Journal of Chromatography, 267 (1983) 242–245
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 15,949

Note

Rapid breakdown of indole-3-acetic acid on silica gel thin-layer plates

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(Received April 26th, 1983)

It has been known for a number of years that indole-3-acetic acid (IAA) breakdown occurs on silica gel chromatography plates. Mann and Jaworski[1] found almost complete loss of IAA from silica gel-impregnated, chromatography sheets when the developed chromatograms were stored overnight (presumably in darkness), and Sagi[2] reported 50% IAA loss from developed silica gel thin-layer chromatographic (TLC) plates over a similar period in darkness.

Destruction of IAA during application of solutions to the origin of silica gel chromatograms[1], and prior to chromatogram development[3], has also been observed. The latter effect increased with time and the presence of light, although no indication of the rate or extent of breakdown was given[3].

The present study is, to our knowledge, the first quantification of short term destruction of IAA on silica gel TLC plates. The results indicate that IAA breakdown during TLC is potentially more serious than earlier reports have suggested. In the presence of light considerable destruction of IAA can occur on silica gel TLC plates in a matter of minutes, rather than hours.

MATERIALS AND METHODS

Thin-layers (0.25 mm) of silica gel G (Sigma) were prepared on glass according to the manufacturer's directions, except that 0.3% (w/v) carboxymethyl-cellulose (Whatman CM 32) was incorporated as a gel hardener[4]. Activation, when employed, was at 100°C for 30 min. One hundred and fifteen Bq of [2-^{14}C]IAA (1.998 GBq/mmol) (Radiochemical Centre, Amersham, Great Britain) dissolved in methanol was applied under N_2 in 15-mm wide bands to the origin of duplicate TLC plates (20 × 20 cm). The plates were spotted and incubated on a bench under normal laboratory light, comprising natural, summer daylight supplemented with color 33 fluorescent lighting. The [^{14}C]IAA was applied at intervals prior to development of the plates in darkness at 20°C in isopropanol–ammonia (sp.gr. 0.91)–water (10:1:1, v/v/v). The developed plates were autoradiographed using Kodak X-ray film, and the radioactivity eluted in 50% (v/v) aqueous ethanol. After 30 min with occasional agitation, 10 ml of toluene–Triton X-100 (2:1, v/v) containing 2,5-diphenyloxazole (PPO, 4 g/l) and 1,4-bis(5-phenyloxazdyl-2)benzene (POPOP, 0.1 g/l) were added. The radioactivity was measured using a Philips PW 4540 liquid scintillation spectrometer. Correction was made for inefficiency in counting.

0021-9673/83/$03.00

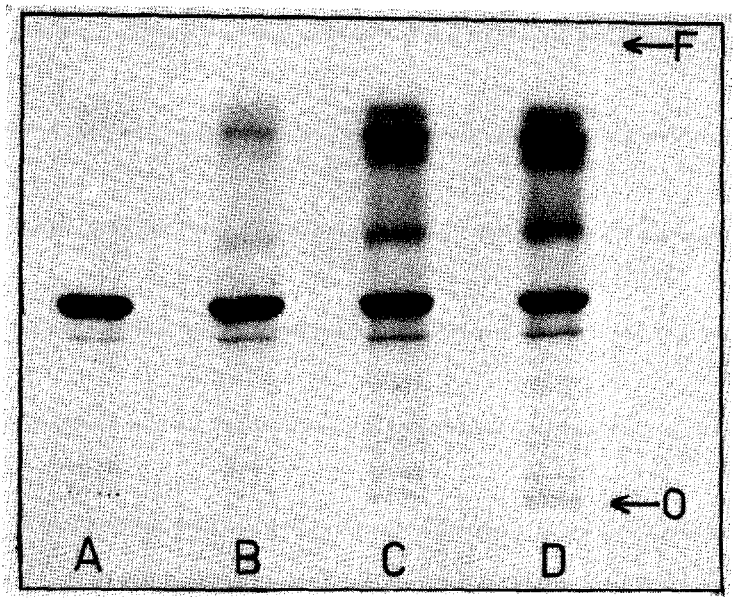

Fig. 1. Time-course of breakdown of [2-^{14}C]IAA on silica gel TLC plate incubated in laboratory light. Each band of [^{14}C]IAA (115 Bq) was applied to the origin at a different interval prior to development of the plate. Immediately after application of the final band, the plate was developed in isopropanol–ammonia–water (10:1:1) and then autoradiographed. The time on the plate prior to development was as follows: A, 0 min; B, 10 min; C, 30 min; D, 60 min. O signifies the position of the origin; F, the position of the solvent front.

RESULTS

[^{14}C]IAA was destroyed rapidly on the silica gel plate during the period prior to development. At least nine major breakdown products were evident and all increased in amount as the length of the pre-development period was extended (Fig. 1). These products were located at R_F 0.00, 0.06, 0.12, 0.26, 0.36, 0.58, 0.74, 0.78 and 0.83, where IAA had an R_F of 0.43. The proportion of the total applied radioactivity recoverable in the [^{14}C]IAA band following each incubation period is shown in Fig. 2. Even when development took place immediately after IAA application, only 93.4% of the radioactivity was recovered as [^{14}C]IAA. Breakdown was directly proportional to the time elapsed between spotting the plates and their development, up to 30 min, when more than half the applied [^{14}C]IAA had been degraded. The rate of decomposition then decreased slightly until 60 min, after which 30.6% of the applied radioactivity remained as [^{14}C]IAA. These experiments were repeatable, with similar results.

