

ACETYLATION OF OX THYROGLOBULIN WITH
2-N-DIACETYLAMINOCYCLOHEX-2-ENONE

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Diacetylamino-cyclohex-2-enone (DACH) is capable of acetylating free α, ω -diamino acids with the formation mainly of $N\omega$ -acetyl derivatives and being converted into ACH [1]. DACH has been used for acetylating the NH_2 groups of lysine residues in porcine pepsinogen [2]. When pepsinogen was treated with DACH for 2 h, practically all the lysine residues were converted into $N\omega$ -acetyllysine residues. However, such acetylation of pepsinogen by DACH does not appreciably affect the activation of pepsinogen and the activity of pepsin.

In the present work, DACH has been used to acetylate thyroglobulin isolated from cattle thyroid gland by the fractionation of salt extracts of thyroid tissue on a column of Sephadex G-200 [3, 4].

The thyroglobulin was acetylated in 0.1 M phosphate buffer at pH 9.0. DACH was added to a solution of thyroglobulin in an amount of 1.8 mole of DACH per mole of (Lys + Arg). Incubation was carried out at 35-37°C for 1 h. The modified thyroglobulin was subjected to gel filtration on a 2.5 × 45 cm column containing Sephadex G-25 to free it from unchanged DACH and the ACH produced.

Since the acetylation of lysine or arginine residues must lead to a change in their amounts in the protein, acetylation was checked by analyzing the amino-acid composition, which was done on a "Khronom-1200E" amino-acid analyzer. The protein was hydrolyzed with 6N HCL at 105°C for 24 h. The evaporated hydrolyzate was dissolved in citrate buffer, pH 2.2.

The amino-acid analysis of the acetylated thyroglobulin showed a decrease in the number of arginine residues in it by 23-25% as compared with the intact protein, while the number of lysine residues remained practically unchanged.

The result obtained shows that, in proteins, DACH can acetylate arginine residues. The retention of the number of lysine residues in thyroglobulin after its acetylation by DACH apparently shows the absence of access of the modifying agent to them.

Sh. Yunukhanov took part in the determination of the amino-acid composition of the thyroglobulin.

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