Journal of Chromatography, 424 (1988) 445–448
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3972

Letter to the Editor

Use of the dye Janus Green to facilitate quantitative analysis of phosphoinositides*

Sir,

Recent observations on the role of phosphoinositides in various biological systems have focused a great deal of attention on these lipids (see refs. 1–3 for reviews). As a result of this increased research activity, the methodology for analysis of phosphoinositides has been continually refined; however, thin-layer chromatography (TLC) continues to be the method of choice for their quantitation [4–6]. During these analyses, a complete transfer of lipid extracts to a thin-layer chromatographic plate is frequently required but is hampered by the fact that the extract is usually colorless. Under these circumstances, if a portion of lipid extract is spattered onto the side of a tube during transfer or nitrogen blow down, only exhaustive rinsing of the tube will ensure that a quantitative transfer to the TLC plate is obtained.

This report describes a blue dye which can be added directly to the chloroform–methanol–hydrochloric acid extraction system used in phosphoinositide analysis; the dye partitions with the lipids, acts as a visual monitor of the lipid mass and does not interfere with the TLC separation of these phospholipids. The use of this dye greatly facilitates the quantitative analyses of phosphoinositides.

EXPERIMENTAL

Janus Green B, 3-(diethylamino)-7-[(*p*-(dimethylamino)phenylazo]-5-phenylphenazinum chloride (CI 11050), was purchased from Sigma (St. Louis, MO, U.S.A.). Silica gel 60 plates, 0.25 mm thickness, with preconcentration zone (EM 11845) were from E. Merck (Damstadt, F.R.G.). Unisil, acid-washed silicic acid, was from Clarkson (Williamsport, PA, U.S.A.). All other chemicals were of reagent grade or better.

* Publication No. 4895 BCR for the Research Institute of Scripps Clinic.

0378-4347/88/$03.50

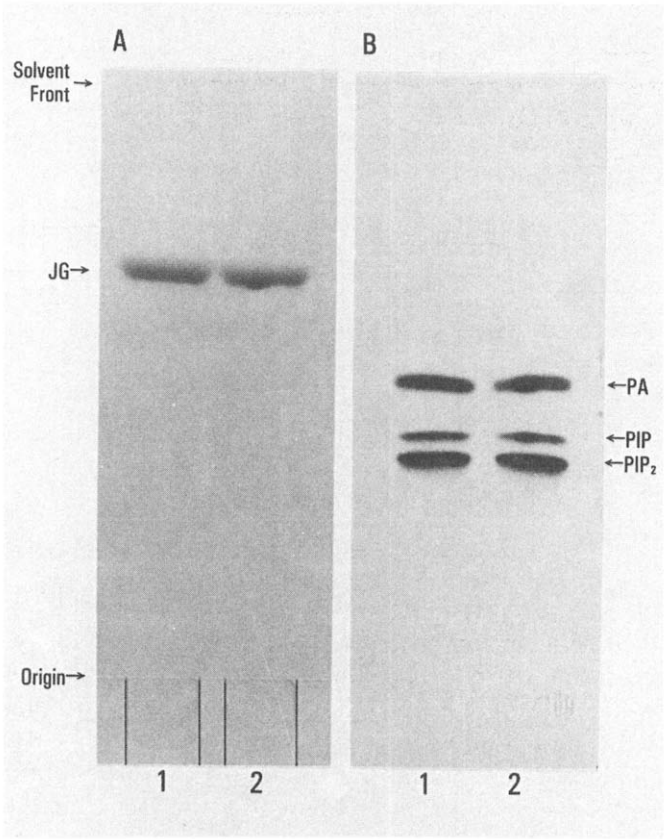

Fig. 1. Chromatography of Janus Green and ^{32}P-labeled lipids from human erythrocytes. Phosphoinositides were extracted in the presence of Janus Green and chromatographed as detailed in the Experimental section. Panel A demonstrates the migration of the Janus Green dye (JG) while panel B shows the migration of the ^{32}P-labeled lipids: phosphatidic acid (PA), phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP_2). Lanes 1 and 2 are identical.

Purification of Janus Green

Janus Green powder (50 mg; 60% dye by weight) was dissolved in 100 ml of chloroform–methanol (95:5) and applied to a column of Unisil (1.5 g of Unisil in a 1 cm I.D. glass column). The run-through was collected, and nitrogen gas was used to evaporate the solvent. Approximately 26 mg of dye were recovered and dissolved at 5 mg/ml in chloroform–methanol (1:1) and stored at −20°C.

