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Note

Rapid and simple method for the detection of nanogram amounts of arachidonate metabolites resolved by thin-layer chromatography

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Increasing interest is devoted to prostaglandins as basic compounds involved in transmembrane cell signalling, and very refined techniques have been developed to isolate, resolve and detect a variety of arachidonate metabolites.

A simplified and sensitive method is presented here for the detection of trace amounts of prostaglandins and unsaturated fatty acids resolved by thin-layer chromatography (TLC) on silica gel plates. The method is based on the formation, in the presence of strong mineral acids at 80°C, of partial oxidation intermediates fluorescent under UV light. The oxidation reaction is monitored with a cholesterol internal standard and stopped when the purple colour of the cholesterol spot tends to brown. The method appears more sensitive than all the other detection procedures so far available for unlabelled prostaglandins, including the classic TLC developments such as charring, iodine vapour, rhodamine-6G, 2′,7′-dichlorofluorescein and 4-methoxybenzaldehyde, as well as high-performance liquid chromatography and mass spectrometry.

General detection methods based on fluorescence have already been described. Segura and Gotto[1] induced fluorescence in a number of organic compounds on silica gel thin-layer chromatograms by exposing them to the vapour of ammonium hydrogen carbonate; Shanfield *et al.*[2] found that a simple gaseous electric discharge in the presence of nitrogen or ammonium hydrogen carbonate vapour can induce fluorescence in a variety of organic compounds separated on silica gel thin-layer chromatoplates.

Our method is a variant of the latter procedures that takes advantage of the particular ability of unsaturated compounds to form partial oxidation intermediates in the presence of strong acids. It has the advantages of rapidity (5 min) and economy (no special reagent or device is needed) and can detect amounts of arachidonate metabolites as small as 1 ng or less.

Standard prostaglandins and unsaturated fatty acids were purchased from Sigma (St. Louis, MO, U.S.A.). High-performance thin-layer silica gel plates (0.25 mm thick) were from Merck (Darmstadt, F.R.G.), and solvents and reagents were analytical grade from Carlo Erba (Milan, Italy).

Essentially, two ascending chromatography methods were used: following Salmon and Flower[3], for arachidonate metabolites with the upper phase of a mixture

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of ethyl acetate, trimethylpentane, acetic acid and water (110:50:20:100, v/v) and following Christie[4] for simple lipids with a mixture of light petroleum (40–60°C), diethyl ether and acetic acid (80:20:30, v/v).

After development by ascending chromatography the plates were dried, sprayed with 50% sulphuric acid and put in an oven at 80°C. The cholesterol internal standard developed a purple colour in *ca.* 3 min, tending to brown 2 min later. The heating was stopped and the plates were ready for visualization using a 240-nm UV lamp (Mineral Light). Spots containing more than 20 ng were visible even in daylight: PGE_1 and PGE_2 were yellow, $PGF_{2\alpha}$ violet, TXB_2 reddish and arachidonic acid reddish-brown. Completely negative areas in daylight were positive under a Wood lamp, showing blue or reddish-blue spots when the compounds were in the microgram concentration range or pale pink shadows in the nanogram range.

For the application of the method to biological samples, human platelets and olive oil were used as sources of arachidonate metabolites and unsaturated fatty acids, respectively. Platelets were isolated from freshly drawn blood from a single donor as previously described[5]. Control and thrombin-aggregated platelets were rapidly extracted with 20 volumes of chloroform–methanol (2:1, v/v) and the organic phase was cold-dried under a flow of nitrogen. Olive oil (Sughericci, Azienda Agricola, Grosseto, Italy) was diluted in hexane (1:10, v/v) and 1 μl of sample was applied to the plate.

Table I shows the sensitivity of the present method compared with the other TLC procedures for visualization of prostaglandins. It is about one order of magnitude more sensitive than the methoxybenzaldehyde method, which is the most sensitive described as yet for prostaglandins.

TABLE I

COMPARISON OF THE SENSITIVITY OF THE VARIOUS METHODS FOR VISUALIZATION OF STANDARD PROSTAGLANDINS ON TLC

Method	*Amount of compound (ng)*						
	64	*32*	*16*	*8*	*4*	*2*	*1*
Charring	+	+	+ –	–	–	–	–
Iodine vapour	+	+ –	–	–	–	–	–
Rhodamine-6G	+	+ –	–	–	–	–	–
2′,7′-Dichlorofluorescein	+ –	+ –	–	–	–	–	–
4-Methoxybenzaldehyde	+	+	+	+ –	+ –	–	–
Present method	+	+	+	+	+	+	– +*

* With the present method the visualization is positive for PGE_1 and PGE_2 as well as TBX_2 at the 1-ng level; $PGF_{2\alpha}$ and arachidonate are at the limit of visualization.

Fig. 1 shows the application to biological samples: it is relevant that trace amounts of prostaglandins are detectable from a number of platelets as small as 10^{-8} and that the appearance of novel aggregation compounds such as TXB_2 is evident. The same figure shows that the method is very suitable even for unsaturated fatty acids from animal and vegetable sources.

