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Note

Distinction of amino alcohols from amino acids by 4-N,N-dimethylamino-azobenzene-4'-isothiocyanate

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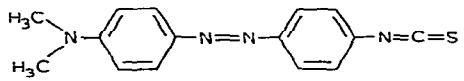
One of the promising chemical methods for the determination of carboxyl terminal of peptides and proteins is the reduction of proteins or esterified proteins, followed by the identification of the released amino alcohols after acid hydrolysis[1–5]. Apart from some disadvantages during the reduction stage, the lack of a simple and sensitive method for identifying the released amino alcohols could account for part of the reason for it not being widely accepted. The use of 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate as a coloured reagent for identifying the amino alcohols is simple and sensitive.



4-N,N-dimethylaminoazobenzene-4'-isothiocyanate

One of the major advantages of employing 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate to identify the released amino alcohols from the C-terminal of peptides is that this reagent forms different coloured products with amino alcohols (blue) and amino acids (red). Coupling amino alcohols and 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate, followed by acid treatment, gives blue 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols on polyamide thin-layer chromatographic (TLC) plates after exposure to hydrogen chloride vapour. However, the same reaction between amino acids and 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate result in the appearance of red 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin amino acids. This property facilitates the identification of amino alcohols in the hydrolyzate without prior separation from the excess amino acids.

MATERIALS AND METHODS

The preparation of 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate was according to our previous report[6]. DL-Alaninolhydrochloride, L-tyrosinol hydrochloride and L-histidinol dihydrochloride were purchased from Sigma, St. Louis, Mo.,

U.S.A. Glycinol was from Mann Research Labs., New York, N.Y., U.S.A. Polyamide sheets were from Chen Chin, Taipei, Taiwan.

The amino alcohol or amino acid (50 nmoles) dissolved in 50 μl of 0.05 *M* sodium hydrogen carbonate–0.1 *M* sodium hydroxide–acetone (100:5:100) buffer was mixed with 25 μl of 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate solution (2 nmoles/μl in acetone). The mixture was stoppered and heated at 50° for 2 h and then dried in a vacuum desiccator. The dried residue was treated with two different acid solutions: (i) 50 μl of 1 *M* hydrochloric acid at 50° for 2 h or (ii) 50 μl of water–acetic acid saturated with hydrogen chloride (1:2) at 50° for 45 min. After the acid treatment, the mixture was dried again and re-dissolved in 50 μl of ethanol for TLC identification.

The techniques and procedures for the micro-TLC identification of 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin amino acids and 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols were essentially the same as those described previously[6,7]. The two solvents used to separate the 4-N,N-dimethylaminoazobenzene-4'-sulphonyl amino acids[8] were employed; water–2-chloroethanol–formic acid (100:60:3.5) for the first dimensional development and benzene–acetic acid (6:1) for the second dimension.

RESULTS

Fig. 1 shows the relative positions of the red coloured 4-N,N-dimethylamino-

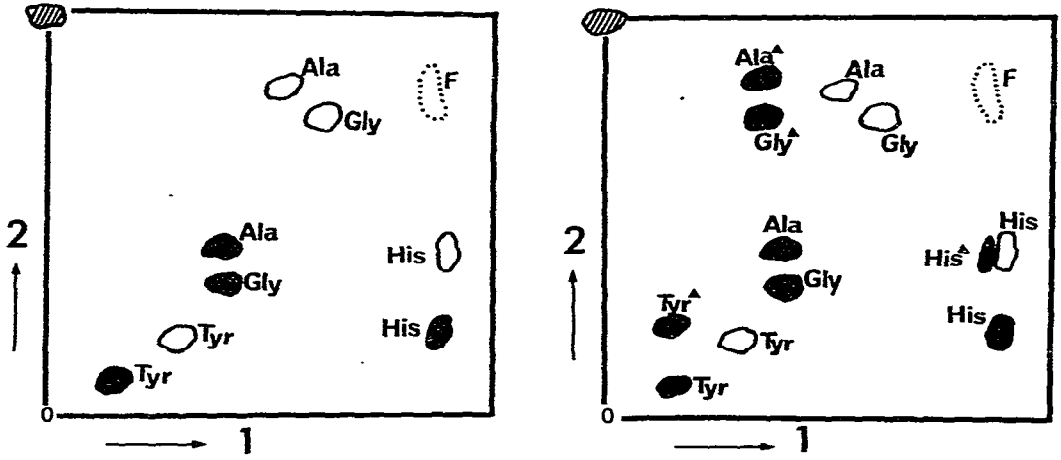


Fig. 1. Two-dimensional separation of red coloured (open) 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin amino acids and blue coloured (solid) 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols. The hatched spot is unchanged 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate and the dotted area F is the hydrolyzed product, 4-N,N-dimethylamino-4'-aminoazobenzene (greenish red). Solvents employed for the first- and second-dimensional separation are described in the text.

Fig. 2. The relative positions of the second blue products (denoted by ▲) derived from 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols after treatment with water–acetic acid saturated with hydrogen chloride (1:2). The nature of these second blue spots is not identified. However, they are believed to be the esterified derivatives between acetic acid and 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols. For details, see text.

azobenzene-4'-thiohydantoin derivatives of glycine, alanine, tyrosine and histidine (open) to the blue coloured 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl derivatives of glycinol, alaninol, tyrosinol and histidinol (solid). Two acid conditions which convert all the 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino acids to their corresponding thiohydantoin derivatives were used to treat the 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols:

(1) Treatment with 1 *M* hydrochloric acid at 50° for 1 h resulted in only partial destruction of the blue 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohol to the purple 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate and the greenish red 4-N,N-dimethylamino-4'-aminoazobenzene (Figs. 1 and 2).

(2) Treatment with water–acetic acid saturated with hydrogen chloride (1:2) at 50° for 45 min converted part of the blue 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohol to another unidentified blue product which was probably the acetyl ester of the 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohol (Fig. 2). In both reactions, however, there were no additional red products generated.

DISCUSSION

A number of methods for the separation of amino alcohols or amino alcohol derivatives were available[1–5,9–11]. However, they all suffer from either low sensitivities, tedious procedures or interferences from amino acid. The sensitivity of our proposed method is in the picomole range. There is no need to extract amino alcohols from the excess amino acid since they form different coloured derivatives with 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate and in fact, those red 4-N,N-dimethylaminoazobenzene-4'-thiohydantoin amino acids can be used as the markers to identify the blue 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols.

The nature of the second blue product after the water–acetic acid treatment of 4-N,N-dimethylaminoazobenzene-4'-thiocarbamoyl amino alcohols was not identified. However, we believe that those second blue products were the esterified derivatives. They presented very similar TLC behaviour to their original amino alcohol derivatives. Indeed, we prefer the water–acetic acid treatment, because the appearance of two blue spots facilitates their TLC identifications.

Although only four amino alcohols were illustrated, the result indicates that it is possible to achieve a complete separation of all the amino alcohols derived from their corresponding amino acids. 4-N,N-dimethylaminoazobenzene-4'-isothiocyanate, first devised to improve the ease and sensitivity of the N-terminal sequence determinations, has been found to be a very versatile reagent in a series of our studies[6,7,12]. The presented results demonstrated that it could also be a very suitable reagent for identifying the amino alcohols during the C-terminal determination.

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