# SPECIFIC INHIBITIONS OF THE ACID PROTEINASE FROM *Aspergillus awamori* BY DICYCLOHEXYLCARBODIIMIDE

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We have used dicyclohexylcarbodiimide labelled with tritium (DCC-T) [1] to modify the acid proteinase from *Aspergillus awamori* [2]. In order to increase the solubility of the DCC-T, the reaction was performed in 30% ethanol.

The acid proteinase (15 μM) was treated at pH 5.9-6.0 with DCC-T at various concentrations (from 60 to 675 μM; 4- to 45-fold excess with respect to the protein) for 5 h. Preliminary experiments showed that in 30% ethanol the fall in the proteolytic activity of the enzyme with respect to hemoglobin had ceased after only an hour (apparently, because of the absence of DCC-T, consumed in reaction with the protein and by hydration). The dicyclohexylurea formed as a result of the reaction was separated by gel filtration in an aqueous solution of Sephadex G-25. The amounts of proteinase in the fractions containing protein were determined from their absorption at 280 nm, using a molecular weight of the enzyme of 35,000 and $\varepsilon_{280}$ = 46,500 [2], and the inclusion of DCC-T in the protein was determined from the radioactivity of the solution. In some cases, the protein fractions were rechromatographed under the same conditions.

At a 20- to 45-fold excess of DCC-T, the modification was specific and led to the inclusion of one DCC-T residue in the macromolecule and to the complete inactivation of the enzyme (Fig. 1). A control experiment showed that the inactivation of the proteinase on incubation in 30% ethanol in the absence of DCC-T did not exceed 5-10%. On isoelectric focusing in polyacrylamide gel, the isoelectric point of the native enzyme (pI 4.2) did not change when one DCC-T residue was included in its molecule which shows the absence of anomalously acidic carboxy groups in the proteinase. Under these conditions of modification, porcine pepsin changes its isoelectric point from pI 2.1 to 4.4.

The complete inactivation of the enzyme on the inclusion in its molecule of only one residue of the inhibitor and also the retention of the specificity with fairly large excesses of DCC-T permit the assumption that the molecule of the *Aspergillus awamori* proteinase has a carboxy group with a high reactivity which apparently plays the decisive role in the functioning of the catalytically active center of the enzyme.

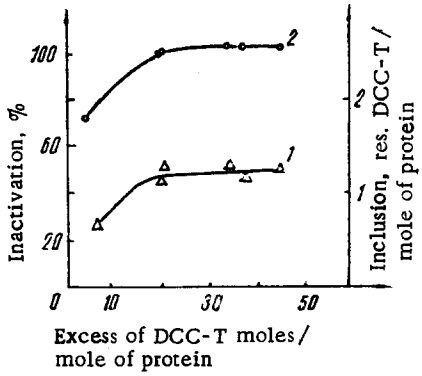


Fig. 1. Dependence of the inclusion of DCC-T (1) and of the inactivation of the acid proteinase (2) on the excess of DCC-T. Concentration of protein 1 mg/ml; reaction in 30% ethanol for 5 h; the proteolytic activity was determined from the cleavage of hemoglobin.

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Our results, and also those obtained previously on the complete inactivation of the proteinase by the addition of 1-2 residues of the water-soluble N-(N,N-dimethylaminopropyl)-N'-(p-phenylazophenyl)carbodiimide [3, 4], permit carbodiimides to be considered as specific inhibitors of the acid fungal proteinase from *Aspergillus awamori*.

## LITERATURE CITED


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