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Cell bound proteolytic enzymes of Arthrobacter

Extracts of many bacteria catalyze the hydrolysis of various proteins and peptides, but few cell-bound proteinases and peptidases have been purified and characterized. Proteolytic organisms must obviously possess a variety of enzymes, both extracellular or surface-located proteinases and cell-bound peptidases, to be able to utilize exogenous proteins. However, proteolytic enzymes probably also play an important role in intracellular protein turnover and in the formation of cell walls.

For some years we have studied the formation and properties of an extracellular proteinase, which is formed by a proteolytic strain of Arthrobacter[1-3]. This enzyme is only found in the cell-free culture medium from which it can be isolated. The cell extract contains several other proteolytic enzymes, and we have now carried out some fractionation experiments on these. We have found an amino peptidase of broad specificity, a specific proline iminopeptidase and an imidodipeptidase as well as a caseinolytic endopeptidase.

When washed cells of Arthrobacter, grown either in complex media containing protein or in a synthetic salts medium, are disintegrated by sonication, an extract with casein-digesting activity is obtained. All this activity remains in the supernatant after centrifugation at 105 000 × g for 90 min. The solubilized enzyme does not clot milk which is a characteristic property of the extracellular proteinase.

Fig. 1 illustrates a gel filtration experiment in which an extract of sonicated Arthrobacter cells was passed through a column of Sephadex G-100. A peak of caseinolytic activity was eluted with the high molecular weight material which left the column with the void volume. 0.7–0.9 void volume later, another proteinase peak was eluted, but no activity was obtained in later fractions where the extracellular proteinase (mol. wt. 23 000) was eluted if this was gel filtered[2]. When the void volume peak was rechromatographed, the caseinolytic activity was obtained in a much broader region, indicating that part of the cell-bound proteinase was liberated as a polymer or complex which might dissociate into smaller molecules. Metal-activating agents had no effect on the stability or activity of the extracellular proteinase, whereas the cell bound proteinase was rapidly inactivated by $1 \cdot 10^{-4}$–$1 \cdot 10^{-5}$ M EDTA. Attempts to isolate pure cell-bound proteinase are in progress so that its chemical and catalytic properites can be compared with those of the extra-

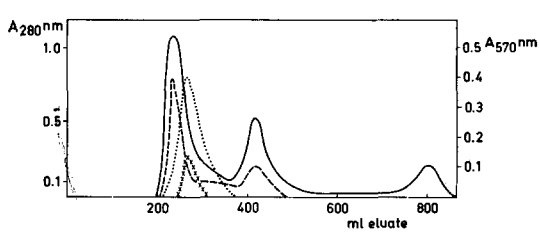

Fig. 1. Gel filtration on Sephadex G-100 of an extract of Arthrobacter in 0.1 M Tris–HCl (pH 8.0). ———, material distribution ($A_{280\ nm}$); — — —, caseinolytic activity; · · · ·, Leu–Gly hydrolyzing activity; × × ×, Pro–Gly hydrolyzing activity.

cellular enzyme. Gel diffusion experiments with antisera prepared against the extracellular proteinase and the cell extract, respectively, have failed to demonstrate any immunochemical relation between this enzyme and the cell-bound proteinase. It therefore does not appear likely that the latter enzyme is a precursor of the extracellular proteinase. It is more probable that it has a function in intracellular protein turnover.

A large variety of simple peptides were hydrolyzed by extracts of Arthrobacter cells grown either in the presence or absence of peptides in the culture medium. The different enzymes which catalyze their hydrolysis could not be separated by gel filtration since they were of very similar molecular size (Fig. 1). Chromatography of cell extracts, on DEAE-Sephadex at pH 8 with stepwise increasing NaCl concentration, gave very complex diagrams with poor resolution between the peptidases. Much better results were obtained on DEAE-cellulose at pH 5 with linear salt gradient. For the experiment illustrated in Fig. 2, the crude cell extract was first

