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A modified spray reagent for the detection of amino acids on thin layer chromatography plates

Short Communication

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Summary. Identification of amino acids is extremely important for the evaluation of protein structure. Thin layer chromatography is an important tool for detecting amino acids by variety of spray reagents. Among these ninhydrin is the most popular due to its high sensitivity. However, ninhydrin produces the same purple/violet color with most amino acids. A spray reagent with high sensitivity for easy and rapid identification of amino acids on thin-layer plates has been introduced.

Keywords: Amino acids – Ninhydrin – N-Cyanoguanidine

Introduction

Identification of amino acids is most important in the evaluation of protein structure as they are structural units and also found in numerous natural products. Several spray reagents for the selective and non-selective detection of amino acids (Basak and Laskar, 1990; Basak et al., 1993; Devaux and Mesnard, 1971; Distler, 1981; Lorentz and Flatter, 1970; Laskar and Basak, 1988, 1990a, 1990b; Laskar et al., 1991; Mahler and Cordes, 1968; Sinhababu et al., 1994; Stahl, 1969; Wolski et al., 1980) have already been described, among which ninhydrin is mostly used for its remarkable high sensitivity, but it produces very similar colors (violet or purple) with all amino acids except proline and hydroxyproline. Such type of color formation leads to a difficult problem for their identification on thin-layer chromatography plates. In order to resolve this problem, this communication deals with a modification of ninhydrin reagent which produces several distinguishable colors with amino acids and proves useful for their detection and identification on silica gel "G" thin layer plates.

Materials and methods

Apparatus

Chromatographic plates (20 × 20 cm) of thickness 0.1 mm were prepared from silica gel "G" (E. Merck, India) using the Unoplan apparatus (Shandon, London, U.K.). Standard solutions (1 μg/μl) of the amino acids (Sigma, St. Louis, MO, U.S.A.) were made in 0.01 M phosphate buffer (pH 8.0).

Reagents

Reagent I: 0.25% ninhydrin (Sigma, St. Louis, MO, U.S.A.) in acetone.
Reagent II: 0.1% aqueous alkaline (pH 10.5) N-cyanoguanidine (Koch-Light Lab. Ltd., U.K.) solution.

Detection on TLC plates

Standard solutions of amino acids were spotted onto the TLC plates with a graduated micropipette (25 μl) and the plates were subjected to TLC using n-propanol : water (70 : 30) as mobile phase after proper drying. After development plates were sprayed with reagent I and kept at room temperature to complete dry for 40 min in air. TLC plates were then sprayed with reagent II and again dried on air and colors so obtained were noted at this stage. Plates were heated at 110°C for 10 min in an oven and colors were recorded (Table 1) again. For complex mixture two dimentional chromatography is preferred using n-propanol : water (70 : 30) and methanol : chloroform (3 : 1) as mobile phases.

Results

It was observed from Table 1 that various distinguishable colors were developed after spraying with reagent II and the detection limits are also substantially low before (0.1–1.0 μg) and after heating (0.03–0.8 μg). Color development is somewhat different after heating (Table 1).

Discussion

Despite its inability to distinguish between amino acids (except proline and hydroxyproline, all of them give similar violet/purple color) by color reaction, ninhydrin is, owing to its remarkable high sensitivity it provides, still the most widely used reagent for detection of amino acids. The method presented here produces several distinguishable colors before and after heating (Table 1) to differentiate most of the amino acids and, as detection limits are comparable with those obtained using ninhydrin alone, has proved to be useful for the detection as well as identification of amino acids on TLC plates. The simplicity and rapidity of the method is, moreover, comparable with those using ninhydrin.

The chemistry leading to such color formation is uncertain but a possibility may be ascertained as given below:

α-Amino acids produce Ruhemann's violet-blue complexes (Stroev and Makarova, 1989) with ninhydrin even at room temperature and the colors are

Table 1. Color formations of amino acids with ninhydrin in the presence of N-cyanoguanidine on TLC plates

Amino acid	Color observed before heating	Detection limit (μg)	Color observed after heating	Detection limit (μg)
Glycine	Pale Lilac	1.0	Yellowish pink	0.1
Alanine	Reddish pink	0.1	Reddish pink	0.1
Valine	Reddish pink	0.1	Reddish pink	0.1
Leucine	Reddish pink	1.0	Reddish pink	0.3
Isoleucine	Reddish pink	1.0	Reddish pink	0.1
Serine	Reddish pink	1.0	Reddish pink	0.1
Threonine	Deep pink	1.0	Brownish pink	0.1
Aspartic acid	Lilac	0.1	Violet	0.1
Asparagine	Brownish yellow	1.0	Light brown	0.1
Glutamic acid	Reddish pink	0.1	Pinkish violet	0.09
Glutamine	Reddish pink	1.0	Pinkish violet	0.1
Lysine	Pinkish violet	0.1	Brownish pink	0.09
Histidine	Dirty pink	0.1	Dirty pink	0.04
Arginine	Pinkish violet	0.1	Violet	0.1
Phenyl alanine	Pinkish violet	1.0	Dirty pink	0.09
Tyrosine	Light pink	1.0	Rosy pink	0.1
Tryptophan	Light brown	1.0	Brown	0.8
Cysteine	Pale lilac	0.1	Light pink	0.1
Cystine	Light violet	0.1	Reddish pink	0.1
Methionine	Deep pink	1.0	Reddish pink	0.08
Proline	Yellow	1.0	Pinkish yellow	0.1
Hydroxy proline	Brownish yellow	1.0	Greyish yellow	0.03

ultimately changed after spraying with reagent II (N-cyanoguanidine – an electron deficient in – comparable to previous product) due to the possible formation of "Charge-transfer" type complexes.

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