Journal of Chromatography, 90 (1974) 362–364
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 7277

Note

Detection of sub-nanomole amounts of phenylthiohydantoins of amino acids

A. S. INGLIS, P. W. NICHOLLS and L. G. SPARROW

Division of Protein Chemistry, CSIRO, Parkville (Melbourne), Victoria 3052 (Australia)

(Received November 14th, 1973)

Thin-layer chromatography[1,2] of phenylthiohydantoins (PTHs) of amino acids provides a simple, rapid method for the identification of amino acid residues obtained from either manual Edman degradations or sequenators[3]. Improvements in the sensitivity of this detection can to some extent compensate for the gradual decrease in yield of the amino acid derivative through successive cycles of the sequenator and so enable longer sequences to be determined. Recently, chromatography on small (5 × 5 cm) polyamide sheets[4] was used to identify 0.05–0.20 nmoles of PTHs, the detection being made by the quenching of a fluorescent indicator on the plate. Also, 0.3 nmole quantities of PTHs were detected on small (6.3 × 6.3 cm) silica gel plates using a starch–iodine–azide reaction[5]. Edman[1] estimated that 0.8 nmole and less than 0.4 nmole could be detected on 20 × 20 cm plates by the fluorescent indicator and the iodine–azide reactions, respectively. Presumably the increase in sensitivity observed with the smaller plates is largely derived from the smaller, more compact spots obtained since the PTHs move only short distances in very short times and do not diffuse as much as on the bigger plates.

We wish to report that the sensitivity using the larger plates[2] can be increased by at least a factor of ten simply by exposing the plates , after solvent removal, to iodine vapour and quickly photographing under UV light. This increase in sensitivity should apply to the smaller scale plate systems giving even lower levels of detection.

Fig. 1 illustrates the increase in sensitivity observed after a short exposure (15 min) to iodine vapour. The PTHs can be located at the 1-nmole level by UV irradiation (Fig. 1A), but with prior exposure to iodine vapour (Fig. 1B), even PTHs at the 0.2-nmole level are detected easily and are approaching the degree of intensity obtained otherwise at the 5-nmole level.

Iodine treatment has long been used for locating organic substances on thin layer plates[6,7], although the results found for PTHs were a little surprising in that Truter[8] does not regard the procedure as being a particularly sensitive one. The visible spots (orange-yellow) obtained from the PTHs on exposure to iodine vapour fade rather quickly but facilitate detection of PTHs down to the 1-nmole level. These spots may be stabilized by carefully spraying with an aqueous 1% starch solution[9] to give dark blue colours, although there is no advantage in this step unless UV detection methods are not available.

The uptake of iodine by the silica gel plate necessitates a longer exposure time in the camera because the presence of iodine reduces the amount of light emitted by the fluorescent indicator. This effect is partially responsible for the greater

