CHEMICAL MODIFICATION OF PORCINE PEPSIN WITH DICYCLOHEXYLCARBODIIMIDE LABELLED WITH TRITIUM

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Water-soluble carbodiimides which are capable of reacting with carboxy groups, converting them into the corresponding N-acylurea derivatives or, in the presence of amines, into amides are widely used for the modification of protein [1].

Dicyclohexylcarbodiimide (DCC), which is widely used in peptide chemistry [2] has hitherto been used only to a limited extent for the modification of proteins [3, 4] which is apparently due to the low availability of the labelled reagent. Nevertheless, the use of labelled DCC will open up new possibilities for the modification of proteins: this reagent does not contain ionogenic groups the presence of which can complicate the modification process, and it should possess an increased affinity for the hydrophobic sections of protein molecules.

For the modification of porcine pepsin we chose DCC labelled with tritium, i.e., DCC-T that we had obtained with a specific radioactivity of 2.4 μCi/mmole and a radiochemical purity of 98%. To perform the reaction, 3.7 mg (18 μmole) of DCC-T was dissolved in 0.5 ml of ethanol, and 4.5 ml of an aqueous solution containing 5.2 mg (0.15 mmole) of porcine pepsin was added. The slightly opalescent mixture was left at 20°C and pH 5.3-5.4 for 24 h. The dicyclohexylurea that had precipitated was filtered off. It had mp 230-232°C (literature data: mp 231-232°C [5]) and had the same specific radioactivity as the initial DCC-T.

The excess of DCC-T with respect to the pepsin had fallen from 120-fold at the beginning of the reaction to 50-fold in the filtrate. The filtrate was deposited on a 140-ml column of Sephadex G-25 Superfine, and gel filtration was performed in an aqueous solution, the amount of protein in the eluate being followed by UV absorption at 280 nm and by the radioactivity of aliquots of the solution, which was measured in a liquid scintillation counter. The fractions containing the pepsin were rechromatographed under the same conditions, which ensured the complete separation of low-molecular-weight radioactive products. The modified pepsin contained 2.28-2.84 residues of DCC per mole of protein, while its proteolytic activity with respect to hemoglobin had fallen to 22-30% of the initial activity.

It has been shown previously [6] that porcine pepsin is capable of adding several residues of a water-soluble carbodiimide without loss of activity. Thus, in its action on pepsin DCC differs substantially from the water-soluble carbodiimides, and it may be assumed that this difference is due to the specific interaction of the hydrophobic DCC molecule with the carboxy groups located in the molecule of pepsin close to the hydrophobic zone of the binding of the substrates.

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