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Note

Improved procedure for peptide characterization using thin-layer chromatography and a fluorescamine indicator

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Fluorescamine and *o*-phthalaldehyde are highly sensitive and rapidly reacting reagents used to detect peptides and amino acids[1,2]. The optimal conditions for the intensity and stability of fluorescent spots have already been reported[3,4], and fluorescamine has also been utilized in the quantitative analysis of amino acids[5].

We have extended the fluorescamine technique to the detection of tryptic peptides on thin-layer chromatograms. These visualized fluorescent tryptic peptides could be further characterized by the determination of their amino acid composition using a conventional amino acid analyzer. The recovery of the N-terminal amino acid of a once-fluorescamine-stained peptide has been shown to be as good as that of a purified identical peptide without staining. We here report an improved procedure for peptide characterization using fluorescamine staining.

EXPERIMENTAL

Fluorescamine (Fluram), 4-phenylspiro(furan-2(3*H*),1′-phthalan)-3,3-dione (Hoffmann-La Roche, Basel, Switzerland) was used as a freshly prepared solution in acetone. Silica gel 60 thin-layer plates (without fluorescent indicator; 20 × 20 cm, thickness 0.25 mm) were purchased from E. Merck (Darmstadt, G.F.R.).

The lyophilized tryptic digests were dissolved in water and 1–1.2 mg of protein were applied to a silica gel plate. Separation in the first dimension was achieved by electrophoresis in 2 *M* acetic acid–0.6 *M* formic acid (pH 1.9) at 50 V/cm for 60 min. Separation on the second dimension was by ascending chromatography in pyridine–acetic acid–*n*-butanol–water (40:14:68:25) for 15 h.

First the plates were sprayed with 0.2 *M* borate buffer (pH 8.0)–acetone (1:1) and then with a solution of fluorescamine in acetone (50 mg per 100 ml). Fluorescent spots were scraped off the plates during the first 3–4 min after the tryptic peptides had been visualized under long-wavelength (366-nm) UV light. The fluorogenic reaction with scraped tryptic peptides was stopped by adding 5.7 *N* hydrochloric acid and then hydrolyzing at 110° for 20 h.

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RESULTS AND DISCUSSION

In the course of sequence analysis of the α subunit of DNA-dependent RNA polymerase from *Escherichia coli*, we purified a tryptic peptide by conventional methods, using column chromatography and then silica gel thin-layer chromatography. The amino acid composition was determined using the amino acid analyzer. In a separate experiment, we isolated an analogous peptide from tryptic fingerprints on silica gel thin-layer chromatograms after staining either with fluorescamine or with ninhydrin. The amino acid composition of samples of this peptide isolated in the three ways described are compared in Table I. Although the N-terminal amino acid determined by the dansyl chloride technique in each case was threonine, the stoichiometric amount of threonine after staining with ninhydrin was 30% lower than the value obtained after fluorescamine staining. Thus, the fluorogenic reaction with the N-terminal amino acid does not have an adverse effect on the amino acid composition.

TABLE I

AMINO ACID COMPOSITION OF AN IDENTICAL TRYPTIC PEPTIDE

The values represent the number of amino acid residues per peptide. The background of a silica gel blank was deducted from the amount of each amino acid residue. Values for threonine were extrapolated to zero time.

Amino acid	*Purified peptide without staining*	*After staining with fluorescamine*	*After staining with ninhydrin*
Lysine	1.0	1.0	1.2
Threonine	1.0	1.0	0.7
Glutamic acid	2.0	2.0	2.2
Valine	0.9	0.8	0.9
Leucine	2.1	2.2	2.0

Although the fluorescamine reagent is known not to react with secondary amino acids such as proline and hydroxyproline, the tryptic peptides of various proteins containing these residues can still be characterized, since trypsin does not cleave lysine–proline or arginine–proline bonds. For example, it was used to find an amino acid substitution in the α subunit of a mutant RNA polymerase[6], or for the investigation of the phylogeny of lactate dehydrogenase molecules[7].

The fluorescamine-treated tryptic peptides could be further purified by rechromatography on thin-layer plates and, after a second fluorescamine spraying, it is possible to repeat the analysis as described above.

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