When plates were incubated in darkness at 25°C prior to development, IAA breakdown was reduced considerably, but not eliminated. Storage under a stream of N_2 during a 15-min exposure to laboratory light did not reduce breakdown, and activation of the plates prior to use had no significant effect.

Experiments using 20-μg applications of unlabelled IAA indicated that, except for a small amount of brown material at the origin, all the decomposition products were both Salkowski and Ehmann reagent[5] negative.

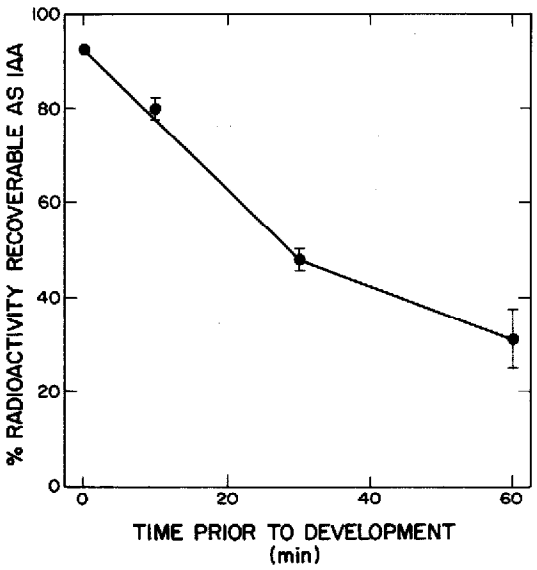

Fig. 2. Quantification of the [^{14}C]IAA breakdown shown in Fig. 1. For each incubation time the proportion of the total radioactivity remaining as IAA was determined by liquid scintillation counting. The data shown represent mean of two separate chromatograms, ± S.E. Where no error term is shown the S.E. is less than or equal to the symbol used to designate the mean.

DISCUSSION

The results show that the problem of IAA destruction during silica gel chromatography may be more serious than was indicated previously[1–3]. The rate of breakdown observed here is more in accord with that published recently by Iino *et al.*[6]. It is apparent that even short delays in development of silica gel TLC plates can result in considerable destruction of IAA. This could cause serious errors in the estimation of endogenous levels of IAA in plant extracts, although the inclusion of an internal standard of radioactively-labelled IAA enables correction for such losses[6–10]. Delays prior to plate development could also lead to incorrect conclusions regarding IAA metabolism.

In quantitative work, however, destruction of IAA on the TLC plate *after* development is potentially more serious than prior to development, especially when silica gel TLC is the final purification step before estimation of IAA levels. In such instances, IAA may be measured either directly by chromogenic sprays[11] or, after elution from the plate, by UV absorbance[12,13], colorimetry[12,13] or fluorimetry[6]. With these procedures an internal standard may not provide an accurate estimation of IAA decomposition on the plate after development because, if [2-^{14}C]IAA is used as the standard, the label is retained in the decomposition products. Counting the radioactivity in the "IAA" spot will show complete recovery of the label. However, the IAA assays described above will detect only the IAA component of the spot, and not the decomposition products[1,2,6]. Under these circumstances a [2-^{14}C]IAA standard will not indicate that IAA destruction has occurred. Actual IAA losses on the silica gel plate will be underestimated.

Photodecomposition of IAA in aqueous solution[14], and when dry[6], involves decarboxylation. Some or all of the degradation of IAA on silica gel plates may also involve loss of the 1-carbon, and therefore [1-^{14}C]IAA might be a more useful standard than [2-^{14}C]IAA for determining IAA losses during TLC. However, this was not established in the present study. The benefits of using 1-labelled IAA as an internal standard during extraction and purification of IAA-containing extracts have been discussed previously by Iino *et al.*[6].

Breakdown of IAA on silica gel probably involves oxidation, and losses have been reduced considerably by applying antioxidant to the origin prior to sample loading[1], and by including it in the chromatography solvent[6]. However, the solvent itself causes little or no destruction (refs. 1, 6 and this study), and most of the breakdown of IAA is the result of incubation on the silica gel. Moreover, breakdown appears to be a property specifically of silica gel, since little decomposition of IAA occurs if cellulose[2,6] or polyamide gel[6] is used as the solid support. Therefore, it is doubtful whether IAA is oxidised simply because of the large surface area presented by the silica gel, as was suggested by Sagi[2].

Light accelerated the rate of IAA breakdown on the plate. Moreover, there appears to be synergistic interaction between light and the silica gel, since Iino *et al.*[6] observed only 3% loss of radioactivity when [1-^{14}C]IAA dried on glass was exposed to laboratory light for 3 h, whereas almost 70% loss occurred from illuminated plates in just 1 h in this investigation.

None of the breakdown products were identified, and this has been the case in all of the studies to date. Consistent with the results of Sagi[2] and Mann and Jaworski[1] these products (with the exception of a minor spot at the origin) did not react with Salkowski reagent. This fact has probably prevented many workers from noting that IAA destruction was occurring during TLC.

Research by Iino *et al.*[6] indicates that although cellulose solid support provides better recoveries of IAA than silica gel, polyamide gel is the best solid support for TLC of IAA. Their data show that it is superior to cellulose and silica gel in terms of IAA recovery, spot resolution, sample loading capacity and speed of development. If other precautions are taken, such as the use of antioxidants, then 99% recovery of IAA can be obtained[6].

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