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The detection of free and N-protected peptides

The detection of cystein peptides[1,2] as well as various lactams[3,4] on thin-layer chromatograms, either by exposure to iodine vapors or by spraying with iodine solution, has been described. Recently, the same method was used for the detection of various peptides[5]. These findings do not give any data about the quantitativity or the limitation of the method.

We would like to report here some of our findings regarding this method. Exposing paper or thin-layer chromatograms of free and protected peptides to iodine vapors results in the formation of yellow-brown spots. These spots disappeared after being in the free atmosphere for some time but reappeared upon a second exposure.

0.1–0.2 μmole of free peptide can be detected on thin-layer chromatograms (using 20 cm × 5 cm glass plates coated with a 0.25 mm layer of Silica Gel G, Merck, Darmstadt, Germany), while 0.07–0.1 μmole can be detected on paper chromatograms (using Whatman No. 1 paper). The following peptides have been detected: glycylvaline, glycylglycine, alanylalanine, glycylphenylalanine, prolylglycine and phenylalanylalanylglycine, using *n*-butanol–acetic acid–5% NH_4OH–water (6:1:1:2) and butanol–acetic acid–water (4:1:5) as solvent systems. The same sensitivity was found in the case of N-protected peptides. The following derivatives have been detected (using the same solvent systems as above): cyclodiglycyl, cyclophenylalanylglycyl, cycloalanyl-ε-*tert.*-butyloxycarbonyllysyl, benzyloxycarbonylglycylglycine ethyl ester, benzyloxycarbonylserylglycine ethyl ester, benzyloxycarbonyl-β-chloroalanylglycine amide, *tert.*-butyloxycarbonylprolylglycine and *tert.*-butyloxycarbonylalanylglycine. Presence of the hydrazide group increases the sensitivity over 10-fold thus 0.003–0.007 μmole of benzyloxycarbonylglycine hydrazide, benzyloxycarbonylglycylglycine hydrazide and *tert.*-butyloxycarbonyl serine hydrazide were detected.

The sensitivity and simplicity of this detection method enabled us to use it routinely instead of the chlorine method[6].

As other nitrogeneous compounds could be detected by this method (*e.g.* N,N'-dicyclohexylurea, N-acyl-N,N'-dicyclohexylurea, N,N'-dicyclohexylcarbodiimide, N-hydroxysuccinimide) we now use this method to follow up the course of the peptide synthesis reaction as well as to check the purity of complex peptides obtained by the N-hydroxysuccinimide or the N,N'-dicyclohexylcarbodiimide method. This method was adapted to a spot test on paper and is also used by us for the detection of peptides and peptide derivatives eluting from columns.

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