Phosphoinositide analysis

Human erythrocytes were labeled with $^{32}PO_4$ and the membrane isolated as described previously [6]. ^{32}P-Labeled erythrocyte membranes (up to 3 mg of stromal protein in a maximum of 500 μl of buffer) were extracted with 3 ml of chloroform–methanol–concentrated hydrochloric acid (20:40:1) containing 5 μg purified Janus Green (1.7 mg purified Janus Green per litre extraction mixture; the dye is stable in this acidic extraction mixture for up to two months). After vortexing, the extract should be perfectly clear; if not then additional extraction

solvent is added until clarity is achieved. For each 3 ml of extraction solvent, 1 ml of water and 1 ml of chloroform are added; the mixture is vortexed and centrifuged at approximately 30 *g* in a benchtop centrifuge for 10 s. A calibrated, gas-tight, glass syringe with a 10-cm metal needle is used to remove 1.5 ml of the lower phase. This chloroform phase is blown completely dry with nitrogen gas; any residual water will lower the yield of phosphoinositides upon TLC.

The dried, blue extract is dissolved in 100 μl of chloroform–methanol (1:1) and quantitatively transferred to the preconcentration zone of the TLC plate with drying by a warm air blower. The completeness of the transfer is easily monitored by observing the blue dye. Chromatography was in chloroform–methanol–5 m*M* EDTA containing 3.3 *M* ammonia (100:80:25). Autoradiography was performed as previously detailed [6].

RESULTS AND DISCUSSION

Commercially available Janus Green B is a relatively pure dye with a few minor impurities, some of which are more polar than the main component. Fractionation of this mixture over silicic acid allows isolation of the main component of the dye without retention of the polar impurities.

Purified Janus Green B is useful in the analysis of phosphoinositides since it is intensely colored and partitions with the lipids during organic solvent extraction. As detailed in the Experimental section, purified Janus Green B was added directly to the chloroform–methanol–hydrochloric acid extraction solvent used for phosphoinositide isolation. The dye partitions entirely into the chloroform phase and serves its most useful function during the subsequent drying and TLC application steps. With an intense blue color, the Janus Green is a visual indicator of the lipid extract so that the investigator has confidence that 100% of the lipid extract has been transferred from the blow-down tube to the TLC plate.

Fig. 1 demonstrates the chromatographic migration of Janus Green and the [^{32}P]phospholipids from erythrocyte membranes. The R_F for the dye in panel A is 0.68. Panel B shows an autoradiogram of this same TLC plate demonstrating the migration of phosphatidic acid, phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-bisphosphate; the R_F values for these lipids are 0.50, 0.41, and 0.36, respectively. When this autoradiogram is compared to panel A, it is clear that the purified Janus Green migrates well ahead of these radioactive lipids and will not interfere with quantitation of the radioactivity [7] or phosphate content [8] of the lipids.

ACKNOWLEDGEMENTS

This work was supported in part by Grants DK 35220, HL 25552, and RR 00833 from the National Institutes of Health.

Department of Basic and Clinical Research,
Research Institute of Scripps Clinic,
10666 North Torrey Pines Road,
La Jolla, CA 92037 (U.S.A.)

GEORGE L. DALE

1 Y. Nishizuka, Science, 225 (1984) 1365.
2 P.W. Majerus, D.B. Wilson, T.M. Connolly, T.E. Bross and E.J. Neufeld, Trends Biochem. Sci., 10 (1985) 168.
3 M.J. Berridge, Biochem. J., 220 (1984) 345.
4 J.E. Ferrell, Jr. and W.H. Huestis, J. Cell Biol., 98 (1984) 1992.
5 C.J. van Dongen, H. Zwiers and W.H. Gispen, Anal. Biochem., 144 (1985) 104.
6 G.L. Dale, Blood, 66 (1985) 1133.
7 S.D. Crosby and G.L. Dale, J. Chromatogr., 323 (1985) 462.
8 Y.H. Itoh, T. Itoh and H. Kaneko, Anal. Biochem., 154 (1986) 200.

(First received June 25th, 1987; revised manuscript received September 26th, 1987)