ACETYLATION OF PORCINE PEPSINOGEN WITH 2-(DIACETYLAMINO)CYCLOHEX-2-ENONE

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It has been shown previously that 2-(diacetylamino)cyclohex-2-enone (DACH) selectively acetylates the terminal amino groups of α,ω-diamino acids, the reaction taking place under exceptionally mild conditions [1].

We have used this reagent to acetylate the ε-NH_2 groups of the lysine residues in porcine pepsinogen in order to study the change in the activation of the zymogen modified in this way.

The porcine pepsinogen was isolated by chromatography on DEAE-cellulose [2, 3]. Acetylation was performed at room temperature for 2 h, the pH of the medium being checked continuously. A solution of 36-45 mg of DACH in 1 ml of 0.01 M Na_2HPO_4 was added to a solution of 12-15 mg of the pepsinogen preparation in 2 ml of 0.1 M Na_2HPO_4, and the pH of the reaction mixture was brought to 9.0 by the addition of the necessary amounts of 1 N NaOH. After 30 min, 1 h, and 2 h, 1-ml samples were taken from the reaction mixture, 4 M sodium acetate (pH 5.0) was added to a pH of 7.0-7.5 to stop the reaction, and dialysis was carried out against distilled water (pH 7.0-7.5) to eliminate the reaction products. For each of the samples taken, the activity of the pepsinogen at pH 2.0 in the hemoglobin cleavage reaction [4] was determined and the number of free ε-NH_2 groups of lysine residues was determined by dinitrophenylation [5, 7]. The amount of protein was determined by Lowry's method [6]. The water-soluble DNP-amino acids were separated and identified by thin-layer chromatography (Silufol U_{254} in the butanol–acetic acid–ammonia system). The numbers of free ε-NH_2 groups (calculated to one mole of protein) for the first two samples taken were 2 and 1. Pepsinogen treated with DACH for 2 h contained practically no free ε-NH_2 groups. In control experiments, the number of ε-NH_2 groups of lysine residues was 10. The degrees of activation of the pepsinogen for all three samples and for a control sample were the same. Thus, it has been established that even the complete acetylation of the ε-amino groups of pepsinogen with DACH has no appreciable effect on the activation of pepsinogen and the activity of the pepsin.

There is information in the literature concerning a decrease or even the complete loss of the capacity for activation of porcine pepsinogen after its treatment with certain chemical reagents: N-acetylimidazole [7], succinic anhydride [8], anhydrides of N-carboxyamino acids [8], and sodium cyanate [9]. The loss of the capacity for activation in acetylated pepsinogen in an acid medium (pH 2) and the change in the constants of the optical rotatory dispersion [8, 9] permitted the authors concerned to speak of a definite value of the ε-NH_2 groups of the lysine residues in stabilizing the native structure of pepsinogen. It is quite possible that the change in the activation of pepsinogen caused by the use of the reagents mentioned is connected with side effects brought about by the groupings introduced (succinylation or the action of anhydrides of N-carboxyamino acids) or by the acetylation of other functional groups (N-acetylimidazole).

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