SPECIFIC INACTIVATION OF THE ACID PROTEINASE FROM *Aspergillus awamori* BY A COLORED WATER-SOLUBLE CARBODIIMIDE

G. N. Balandina, E. N. Lysogorskaya, and V. M. Stepanov

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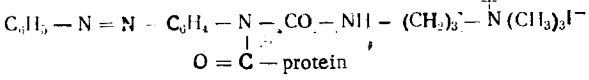
We have used the colored water-soluble N-(dimethylaminopropyl)-N'-phenylazophenylcarbodiimide methiodide [1] to modify the acid proteinase from Aspergillus awamori [2]. For this carbodiimide and the urea corresponding to it, λ_{max} is 355 nm (Fig. 1). Incubation of the proteinase with various concentrations of carbodiimide gave protein derivatives with an absorption maximum at 355 nm. The most probable product of the reaction may be the corresponding substituted urea acylated at the least basic nitrogen atom:



In favor of this assumption is the fact that the model compounds p-phenylazophenylsuccinimide and 5-phenyl-3-phenylazophenylhydantoin have λ_{max} 325 nm, like the modified protein [1].

The acid proteinase (28.6 μmole) was treated in aqueous solution at pH 5.6 with various concentrations of carbodiimide (from 28.6 to 856 μmole) for 1 h with subsequent gel filtration on Sephadex G-25. The number of residues of the reagent in the protein molecule was calculated by using a value of ε_M^{325} of 22,000. The treatment of the enzyme with an equimolar amount of the carbodiimide led to the inclusion of 0.6 of a residue in the protein molecule, causing 60% inactivation. With a twofold excess of the carbodiimide, one residue of the reagent was included in the enzyme molecule, and the activity of the protein was lost com-

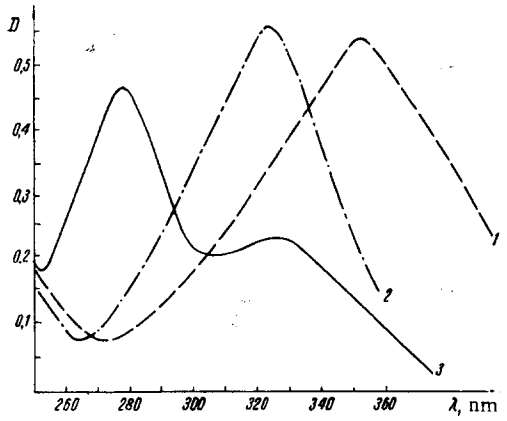


Fig. 1. IR absorption spectra of N-(dimethylaminopropyl)-N'-phenylazophenylcarbodiimide (1), 5-phenyl-3-phenylazophenylhydantoin (2), and the proteinase from Asp. awamori modified with one carbodiimide residue (3).

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pletely. The modified protein was eluted as a single peak when chromatographed on DÉAÉ-cellulose (pH 5.6, concentration gradient of NaCl from 0 to 1 M). The colored water-soluble carbodiimide is apparently capable of selectively blocking some functional group that is essential for the activity of the enzyme.

A pepsin enzyme similar to the acid proteinase from Aspergillus awamori is not inactivated completely even by the inclusion of six molecules of the carbodiimide [1]. Thus, an inhibition reaction specific for an acidic fungal proteinase has been discovered for the first time.

LITERATURE CITED

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2. L. S. Lobareva, G. G. Kovaleva, M. P. Shimanskaya, and V. M. Stepanov, Biokhimiya, 37, 198 (1972).