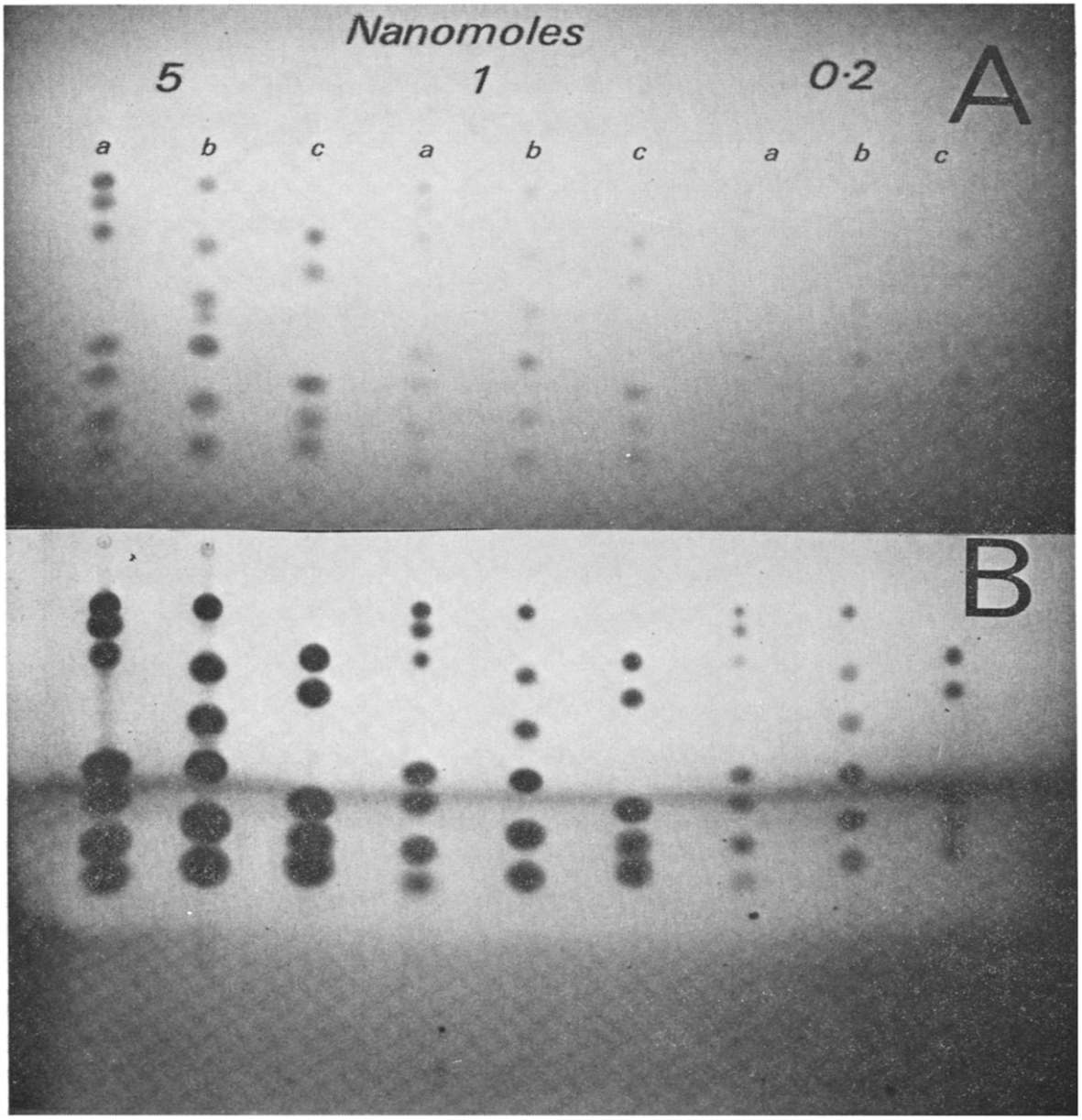

Fig. 1. Photographs under 254 nm radiation of PTHs on silica gel plates containing a fluorescent indicator. (A) Conventional TLC plate[2]; (B) same plate after exposure to iodine vapour. Standard a contains PTHs of Asn, Cys(CM), Ser, Gly, Ala, Val and Pro. Standard b contains PTHs of Gln, Thr, Tyr, N^{ε}-PTC-Lys, Met and Leu. Standard c contains PTHs of Asp, Glu, Trp, Phe and Ile. (CM = S-Carboxymethyl; PTC = phenylthiocarbamyl.)

contrast observed in Fig. 1B. Also, the addition complex between the PTH amino acid and iodine is a more efficient quencher of fluorescence than the free PTH amino acid, presumably because the iodine complex is more effectively absorbing the

exciting radiation at 254 nm. Accordingly, the UV absorption spectra of PTH amino acid–iodine mixtures in solution exhibit increased absorption below 270 nm.

It should be noted that there are important differences between this procedure and the iodine–azide reaction[10]. The latter has been used extensively[1,5,11] for increasing the limits of detection of PTHs. Edman[1] prefers not to use the iodine–azide method, if possible, because the intensity of a spot bears no relation to the amount of PTH present. As is evident from Fig. 1, the fluorescence method suffers to some extent from the same problem and only an estimation of the concentration of the spots can be made from a visual assessment of the plate. However, visual comparisons from lane to lane of a 20×20 cm plate[1] usually give a reasonable indication of the existence of overlap, multiple end groups, etc. during a prolongend sequence determination. As is evident from the figure, the iodine complex should be at least as good in this respect.

Finally we should emphasize that this iodine reaction is non-destructive and reversible[7]. If other means of detection are desirable, then these can be made after the removal of iodine by warming or allowing to stand in a well ventilated position.

REFERENCES

1 P. Edman, in S. B. Needleman (Editor), *Protein Sequence Determination*, Springer, Berlin, 1970.
2 A. S. Inglis and P. W. Nicholls, *J. Chromatogr.*, 79 (1973) 344.
3 P. Edman and G. Begg, *Eur. J. Biochem.*, 1 (1967) 80.
4 M. R. Summers, G. W. Smythers and S. Oroszlan, *Anal. Biochem.*, 53 (1973) 624.
5 M. Cohen Solal and J. L. Bernard, *J. Chromatogr.*, 80 (1973) 140.
6 G. Brante, *Nature (London)*, 163 (1949) 651.
7 G. C. Barrett, *Nature (London)*, 194 (1962) 1171.
8 E. V. Truter (Editor), *Thin-Film Chromatography*, Cleaver-Hume Press, London, 1963.
9 K. Randerath (Editor), *Thin-Layer Chromatography*, Verlag Chemie, Weinheim/Bergstr. and Academic Press, New York, 2nd ed., 1966.
10 F. Feigl (Editor), *Spot Tests in Organic Analysis*, Elsevier, Amsterdam, 7th ed., 1966.
11 J. M. Boigne, N. Boigne and J. Rosa, *J. Chromatogr.*, 47 (1970) 238.