Chromatographic separation on silica gel glass fiber paper of compounds from yeast labeled by aspartate-^{14}C

As part of an investigation of the metabolism of aspartate-^{14}C by a yeast mutant lacking the enzyme dihydroorotic dehydrogenase[1], it became necessary to separate and measure, quantitatively in a large number of samples the levels of the small molecules of the cell that receive ^{14}C from uniformly labeled aspartate. By means of conventional two dimensional chromatography it was found that the chief labeled components of the extract are aspartate, glutamate, ureidosuccinic acid and dihydroorotic acid. It was desired to separate these on paper over a short distance so that large numbers of strips could be rapidly scanned and the activities of the components recorded by a radiochromatogram scanner and integrator.

The yeast employed was a haploid strain of *Saccharomyces cerevisiae*, S 1237, with biochemical deficiencies for adenine, uracil and histidine. The yeast was grown in liquid synthetic medium[2] at 30° with aeration until the mid log phase of growth (10^7 cells/ml) was reached. After uptake of aspartate-^{14}C, samples were prepared for chromatography by extraction of $5 \cdot 10^7$ cells in 3 ml 95 % ethanol at 50° for 3 min, followed by dilution to 6 ml with water and extraction again at 50° for 3 min. The extract was vacuum dried and then dissolved in 25 μl of aqueous buffer.

Various thin layer chromatographic techniques were explored with little success until the recently available Gelman* silica gel ITLC glass fiber paper was used. For our application it was found that this must be activated for 16 h at 200° before use. After activation it can be kept up to a month in a desiccator and used, provided it is reheated at 200° for 30 min or more just before use.

Many solvents involving combinations of ethanol, butanol, ammonia, formic acid, etc. were tried (all those listed by RANDERATH[3] page 93, were tried) with the invariable result that most of the compounds appeared in a streaked spot near the solvent front. The one exception was liquefied phenol 80 %, H_2O 20 %, KCN 20 mg per 100 ml. Ascending chromatography was carried out on 8 × 8 inch paper in the Eastman Chromatogram sandwich-type developing system with 100 ml of solvent in the trough. Two microliter applications (single or multiple) were made using disposable capillary micropipets. The best results were obtained when the extract or pure standards were dissolved in 0.134 *M*, pH 7.5, sodium phosphate buffer before application. If buffer is not used ureidosuccinic acid tends to spread or form two spots. The radioisotope ^{54}Mn was added to all yeast samples before extraction and was used as an internal radioactive standard to facilitate determination of loss of material during purification.

Fig. 1 shows the radioactivity profile of a mixture of aspartate-^{14}C ($2.2 \cdot 10^{-3}$ μg), glutamate-^{14}C (0.64 μg), ureidosuccinic acid-^{14}C (US) ($9.4 \cdot 10^{-2}$ μg), dihydroorotic acid-^{14}C (DHO) ($4 \cdot 10^{-2}$ μg), and ^{54}Mn. All are well resolved after 50 min development at 25° with the solvent front at 10.5 cm. R_F's are as follows: DHO (No. 5) 0.85, glutamate (No. 4) 0.68, aspartate (No. 3) 0.57, US (No. 2) 0.36, and ^{54}Mn (No. 1) at the origin. Distances from the origin to the tops of the peaks were used in computing R_F's.

Especially noteworthy is the clear separation of aspartate and glutamate by this simple and rapid method. When run singly each compound shows one peak only.

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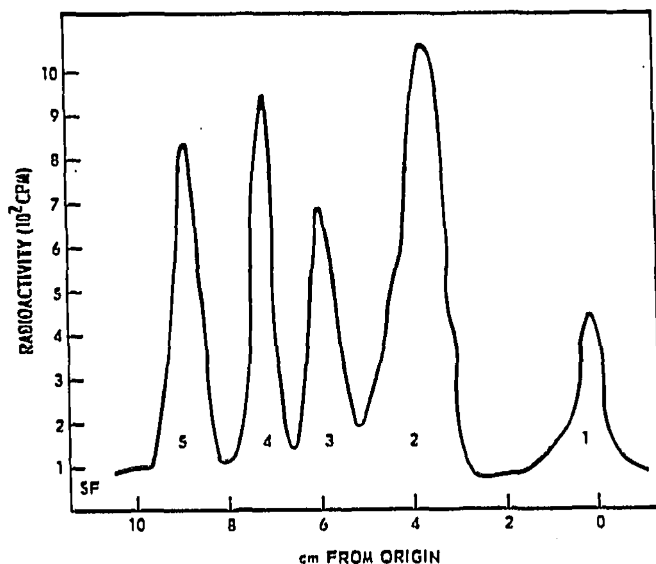
* Gelman Corp., Chelsea, Michigan.



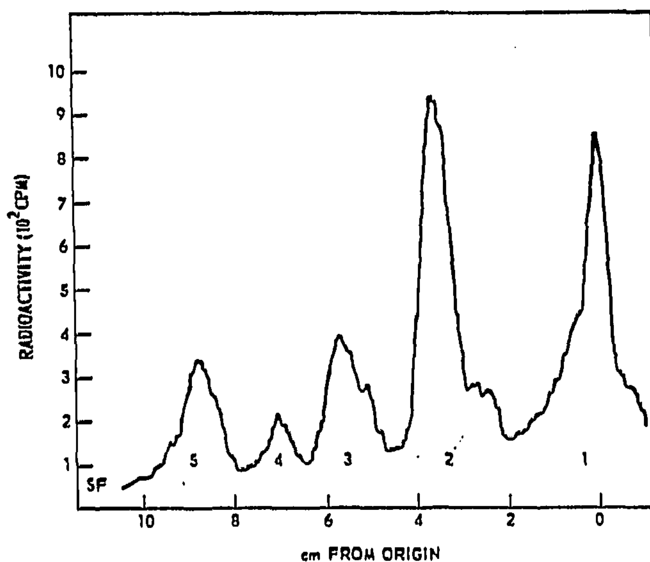


Fig. 1. Radiochromatographic separation of 1 = ^{54}Mn; 2 = ureidosuccinic acid; 3 = aspartic acid; 4 = glutamic acid; 5 = dihydroorotic acid. Development time, 50 min.

Fig. 2. Radiochromatographic separation of labeled compounds from yeast S 1237. Peaks are numbered as in Fig. 1.

Fig. 2 shows the profile of an extract of yeast S 1237 that has been incubated with aspartate-^{14}C in the absence of uracil. This yeast accumulates US and DHO in the absence of uracil. It is seen that the compounds in extract are well resolved and have R_F's close to those of the corresponding standards. The results are quite reproducible and the method is well suited for routine quantitative analysis.

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