Journal of Chromatography, 88 (1974) 425–427

CHROM. 7045

Note

Detection of fatty acids, fatty aldehydes, phospholipids, glycolipids and cholesterol on thin-layer chromatograms stained with malachite green

R. J. TEICHMAN, G. H. TAKEI and J. M. CUMMINS

Department of Anatomy and Reproductive Biology, University of Hawaii School of Medicine, Honolulu, Hawaii 96822 (U.S.A.)

(Received August 3rd, 1973)

The detection of lipid materials on thin-layer chromatograms has been accomplished by numerous techniques. Malachite green, a cationic stain, has been employed by previous investigators for the biochemical determination of phosphate[1] and for the detection of lysophosphatides on paper chromatograms[2]. Recently, this stain was employed as a histochemical reagent for fine structure studies of the phospholipids of mammalian spermatozoa[3]. Malachite green has been used by us as a detection reagent for numerous lipids and fatty acids on thin-layer chromatograms.

METHODS

Lipid standards (K & K, Plainview, N.J., U.S.A., and Sigma, St. Louis, Mo., U.S.A.) were spotted on glass plates layered with silica G or H and chromatographed in a solvent system consisting of chloroform–methanol–water (65:25:4) and allowed to dry in air completely. The chromatograms were then sprayed with a 0.5% aqueous solution of malachite green until the surface was uniformly covered. The plates were again air dried (40–60 min) and lightly sprayed with 2% sodium bisulfite in 2 *N* HCl to reduce all unbound stain.

1–200-μg samples of numerous biological compounds were applied to chromatographic paper, dried and immersed in the malachite green for 10 min. The compounds included: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysolecithin, oleic acid, myristic acid, stearic acid, palmitoleic acid, myristic aldehyde, palmitaldehyde bisulfite, stearic aldehyde bisulfite, glyceryl tripalmitate, betaine, acetyl-β-methylcholine chloride, acetylcholine, butyrylcholine, carbamylcholine chloride, DL-carnitine, ethanolamine chloride, choline chloride, β-glycerol phosphate, glycerylphosphorylcholine, phosphorylcholine chloride, orthophosphoethanolamine, inosine-5′-monophosphate, 1-inositol, D-glucose, sucrose, N-acetyl-D-galactosamine, D-glycerol, cholesterol, guanosine-5′-triphosphate, cytidine-5′-triphosphate, adenosine-5′-triphosphate, uridine-5′-triphosphate, albumin, β-lipoprotein heparin, glycine, alanine, asparagine, L-proline, L-phenylalanine, tryptophane, L-serine, L-histidine and L-cysteine. Retention of the stain after 10 min in running tap water was considered to be a positive staining reaction.

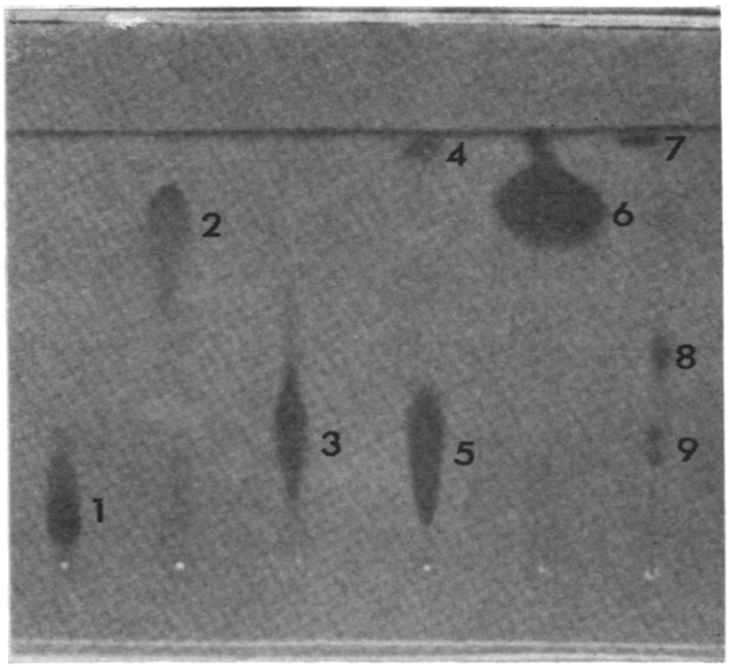

Fig. 1. Thin-layer chromatogram (silica gel G) illustrating various lipids stained with malachite green. The background stain has been reduced with the bisulfite reagent. Solvent system: chloroform–methanol–water (65:25:4). 1 = Lysolecithin; 2 = cardiolipin; 3 = lysophosphatidylethanolamine; 4 = cholesterol; 5 = sphingomyelin; 6 = oleic acid; 7 = cholesterol; 8 = lecithin; 9 = sphingomyelin.

RESULTS

Spot test experiments demonstrated that phospholipids (phosphatidylcholine, lysolecithin, phosphatidylethanolamine and phosphatidylserine), cerebrosides, cholesterol, fatty acids (oleic, myristic, stearic and palmitoleic), fatty aldehydes (myristaldehyde, palmitaldehyde and stearaldehyde), glyceryl tripalmitate and β-lipoprotein stained with malachite green; however, all other biological compounds investigated did not.

Chromatographed lipid materials (choline and cephalin phosphatides, cardiolipin, glycolipids, sphingomyelin, lysolecithin, cholesterol, glyceryl tripalmitate; fatty acids: oleic, myristic, stearic and palmitoleic; and fatty aldehydes: myristaldehyde, palmitaldehyde and stearaldehyde) were observed as intensely green spots on a light yellow background. The sensitivity of the stain is approximately 1 μg of lipid. Malachite green in 90% ethanol may be substituted for the aqueous reagent, however staining is not as intense.

DISCUSSION

The mechanism of malachite green staining is not known, however its affinity for fatty acids and fatty aldehydes, and the lack of affinity for glycerylphosphoryl-

choline and various other phospholipid constituents does suggest that staining requires the presence of long-chain carbon groups. Since stained lipids are easily visualized prior to bisulfite spraying, bisulfite reduction of unbound stain is only necessary if plates are to be photographed. Stained chromatograms have been stored for up to two months without any apparent reduction in the intensity of the stained lipids. Malachite green spraying of thin-layer chromatograms therefore permits a fast detection of lipids without interference from other biological compounds and does not require treatment with caustic reagents in order to visualize stained lipids.

ACKNOWLEDGEMENTS

This study was supported by grant HD-06897 from the U.S. Public Health Service.

REFERENCES

1 H. J. Altmann, E. Fürstenau, A. Gielewski and L. Scholz, *Anal. Chem.*, 256 (1971) 274.
2 L. Hörhammer, W. Hildebert and G. Richter, *Biochem. Z.*, 331 (1959) 155.
3 R. J. Teichman, M. Fujimoto and R. Yanagimachi, *Biol. Reprod.*, 7 (1972) 73.