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Note

Use of o-phthalaldehyde for detection of amino acids and peptides on thin-layer chromatograms

E. GUNNAR G. LINDEBERG

Institute of Biochemistry, Biomedical Center, University of Uppsala, Box 576, S-751 23 Uppsala (Sweden)

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So far, the most widely used reagent for detection of amino acids and peptides on paper and thin-layer chromatograms has been ninhydrin. However, in many cases, such as the assessment of the purity of synthetic peptides, a more sensitive reagent is desirable. The recently introduced[1–4] fluorescamine {4-phenylspiro[furan-2(3H), 1'-phthalan]-3,3-dione} procedure, which allows detection of most amino acids at the 50-pmole level, meets the requirement of increased sensitivity, but the high cost of this versatile reagent precludes its everyday use in many laboratories.

o-Phthalaldehyde, in the presence of a strong reducing agent such as 2-mercaptoethanol, was shown by Roth[5] to produce highly fluorescent compounds with most amino acids. This reaction was later used for amino acid analysis[6] and was recently extended to the pmole range[7]. The work presented in this paper describes its application to detection of amino acids and peptides on chromatograms and was prompted by the recent finding[7] that, in solution, this method was more sensitive than the fluorescamine procedure. Indeed, *o*-phthalaldehyde turned out to be as convenient and sensitive as fluorescamine when used as a spray reagent.

EXPERIMENTAL

Materials

o-Phthalaldehyde and 2-mercaptoethanol were purchased from Fluka (Buchs, Switzerland) and thin-layer plates precoated with silica gel or cellulose and acetone from E. Merck (Darmstadt, G.F.R.). Triethylamine, also obtained from E. Merck, was dried with potassium hydroxide and re-distilled. Bradykinin and arginine-vasopressin were synthetic preparations from this laboratory.

Determination of detection limits

Aliquots (1-μl) containing 5, 10, 25, 50, 100, 250, 500 and 1000 pmoles, respectively, of amino acid or peptide in 0.01 *M* HCl were spotted on precoated plates, dried at 100° for 30 min and allowed to cool to room temperature. The plates were then treated according to the staining procedure below. The detection limits in Table I refer to the smallest amount of the respective compound giving a visible reaction.

TABLE I

MINIMUM QUANTITIES OF SOME AMINO ACIDS AND PEPTIDES DETECTED

1-μl aliquots containing 5–1000 pmoles were spotted on thin-layer plates, dried at 100° for 30 min, treated with the spray reagents and viewed under UV light (350 nm) after the time indicated. Thin-layer chromatography (TLC) was carried out on silica gel with *n*-butanol–acetic acid–water (4:1:1); front migration, *ca.* 10 cm; dried and visualized as above.

Substance	*Detection limit (pmoles per spot)*								
	Cellulose				*Silica gel*				*After TLC*
	10 min	*1 h*	*2 h*	*1 h, 100°*	*10 min*	*1 h*	*2 h*	*1 h, 100°*	*10 min*
Alanine	250	250	250	250	50	250	500	1000	100
Arginine	10	10	25	25	10	50	100	500	25
Asparagine	250	250	250	250	25	100	250	500	50
Aspartic acid	100	100	100	100	25	100	100	1000	100
Cystine	10	10	10	10	100	250	250	100	250
Glutamine	100	100	100	100	25	100	250	1000	50
Glutamic acid	100	100	100	100	25	100	250	1000	100
Glycine	100	100	100	100	25	250	1000	—	100
Histidine	25	50	50	50	25	100	100	100	25
Isoleucine	100	100	100	100	50	500	500	—	100
Leucine	100	100	100	100	50	500	500	—	100
Lysine	25	25	25	25	25	100	250	1000	50
Methionine	100	100	100	100	50	100	250	1000	100
Phenylalanine	50	50	50	50	50	250	500	1000	100
Proline	—	—	—	250	—	—	—	250	250*
Serine	100	100	100	100	10	250	250	1000	25
Threonine	100	100	100	100	25	250	250	1000	50
Tryptophan	25	25	25	50	50	100	100	100	250
Tyrosine	25	25	25	50	50	500	500	500	100
Valine	25	25	25	50	50	500	1000	—	100
Glycyl-glycine	250	250	250	250	100	500	1000	—	250
Bradykinin	50	50	50	50	50	500	1000	250	100
Arginine-vasopressin	100	100	100	500	250	1000	1000	1000	500

* After heating at 100° for 1 h.

o-Phthalaldehyde staining procedure

The plates were sprayed generously with a solution of 0.1 % *o*-phthalaldehyde and 0.1 % 2-mercaptoethanol in acetone followed 5 min later by 1 % triethylamine in acetone. After 10 min the plates were viewed under a long-wave (350 nm) ultraviolet lamp. The spray reagent was stable for several days when kept at room temperature in a closed bottle protected from light but deteriorated rapidly upon addition of base (1 % triethylamine).

RESULTS AND DISCUSSION

The detection limits and stability of the fluorescent spots given in Table I demonstrate that the *o*-phthalaldehyde procedure allows detection of as little as 50–100 pmoles of many amino acids. In most cases the sensitivity is 2–5 times higher on silica gel, but the aromatic amino acids and, especially, cystine are more easily detected

on cellulose. The difference in stability on the two sorbents is most striking. On silica gel the spots decay already within a few minutes at room temperature while on cellulose they are essentially resistant even to heating. Proline, as expected, shows no reaction immediately after spraying. However, heating at 100° for 1 h makes spots containing 250 pmoles clearly visible. Since this treatment causes the disappearance of most other spots on silica gel these need to be marked in advance. Amino acids substituted with labile N-protecting groups (*tert.*-butyloxycarbonyl, 2-phenylisopropyloxycarbonyl, and *p*-methoxybenzyloxycarbonyl) give no reaction when the plate is dried at room temperature but are detected at the 500-pmole level when heated at 100° for 2 h prior to spraying.

Lowering the concentration of *o*-phthalaldehyde in the spray reagent to 0.01% caused a slight decrease in sensitivity whereas increasing above 0.1% had no effect. The optimal concentration of 2-mercaptoethanol was found to be 1–10 times that of the aldehyde. The composition of the triethylamine spray was not critical, but high concentrations of this reagent (10%) increased background fluorescence. The best results were obtained when the base was applied after the phthalaldehyde–mercaptoethanol solution or was mixed with it immediately before spraying. Pre-treatment of the plates with 1% triethylamine[3,4] did not improve sensitivity. The stability was improved by substitution of triethanolamine for triethylamine, however with somewhat reduced sensitivity. The use of sodium hydroxide as the base (1 part of an alcoholic solution containing 0.1% *o*-phthalaldehyde and 0.1% 2-mercaptoethanol mixed with 1 part of 10% sodium hydroxide in 60% ethanol) which afforded essentially the same sensitivity as the recommended formula did not lead to increased stability.

In conclusion, the *o*-phthalaldehyde reagent is comparable to fluorescamine in sensitivity and convenience and has the additional advantage of being considerably less expensive.

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