Role of Sulfur Compounds in the Detection of Amino Acids by Ninhydrin on TLC Plate

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Abstract

Three new sulfur reagents for specific identification of amino acids on thin-layer chromatography plates have been introduced. These three sulfur containing reagents are capable of developing various distinguishable colors with many of them. A probable mechanism for such color formation is proposed.

Introduction

Several specific and nonspecific reagents have been reported in thin-layer chromatography (TLC) for the detection of amino acids (1–9). Such identification is useful because of the occurrence of these compounds as monomeric units of proteins, in the free state in numerous natural products (seeds and leaves) and as the C-terminal determination of degraded proteins. Ninhydrin, a well-known nonselective color reagent for amino acids, is best used in neutral conditions and produces a purple violet color with all amino acids, except proline and hydroxyproline, because of the presence of secondary cyclic amine groups. The present communication deals with the use of ninhydrin as a spray reagent in presence of three sulfur reagents representing three different class of organic compounds [i.e., thiophenol (aromatic), 2-marcaptoethanol (saturated aliphatic) as well as carbon disulphide (unsaturated aliphatic)]. The most significant aspect of this study is that it readily produces different distinguishable colors on TLC plates. It is very easy and simple to perform.

Experimental

Chemicals and chromatographic equipment

Chromatography plates (0.1 mm) were prepared using silica gel G (E. Merck, Mumbai, India) and an Unoplan apparatus (Shandon, London, U.K.). The samples were spotted on the plates with graduated capillary tubes (5 µL) (Spectrochem, Mumbai, India). *n*-Butanol was used as mobile phase. The standard amino acid solution was made in 0.01M phosphate buffer (pH 7.8–8.0).

Sample preparation

Reagent I consisted of (*a*) 1% thiophenol (Sigma, St. Louis, MO) in acetone, (*b*) 1% 2-mercaptoethanol (Sigma) in acetone, and (*c*) 1% carbondisulphide (E. Merck) in acetone. Reagent II consisted of 0.25% ninhydrin (Sigma) in acetone.

Results and Discussion

Standard amino acid solutions (Sigma) were spotted on TLC plates by graduated capillary tubes (capacity: 5 µL volume) (Spectrochem) and, after TLC, the plates were sprayed successively with the reagents as follows:

The plates were developed in *n*-butanol, dried, and sprayed first with reagent I (a, b, and c differently). After heating at 110°C for 5 min in an oven, the plates were cooled and then sprayed with reagent II. The plates were then air-dried and the colors were observed immediately thereafter. The plates were then heated again at 110°C for 5 min; in the case of reagent I (b), (i.e., 2-mercaptoethanol), the heating time was 10 min. The developed colors were recorded in Table I. Colors developed by various amino acids when thiophenol was used as the spray reagent are shown in Figure 1 (see page 3A). The exact reaction pathways are uncertain, but it may be presumed that at first amino acids formed an additional compound with different sulfur-containing reagents. This additional compound reacted with ninhydrin to form cyclic compounds that were responsible for the development of such different colors. The limit of detection (LOD) of all reagents was 1 ng for all amino acids, except for aspartic acid (in cold), asparagine, and proline, which had LODs of 2 ng when thiophenol–ninhydrin was used as the spray reagent. For carbondisulphide–ninhydrin, the LODs for asparagine and cysteine were 2 ng, whereas for aspartic acid and cystine they were 3 ng.

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Table I. Color Reactions of Amino Acids on Silica-Gel Thin-Layer Plates with Three Spray Reagents*

Amino acid	Thiophenol–ninhydrin	2-Mercaptoethanol–ninhydrin	Carbondisulfide–ninhydrin
Glycine	No color	No color	No color
Alanine	No color	No color	No color
Valine	No color	Light rose	No color
Leucine	Deep violet (deep violet)	Rose	Purple (grey)
Isoleucine	Deep violet (violet)	Pinkish grey	Deep purple (grey)
Serine	Deep blue (deep violet)	Pinkish grey	Deep purple (violet)
Threonine	No color	No color	No color
Aspartic acid	Violet (saffron)	Light grey	Reddish purple (pink)
Asparagine	Saffron (dirty orange)	Light grey	Light orange (orange)
Glutamic acid	Violet (yellowish saffron)	Light saffron	Saffron (violet)
Glutamine	Deep grey (deep grey)	Rose	Saffron (deep violet)
Lysine	Light grey (light grey)	Grey	Light grey (purple)
Histidine	Light violet (yellowish purple)	Deep grey	Deep grey (purple)
Arginine	Grey (purple)	Violet	Grey (grey)
Phenylalanine	Yellowish violet (saffron)	Rose	Yellowish violet (deep violet)
Tyrosine	Light violet (violet)	Faint grey	Light rose (light grey)
Tryptophan	Deep grey (grey)	Light grey	Violet (violet)
Cysteine	Pinkish red (light saffron)	Yellowish grey	Light red (reddish grey)
Cystine	Deep pink (yellowish saffron)	Light violet	Light violet (yellowish violet)
Methionine	Deep violet (deep grey)	Violet	Purple (violet)
Proline	Lemon yellow (pale yellow)	Lemon yellow	Lemon yellow (lemon yellow)
Hydroxyproline	Dirty grey (deep grey)	Orange	Light saffron (deep rose red)

* Colors in parenthesis were observable before the TLC plates were heated.

Conclusion

This method is rapid, easy, and inexpensive by which almost all amino acids can easily be identified by their easily-detectable colors. Moreover, it responded quite well for protein hydrolysate.

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