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Note

Improved conversion of thiazolinones to phenylthiohydantoins during amino acid sequence analysis

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In a recent paper[1] a simple method was described for converting 2-anilino-5-thiazolinone derivatives of amino acids (ATOs) to the more stable 3-phenyl-2-thiohydantoins (PTHs) prior to thin-layer chromatography (TLC). A concentrated sample of the ATO extracted from the protein sequenator was applied to a TLC plate of silica gel, 1 μl of heptafluorobutyric acid was applied at the same point and the PTH was formed on heating the plate at 140° for several minutes. The heat conversion procedure has been found well suited to routine sequencing of proteins and is economical in both labour and materials compared with the HCl method[2]. However, the HCl procedure was preferable for conversion of ATO-N_ϵ-phenylthiocarbamyl (PTC) lysine (which was converted to three products) and ATO-tryptophan; also, when using the procedure developed for peptides[3] the larger amounts of Quadrol salts present in the samples adversely affected the heat conversion process.

The following modified procedure overcomes the earlier limitations of the heat conversion method. The extract from the sequenator was dried in a stream of nitrogen and dissolved in 100 μl of 1,2-dichloroethane plus 1 μl of pentafluoropropionic acid containing 5 mg/ml dithiothreitol. An appropriate aliquot was slowly loaded onto an aluminium-backed silica gel plate (Kieselgel 60F254, Merck) using a multispotter (Unimetrics, Joliet, Ill., U.S.A.) with the heating strip at approximately 70°. After loading, the TLC plate was covered with a 20 × 20 × 0.4 cm glass plate and heated at 140° for 10 min.

The presence of dithiothreitol and the exclusion of the atmosphere from the silica gel plate should significantly decrease the possibility of desulphuration of the thiocarbonyl groups by oxidation[2] either during loading or during heating at 140°. The lysyl derivative, which also has a thiocarbonyl group on the side chain, would be more susceptible to such oxidation than the other amino acids. Addition of the acid catalyst to the concentrated solution of the ATO prior to spotting on the plate eliminated the problem associated with peptide sequencing. Presumably with the earlier method the samples were decomposing during loading onto the plate. It should also be noted that the more volatile pentafluoropropionic acid, which is now used routinely in the sequenator[4], was substituted successfully for the heptafluorobutyric acid. 1,2-Dichloroethane containing the perfluoro acid was found to extract the ATOs of histidine and arginine satisfactorily from the dried extract with the advantage that it does not extract other polar material; the latter otherwise shows up at the

origin of the silica gel plate or, after hydrolysis of the extract in hydriodic acid, on the amino acid chromatogram.

