CHROMATOGRAPHIC SEPARATION OF DANSYLATED IODO(AMINO ACID)S

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The study of the structures of iodine-containing peptides and proteins is complicated to a considerable degree by the fact that for the performance of analysis by stepwise degradation using Edman's method [1-3] one must have as standards the dansyl (Dns) derivatives of all the amino acids, including iodo(amino acid)s.

The difficultly in the synthesis of Dns derivatives of iodo(amino acid)s consists in the fact that they are insoluble at the pH values at which dansylation is carried out. In view of this, the iodo(amino acid)s — triiodotyrosine (T_3) and thyroxine (T_4) — are dissolved in a mixture of 70% ethanol and 0.2 N $NaHCO_3$, while diiodotyrosine (DIT) is dissolved in 0.2 N $NaHCO_3$. We have investigated the following variants of the Dns derivatives of amino acids: 1) iodo(amino acid)s + dansylation; 2) Dns-iodo(amino acid)s + hydrolysis with 5.7 N HCl; and 3) iodo(amino acid)s + hydrolysis with 5.7 N HCl + dansylation.

Dansylation was carried out by the generally adopted method with a slight modification. The Dns derivatives of the iodo(amino acid)s were identified by thin-layer chromatography on polyamide plates, using the following solvent systems: 1) 1.5% formic acid; 2) benzene—acetic acid (9:1) and 3) ethyl acetate—methanol—acetic acid (10:1:1). The Dns derivatives of the iodo(amino acid)s migrate in systems 2 and 3.

The behavior of the Dns-iodo(amino acid)s on chromatography and the nature of the spots were identical in all the series of experiments. The Dns derivative of DIT on a polyamide film was located beyond the Dns derivative of di-tyrosine. The Dns derivatives of thyroxine and triiodotyrosine were detected in the form of two spots with pale blue fluorescence in UV light but differing in mobility.

Hydrolysis did not affect the distribution of the spots on the polyamide film. However, the presence of two spots for the Dns derivatives of T_3 and T_4 is apparently explained either by the appearance of a by-product as the result of the whole process of hydrolysis and dansylation or by the partial deiodination of these amino acids.

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