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Spray reagent for the detection of amino acids on thin layer chromatography plates

Short Communication

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Summary. A spray reagent for easy identification of amino acids on thin-layer chromatography plates has been introduced. The reagent is capable of developing various distinguishable colours with many of them. A probable mechanism for such colour formation has also been proposed.

Keywords: Amino acids – 5-Sulphosalicylic acid – Ninhydrin

Introduction

Evaluation of protein structure is greatly dependent on the amino acid detection as they are structural units of proteins and also found in free state in various natural products. Several specific and non-specific reagents are used in thin-layer chromatograms (Basak and Laskar, 1990; Devaux and Mesnard, 1971; Distler, 1981; Lorentz and Flatter, 1970; Laskar and Basak, 1988, 1990; Laskar et al., 1991; Mahler and Cordes, 1968; Stahl 1969; Wolski et al., 1980) for their detection and identification. It is well known that ninhydrin is widely accepted as a non-specific reagent for its remarkable high sensitivity. But the formation of identical colour (purple/violet) with almost all amino acids (except proline and hydroxy proline) leads to a difficult problem for their identification on TLC plates.

Several attempts were made by the authors for the detection of amino acids or group of amino acids directly from their different colours. This communication presents a reagent which will throw some light in this endeavour.

Materials and methods

Apparatus

Thin-layer chromatography plates (20 cm × 20 cm; thickness 0.1 mm) were prepared from silica gel 'G' (E. Merck, India) using the Unoplan apparatus (Shandon, London, U.K.) Standard solutions of amino acids (Sigma, St. Louis, MO, U.S.A.) were made in 0.01 M phosphate buffer (pH. 8.0).

Reagents

Reagent I: 2% 5-sulphosalicylic acid, dihydrate (Aldrich Chemical Co., U.S.A.) in absolute alcohol: 1 M sodium hydroxide solution (1 : 1, v/v).
Reagent II: 0.25% ninhydrin (Sigma, St. Louis. MO. U.S.A.) in acetone.

Detection on TLC plates

Standard amino acid solutions were spotted on 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. Chromatoplates were sprayed with reagent I and heated at 110° for 10 min. Plates were then cooled, followed by spraying with reagent II. TLC plates were then dried in air and colours were noted immediately. Plates were again heated at 110° for 10 min in an oven and colours so developed were recorded (Table 1). For complex mixtures

Table 1. Colour reactions of amino acids on *TLC* plates using sulphosalicylic acid-ninhydrin as spray reagent

Amino acid	Colour observed: Before heating	Detection limit (μg)	Colour observed: After heating	Detection limit (μg)
Glycine	Light orange	0.1	Rosy pink	0.1
Alanine	Pinkish violet	0.2	Violet	0.2
Valine	Light violet	0.2	Violet	0.1
Leucine	Pinkish violet	0.2	Violet	0.2
Isoleucine	Pink	0.2	Deep pink	0.2
Serine	Light pink	0.2	Pinkish violet	0.2
Threonine	Light violet	0.2	Pale rose	0.2
Aspartic acid	Grey	0.2	Greyish violet	0.2
Asparagine	Pale cream	0.2	Pale cream	0.2
Glutamic acid	Pink	0.2	Deep pink	0.2
Glutamine	Lilac	1.0	Pinkish violet	0.5
Lysine	Violet	0.1	Chocolate	0.1
Histidine	Violet	0.2	Grey	0.2
Arginine	Violet	0.2	Chocolate	0.2
Phenylalanine	Greyish pink	0.2	Pinkish violet	0.2
Tyrosine	Pink	2.0	Deep pink	2.0
Tryptophan	Greyish pink	0.5	Greyish pink	0.3
Cysteine	Light grey	2.0	Light grey	2.0
Cystine	Yellowish pink	2.0	Pinkish grey	2.0
Methionine	Light pink	0.6	Pinkish violet	0.2
Proline	Lemon yellow	0.2	Brownish yellow	0.1
Hydroxyproline	Buff colour	0.2	Grey	0.2

two dimensional chromatography is preferred using n-propanol-water (70 : 30) and methanol-chloroform (3 : 1) as mobile phases.

Results

From Table 1, it was observed that the detection limit for this reagent is substantially low (0.1–2.0 μg) and various distinguishable colours were developed. Actually there is no change in detection limit (barring in very few cases) but a slight changes in colour is observed after heating.

Discussion

Formation of similar colour (violet/purple) with all amino acids (except proline and hydroxyproline) by ninhydrin alone possesses a serious problem for their identification. But due to its high sensitivity ninhydrin has wide application for amino acid detection. On the other hand, the original colour produced by ninhydrin itself is changed to various distinguishable colours in presence of sulphosalicylic acid and thereby proves its worth for detection and identification of amino acids rapidly.

The mechanism leading to such colour formation is uncertain but a possibility may be ascertained as follows:

Under the experimental condition, sulphosalicylic acid reacts with an amino group of an amino acid to give a secondary amide which will further react with ninhydrin (when sprayed over the chromatogram) to form an intermediate with the elimination of a water molecule. A stable five membered lactone will be immediately formed due to possible intermolecular rearrangement of the intermediate which generates various colours depending on the nature of R-group of amino acid (the group attached to α-carbon atom of an amino acid). A probable formation of seven membered lactone may be discarded as the formation of ν-lactone occurs with great readyness and predominates over others.

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