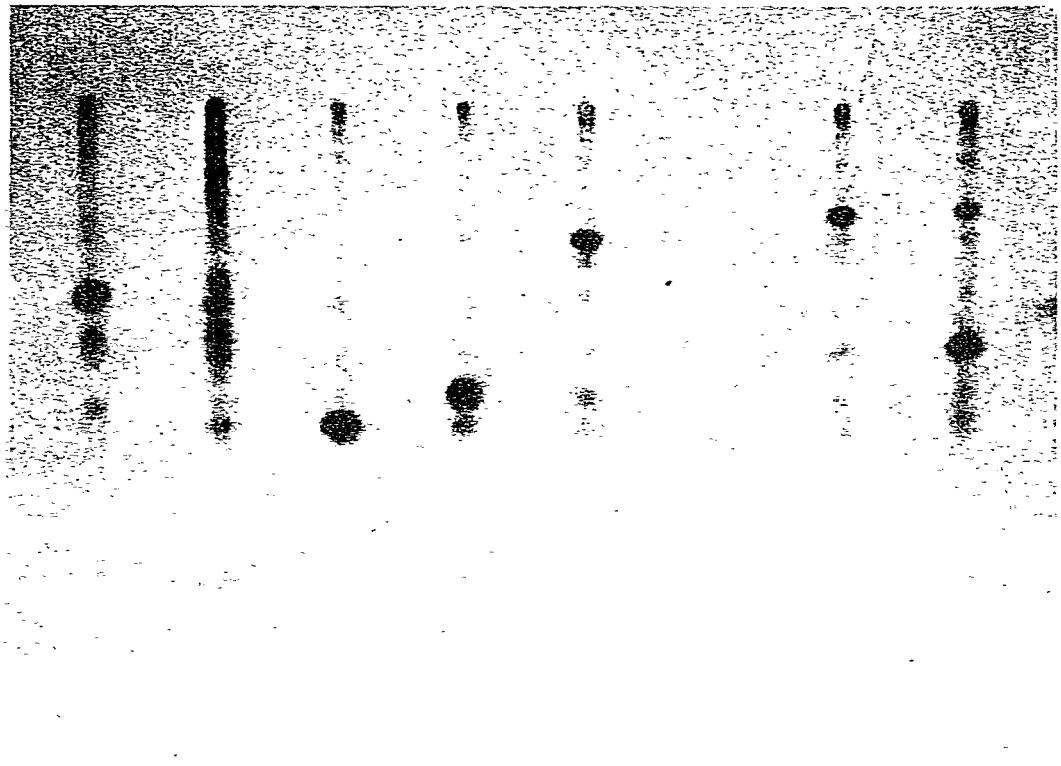

Fig. 1. UV photograph of TLC plate after chromatography of heat-converted ATOs from the sequenator. The PTHs shown, beginning from the left lane, are those from lysine, serine, isoleucine, phenylalanine, glutamic acid, aspartic acid and tryptophan corresponding with residues 1, 2, 3, 4, 5, 10 and 11 of a duck egg yolk protein (0.4 μmole). Loadings were 10 μl from 100 μl of 1,2-dichloroethane except for residue 2 (serine) which was 30 μl. The plate was developed with 1,2-dichloroethane–acetic acid (60:7) as recommended in ref. 5.

Fig. 1 shows the ultraviolet (UV) photograph of a TLC plate obtained with the modified method using the extracts from selected cycles of a sequenator analysis of apovitellenin I from the egg yolk of the duck[6]. The PTH-derivatives shown in the photograph correspond to residues 1 (Lys), 2 (Ser), 3 (Ile), 4 (Phe), 5 (Glu), 10 (Asp) and 11 (Trp). The minor spot for residue 11 is due to overlap of residue 10. It is clear that the yield of PTH-(N_ϵ-PTC) lysine is comparable with those of other stable amino acids in marked contrast to the earlier method[1]. Better yields of PTH-tryptophan were also obtained with the modified method. The yellow products obtained with the earlier procedure on conversion of tryptophyl and seryl residues were also evident on this plate. The multiple spots obtained for the seryl residue (cycle 2) are characteristic although there is usually more of PTH-dehydroserine (the heaviest spot close to the lysyl derivative) and less of the other products.

A semi-quantitative comparison can be made of the yields of PTHs using the

TLC scans obtained with a Shimadzu dual wave length scanner[6,7] at 270 and 320 nm. The scans of the first three lanes of the plate shown in Fig. 1 are illustrated in Fig. 2. In agreement with the conclusions from the photograph, the peak heights of the scans at 270 nm show that the yields of PTH-(N_ε-PTC) lysine and PTH-isoleucine at cycles 1 and 3 are similar. For the seryl residue (cycle 2) the scan at 320 nm shows that three of the products also absorb strongly at this wavelength, PTH-dehydroserine being the major one. A heating period of 5–10 min at 140° was found to give best results with the earlier method but with the present method the TLC scans showed that at least 10 min heating at 140° was essential for complete conversion of the ATOs of lysine and tryptophan. Yields of the products were not changed markedly after a 15-min conversion period. The profile for the serine residue changed significantly with changes in the heat conversion time. After a 5-min heating period at 140° the three components absorbing at 320 nm were the major products; on heating for 15 min all the 270 nm bands decreased but there was a concomitant increase in the 320 nm absorption of the PTH-dehydro derivative ahead of PTH-(N_ε-PTC) lysine. Identification of S-carboxymetylcysteinyl residues is unsatisfactory after conversion at 140° and the ATO must be converted at 100° for good results.

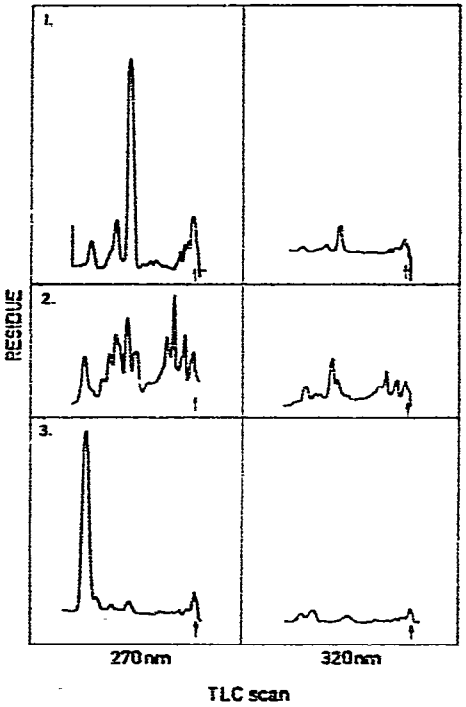

Fig. 2. TLC scans of lanes 1 to 3 of plate shown in Fig. 1 at 270 and 320 nm (sensitivity, × 1). The arrows indicate the position of the origin.

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