IDENTIFICATION OF TRYPTOPHAN BY EDMAN'S METHOD IN COMBINATION WITH DANSYLATION

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The widely known method of Edman in combination with dansylation has not previously permitted the identification of the tryptophan residue because of the degradation of dansyl[DNS]-tryptophan on acid hydrolysis. We have studied the conditions for the hydrolysis of DNS-tryptophan in the presence of a reducing agent – thioglycolic acid.

Like tryptophan [1], DNS-tryptophan is stable on hydrolysis with 5.7 N hydrochloric acid in the presence of 2% of thioglycolic acid. A sample was hydrolyzed in a volume of 0.1 ml (vacuum of 0.050 mm, 20 h, 110°C). The hydrolyzate was evaporated at 60°C, and the dry residue was successively treated with benzene, chloroform, and ether (five times each) and was kept in a desiccator over solid alkali for 20 h. The dansyl-tryptophan was identified by thin-layer chromatography on KSK-3 silica gel in a fixed layer [2]. Chromatography showed an intense spot of DND-tryptophan, and small amounts of DNS-glycine and DNS-amide, which showed the partial degradation of the DNS-tryptophan. Treatment of the sample with organic solvents is necessary to eliminate traces of thioglycolic acid, which distorts the shape of the spots and the mobility of the DNS-(amino acid)s.

The method was illustrated on the synthetic dipeptide Gly-Trp and two pepsin peptides which we obtained from a thermolysin hydrolyzate of a B-2 peptide fraction [3]:

Leu-Asn-Trp-Val-Pro,Leu-Trp-Asp-Gln-Cly

LITERATURE CITED

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