Corning Pyrex Buchner funnels with 30-mm fritted glass filters (glass number 7740) having ultrafine pores (0.9–1.4 μm) were washed as follows. The funnels were sonicated in detergent, rinsed with tap water, distilled water, and methanol, and then dried in a vacuum oven at 95°C. For the binding experiments 1 ml volumes of the indicated organic solvents containing 1 μmole of phospholipid were placed on the fritted glass filter and allowed to remain undisturbed for 5 min at room temperature. Suction was then applied, and three 5-ml volumes of the same solvent were washed through. The funnel was dried in a nitrogen stream, and two 5-ml volumes of methanol–chloroform 4:1 were washed through with suction, combined, and counted in a Nuclear-Chicago well-type scintillation counter (Nuclear-Chicago Corporation, Des Plaines, Ill.). Phosphorus was measured by the procedure of Svanborg and Svennerholm (2). All the solutions were optically isotropic.

TABLE 1 BINDING OF LECITHIN-^{14}C TO GLASS

Solvent	Percentage of lecithin bound	Dielectric constant of Solvent (13)	Position in eluotropic series*	
			Wren (4)	Kaufman and Makus (6)
1,4-Dioxane	81	2.2	N.G.†	5
Toluene	75	2.4	3	1
Benzene	74	2.3	4	2
Chloroform	56	4.8	2	3
Hexane‡	48	1.9	N.G.	N.G.
Diethyl ether	40	4.3	5	4
Carbon tetrachloride	30	2.2	1	N.G.
1-Butanol	7.9	17.8	N.G.	N.G.
Ethanol	7.7	24.3	6	N.G.
Methanol	0.0	32.6	7	N.G.

* From least to greatest effectiveness.
† Not given.
‡ Mixed *n*- and isohexanes.

Of the solvents employed, all except methanol resulted in measurable binding (Table 1). No lecithin was bound in methanol–chloroform 4:1, as tested by elution with pure methanol. Surprisingly, dioxane was associated with the greatest degree of binding. The binding order in the various solvents does not parallel the eluotropic series found by Trappe (3), Wren (4), Strain (5), or Kaufman and Makus (6). These results do not reflect the dielectric constants of the solvents or support the recommendation of the glass manufacturer that carbon tetrachloride will remove "fatty materials" (7).

With a 10-fold increase in phosphatidylcholine concentration no appreciable increase in binding resulted, indicating that saturation had essentially been reached. As a practical consequence, loss of lecithin due to binding to glass in benzene is likely to be of quantitative significance only in dilute solutions.

The funnels were treated once with 1% dimethyldichlorosilane in benzene at 60°C followed by heating at 150°C for 30 min. After the usual washing procedure, the experiment with lecithin in benzene was repeated at the same concentration. The percentage bound fell from 74 to 34. In another experiment the glass was silanized five times with dimethyldichlorosilane and washed with methanol between applications to remove the hydrochloric acid which had formed. Lecithin-^{14}C binding in benzene fell to 3% as a result of this procedure. The resulting increased hydrophobic character of

the glass (8) associated with reduced binding suggests that electrostatic bonds are involved.

Evidence from the work of Izmailova and Deryagin (9) indicated that stearic acid bound to glass surfaces and was not removed by benzene washing. Other investigators have also studied fatty acid binding to glass (10). Lecithin is of particular interest in regard to binding since it has a net charge of zero under usual circumstances and does not form ionic complexes with cytochrome *c*, unlike the acidic phospholipids (11). However, since fatty acids have been reported to bind to glass surfaces it was of interest to assess the binding of oleic acid-^{14}C (Applied Science Laboratories Inc.) under the present experimental conditions. Adding 1 μmole to untreated glass filters resulted in only 6% binding in benzene, 1% in toluene, 0.5% in hexane, and no significant binding in the other solvents. Thus both the magnitude and the order in the various solvents differed from the results with phosphatidylcholine. This probably reflects the differences in the polar end-groups.

Lecithin is thought to be aggregated in benzene and to a lesser extent in the lower alcohols (12), but the apparent molecular size would still be far smaller than the pore size of about 1 μm. The many possible charge and adsorption factors related to this binding are difficult to weigh, but these data indicate that in practice the significant binding of lecithin in benzene solutions can be effectively prevented by thorough treatment of the glass with dimethyldichlorosilane.

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