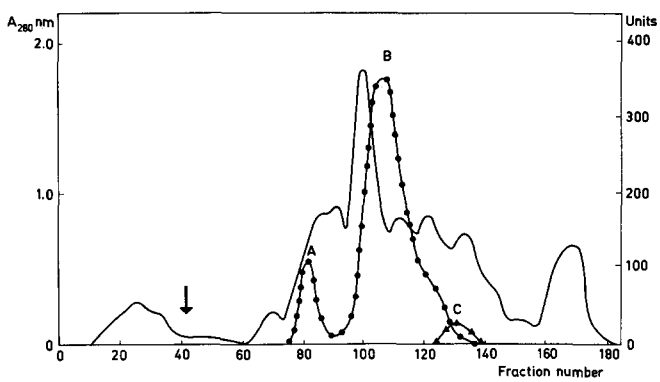

Fig. 2. Chromatography on DEAE-cellulose of an Arthrobacter extract in 0.05 M sodium acetate–0.028 M acetic acid buffer (pH 5.0). The column was developed with a gradient of increasing concentration of the same buffer at the point indicated by the arrow. Fraction volume, 5.5 ml. ———, material distribution ($A_{280\ nm}$); ●—●, activity against Pro–Gly; ▲—▲, activity against Ala–Pro.

dialyzed against the starting buffer which produced a large amount of inactive precipitate. Three different peaks of peptidase activity were obtained in the eluted fractions. The first peak (A) hydrolyzed Pro–Gly, Pro–Leu and, very slowly, poly Pro, but not Ala–Pro. The second peak (B) hydrolyzed Pro–Gly, Pro–Leu and a variety of other peptides, such as Leu–Gly–Gly, Leu–Gly, Gly–Leu, Gly–Gly, and Gly–Ser, but not Ala–Pro. Ala–Pro and Gly–Pro were only hydrolyzed by a few fractions (Peak C), but no activity could be detected against several peptides containing the sequence X–Pro–Y. This enzyme was clearly different from those in earlier fractions because its stability was much lower, and part of its activity was lost already during the initial dialysis step.

Column electrophoresis on cellulose in 0.075 M Tris–citrate buffer (pH 8.8) likewise gave three well-separated peptidase peaks. All attempts to resolve Peak B into different peptidases by rechromatography on DEAE-cellulose, CM-cellulose or hydroxylapatite were unsuccessful.

Most peptidases seem to require divalent metal ions for activity, and the Arthrobacter enzymes are no exceptions. Dialysis against 0.02 M EDTA (pH 8.0) thus reduced the activities of all three peptidases. Subsequent incubation with $1 \cdot 10^{-3}$ M Mg^{2+} or Mn^{2+} partly reactivated peptidases A and B, whereas peptidase C could be reactivated by Co^{2+} to about 30%.

Peptidase A of Arthrobacter appears to be rather similar to the proline iminopeptidase from *Escherichia coli* K described by SARID *et al.*[4], but that enzyme also hydrolyzes long peptides with a free N-terminal proline. Peptidase C may be classified as an imidodipeptidase (prolidase) since it appears to be active only against dipeptides, unlike the proline peptidase from *E. coli* B [5]. Peptidase B appears to have wide specificity and may best be characterized as an amino peptidase since it was not active against peptides with a blocked α-NH_2 group.*

Institute of Biochemistry,
University of Uppsala,
Uppsala (Sweden)

ULF BJARE
BENGT V. HOFSTEN
ANN-CHRISTINE RYDÉN

1 B. V. HOFSTEN AND C. TJEDER, *Biochim. Biophys. Acta*, 110 (1965) 576.
2 B. V. HOFSTEN, H. VAN KLEY AND D. EAKER, *Biochim. Biophys. Acta*, 110 (1965) 585.
3 B. V. HOFSTEN AND B. REINHAMMAR, *Biochim. Biophys. Acta*, 110 (1965) 599.
4 S. SARID, A. BERGER AND E. KATCHALSKI, *J. Biol. Chem.*, 237 (1962) 2207.
5 A. YARON AND D. MLYNAR, *Biochem. Biophys. Res. Commun.*, 32 (1968) 658.
6 A.-C. RYDEN, *Acta Chem. Scand.*, in the press.

* *Note added in proof.* A more detailed description of the proline peptidases will soon be published[6] (Received September 7th, 1970).

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