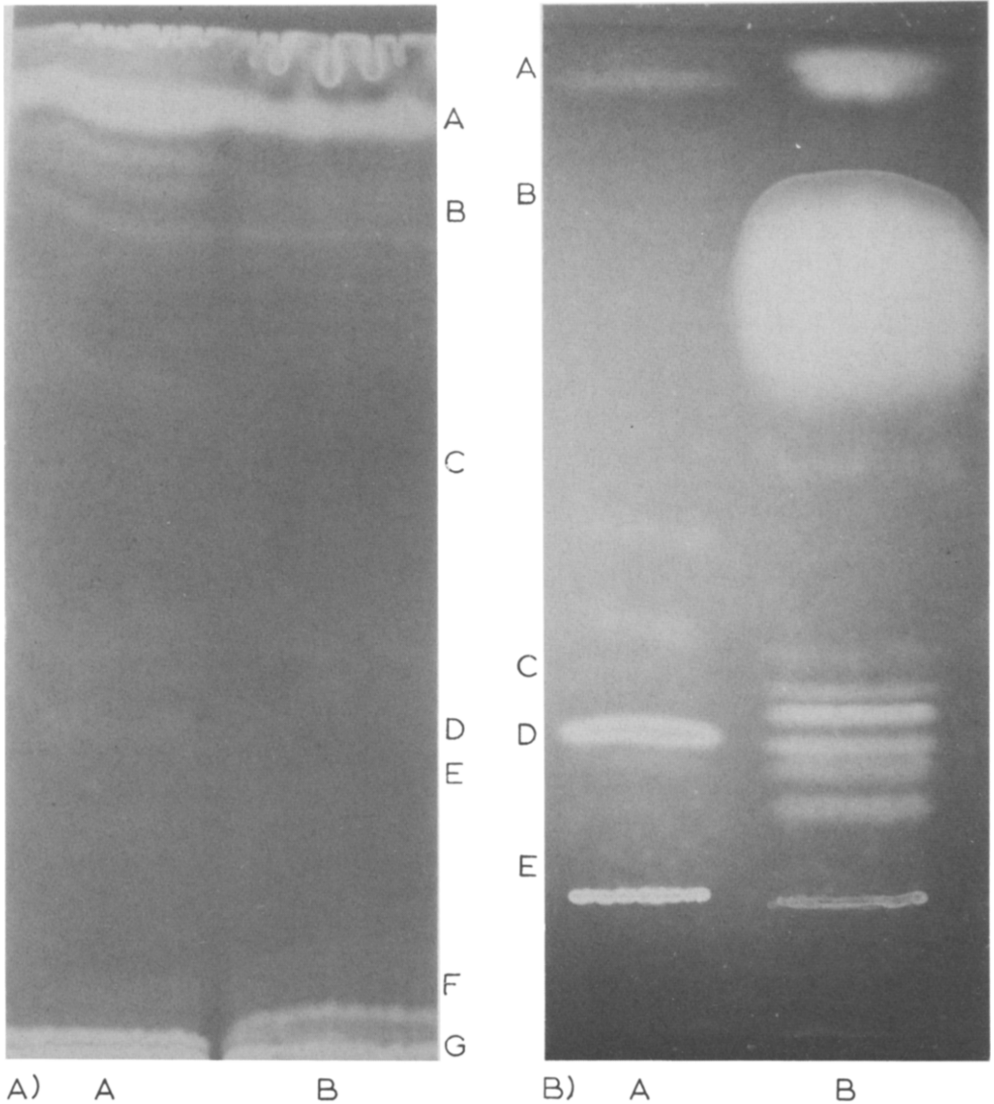

Fig. 1. (A) Visualization of prostaglandins from control and thrombin-aggregated platelets. 10^{-8} Platelets were resuspended in Tyrode saline and divided into two aliquots: one sample was treated with thrombin (10 units of the enzyme × 5^{-8} cells suspended in 4 ml of Tyrode saline) and the other was used as control. After aggregation (3 min at 37°C), the two samples were extracted with chloroform–methanol (2:1, v/v, 20 volumes), and the organic phase was collected and dried in the cold. The extracted lipids were dissolved in 10 μl of chloroform and applied to the plate. Lane A = thrombin; lane B = control. Symbols on the right: A = neutral lipids; B = leukotrienes area; C = PGE_2; D = TXB_2; E = $PGF_{2\alpha}$; F = phosphatidic acid area; G = phospholipids still at the start. (B) Visualization of platelets (lane A) and olive oil (lane B) simple lipids after chromatography with the Christie method. Symbols on the left: A = cholesterol esters in platelets lane and phytosterol esters in oil; B = triolein large spot; C = arachidonic acid in platelets lane; D = cholesterol in platelets and phytosterol in oil accompanied by a variety of unidentified fatty acids; E = phospholipids still at the start.

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