Separation and Measurement of Microgram Amounts of Radioactive Polysaccharides in Metabolic Experiments

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A method is described for the detection and estimation of radioactive polysaccharides separated by zone electrophoresis on glass-fibre paper.

Studies of the metabolism of biological polymers generally depend on the availability of rapid and specific methods for the separation, purification and estimation of small quantities of radioactive polymers. Such requirements have proved difficult to meet for polysaccharides.

Electrophoresis in borate buffers provides a quick and sensitive means of separating neutral polysaccharides (Northcote, 1954; Hocevar & Northcote, 1957; Olaitan & Northcote, 1962), and can be used on a small scale as zone electrophoresis with a glass-fibre or silk support (Fuller & Northcote, 1956; Bouveng & Lindberg, 1958; Northcote, 1965). Acidic polysaccharides are readily separated on glass-fibre paper in pyridine-acetic acid-EDTA buffer, pH 6.5 (Barrett & Northcote, 1965; Northcote, 1966; Stoddart, Barrett & Northcote, 1967).

Wang & Jones (1959) have described a method of detecting radioactive spots on chromatograms and electrophoretograms (on cellulose paper) by dissecting them into strips and counting the strips in a scintillation counter. It has been claimed that no orientation effect occurs (Geiger & Wright, 1960), but this has been contested (Loftfield & Eigner, 1960). Pinter, Hamilton & Miller (1963) have reported that glass-fibre disks, impregnated with radioactive materials, can be estimated with a far higher efficiency than similar cellulose paper disks. Gill (1964) has confirmed this for glass-paper cylinders. We have found that the techniques of zone electrophoresis on glass-fibre paper and liquid-scintillation counting can be combined to yield a highly sensitive means of analysing sub-milligram quantities of radioactive polysaccharides, whether they are acidic, neutral or a mixture of both. The counting procedure is applicable whenever radioactive compounds are separated on glass-fibre paper and whenever it is necessary to estimate the radioactivity of toluene-insoluble material in the absence of aqueous fluors.

MATERIALS AND METHODS

Whatman GF81 glass-fibre paper, heat treated to remove cellulose, was used. The fluor solution was composed of

2,5-diphenyloxazole (3.5 g.) and 1,4-bis-(5-phenyloxazol-2-yl)benzene (50 mg.) in sulphur-free toluene (1 l.). Radioactive materials (D-[U-¹⁴C]glucose, [U-¹⁴C]starch, D[1-³H]-mannose and the standards) were obtained from The Radiochemical Centre, Amersham, Bucks. The radioactive standards were: for ¹⁴C, [1-¹⁴C]*n*-hexadecane in toluene; for ³H, [1,2-³H]*n*-hexadecane in toluene.

The counters used in these investigations were a Packard Tri-Carb model 314A liquid-scintillation spectrometer, a Nuclear-Chicago model 720 liquid-scintillation system (used without pulse-height summation) and a Nuclear-Chicago Unilux TM liquid-scintillation system.

To investigate the characteristics of the counting system solutions of radioactive carbohydrates were applied to 1 cm. × 4 cm. strips of the paper and the strips were dried in a stream of warm air. They were then inserted into 0.5 in. × 2 in. glass specimen tubes and 0.5 ml. of the fluor solution was added to each. The tubes were closed with polythene stoppers and placed inside 20 ml. Packard liquid-scintillation spectrometer tubes for counting. They were cooled for 20 min. before counting in the refrigerated counters. The Packard counter was used in all studies save the measurements of efficiency.

RESULTS

A paper strip impregnated with radioactive glucose was counted at various orientations relative to the line joining the two photomultipliers, to check for a possible dependence of count rate on sample orientation. No such effect was observed. The effect on the count rate of the varying of the volume of fluor solution added to the vial was investigated (Fig. 1) and a slight dependence was found. As a routine we have used 0.5 ml. of solution with strips cut from electrophoretograms and the error in the addition of fluor was below ± 0.05 ml. If the radioactive material was immersed below the fluor some slight loss of efficiency resulted. No dependence of the count rate on the width of the paper strip was found in the range 5–12.5 mm. The background count varied slightly and was not constant from batch to batch of paper. This was probably due to a variation in the potassium content of the glass paper. We have confirmed Gill's (1964) observation that a strip of Whatman no. 1 paper

Fig. 1. Dependence of count rate on fluor solution volume, with a glass-fibre strip and D-[U-¹⁴C]glucose.

Fig. 2. Dependence of count rate on loading of glass-fibre strip with D-[U-¹⁴C]glucose solution.

Table 1. *Efficiency of counting of n-hexadecane standards on glass-fibre strips*

Counter	Efficiency (%)	
	¹⁴ C	³ H
Packard Tri-Carb 314A	60	14.6
Nuclear-Chicago 720	78	15.3
Nuclear-Chicago Unilux TM	85	21.4

inserted beside the glass-fibre strip caused no change in efficiency. We also found that the application of up to 400 μg. of polysaccharide (dextran, mol.wt. 72 600, apple fruit pectin or apple suspension-callus

Fig. 3. Electrophoretic separation of extracted radioactive sycamore pectin (GF81 glass-fibre paper; pyridine-acetate buffer containing 10 mM-EDTA, pH 6.5; 40 v/cm. and 60 mA, run for 25 min.).

pectin) had little or no effect on the efficiency with which an underlying spot of [¹⁴C]glucose was counted. Since we did not normally use more than 250 μg. of polysaccharide for an analytical separation (which gave about 30 strips of paper), there was no risk of quenching by non-radioactive polysaccharide.

Saturation of the glass paper with borate or pyridine-acetate-EDTA buffers, followed by drying and the application of radioactive glucose as before, led to no loss of efficiency by comparison with controls. Thus it was possible to use either (or both) buffers.

The count rate was found to be proportional to the loading for [¹⁴C]glucose (Fig. 2), [¹⁴C]starch and [³H]mannose. The radioactivity present in any zone of an electrophoretogram can thus be calculated directly.

The efficiencies of counting ¹⁴C and ³H were measured on all three counters. Balance-point settings were determined. The efficiencies quoted in Table 1 were obtained by using the organic standards and for ¹⁴C were the same as those found in the absence of the glass-fibre paper. Similar efficiencies were found with radioactive glucose and radioactive starch; self-absorption was very slight indeed.

The separation of an extract of sycamore pectin into neutral arabinan-galactan, weakly acidic pectinic acid and strongly acidic pectinic acid is shown in Fig. 3.

DISCUSSION

By using the two techniques of electrophoresis (one- or two-dimensional) and scintillation counting, it is possible to separate radioactive polysaccharides and to estimate the radioactivity within them with considerable precision and without the

need for elaborate corrections. During a metabolic study of pectin in sycamore tissue we have followed the synthesis of various polymers of the pectin complex by electrophoresis of the labelled material synthesized from D-[U- ^{14}C]glucose (Stoddart & Northcote, 1967). The method is applicable also to the counting of unfractionated polysaccharides before electrophoresis and gives a very high efficiency, especially for ^{14}C . It has an additional merit in that it is economical of fluor solution. The method should be applicable to the counting of other substances.

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