DETERMINATION OF THE C-TERMINAL SEQUENCES IN DANSYL-PEPTIDES

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In the determination of the C-terminal sequence in peptides using carboxypeptidases and the subsequent identification and quantitative determination of the amino acids split off by high-voltage paper electrophoresis [1], it is frequently difficult to distinguish amino acids from shortened peptides on the electrophoregram. In the present paper it is shown that in the determination of the C-terminal sequence of dansyl-peptides it is easily possible to distinguish the shortened peptides formed as the result of the action of carboxypeptidases from amino acids on the electrophoregram. In addition, one and the same sample of peptide can be used not only for the determination of the C-terminal sequence in it, but also for the determination of the N-terminal residue in the form of the DNS-derivative.

The peptide (0.02-0.05 μmole) was dissolved in 30 μl of N-ethylmorpholine buffer with pH 8.5, and 30 μl of DNS-Cl (2.5 mg/ml) was added. The mixture was kept at 37°C for 2 h to convert the excess of DNS-Cl into the sulfonic acid, and then 0.2 ml of the N-ethylmorpholine buffer and 30-40 μl of carboxypeptidase at a concentration of 1 mg/ml were added. After incubation for 4-6 h, the volatile components were eliminated in a vacuum desiccator, and the residue was dissolved in water and subjected to high-voltage electrophoresis and to a quantitative determination of the amino acids split off at pH 1.7 [1]. The residue was again dried and was hydrolyzed in 50 μl of 5.7 N HCl at 105°C for 20 h. The N-terminal amino acid in the form of the DNS derivative was identified by high-voltage electrophoresis at pH 1.9 and 4.4 [2].

The results of the determination of the C-terminal sequence and of the N-terminal residue of a number of peptides obtained after the chymotrypsin hydrolysis of the reduced and carboxymethylated polyhedral protein of Borrelinavirus bombycis are given below:

Peptide C	Amino-acid composition	N-Terminal amino acid	C-Terminal sequence
13	Asp, Glu, Pro, Ala, Val, Leu (Ile), Phe, Tyr	Ala-	-Val—Tyr
27	Asp, Leu (Ile), Tyr	Asp-	-Tyr
28	Asp, Glu, Phe	Glu-	-Phe
33	Asp, Ala, Val, Leu_2	Leu-	-Ala-Leu
36	Thr, Tyr	Thr	-Tyr
37	S-CM-cys, Tyr, Arg	Arg-	-Tyr
38	Asp_2, Tyr, Lys	Asp-	-Tyr
39	Val, Phe	Val-	-Phe
40	Thr_{1-2}, Phe	Thr-	-Phe
41	Thr, Pro, Gly, Ala, Tyr, Phe	Phe-	-Thr-Tyr

LITERATURE CITED

1. V. M. Kavsan, N. V. Taikova, and S. B. Serebryanyi, Ukr. Biokhim. Zh., 41, 601 (1968).
2. W. R. Gray, Methods in Enzymology, 9, 139 (1967).

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