effects of temperature. As shown in Figure 2B, temperature increases, when it rises from 16 to 32 °C; there is the activity of the IAA-destroying system. Further increase in temperature caused rapid inactivation of the system. Assays were carried out at 28 °C. Similar results were obtained for root cells[9].

To test the specificity of the auxin pretreatment, 3 IAA analogs (β-indolyl-carboxylic acid; β-indolylpropionic acid and DL-tryptophan) were used in a preincubation of 12 h, at the same concentration ($1 \times 10^{-5} M$) as IAA. For the study of the IAA destruction, a similar procedure was followed (10 ml of the active mixture, 2 ml of IAA – at 50 µg per ml – were added at zero time). The results summarized in Table II show that most IAA analogs inhibit the in vitro IAA destruction. Such observations are in agreement with those obtained with *Arthrobacter*[3].

Table II. Specificity of the auxin pre-treatment (12 h) on the IAA destruction (60 min; 28 °C; pH = 6.1) by *Lactobacillus*

Pre-treatment ($1 \times 10^{-5} M$)	µg IAA destroyed per 0.1 mg protein	Control (%)	IAA pre-treatment (%)
Control	11.2	0	—16.4
IAA	13.4	+10.7	0
β-indolylcarboxylic acid	9.5	—15.1	—29.1
β-indolylpropionic acid	8.5	—24.1	—36.1
DL-tryptophan	10.9	— 2.7	—18.6

The induction of a β-indolylacetic acid (IAA) inactivating enzyme in *Lactobacillus bulgaricus* was investigated. An IAA pretreatment of bacterial cells, which induces an IAA-oxidase, causes a strong inhibition of growth after 6 h, followed by a slight stimulation after 12 h. The optimal pH for the oxidizing system is 6.0 and the maximal IAA breakdown was observed at 32 °C. The ability of IAA to induce the oxidase was inhibited by some IAA analogues (β-indolyl carboxylic and β-indolyl propionic acids; DL-tryptophan). These results show that the induction system for IAA-oxidase has a high specificity for IAA. Properties of this enzyme-complex were found to be very similar to those of IAA-oxidase prepared from several plant tissues and explain the growth reactions of *Lactobacillus* incubated with IAA.

Résumé. Un traitement à l'acide β-indolylacétique (IAA) a pour effet d'induire – chez *Lactobacillus bulgaricus* – un système inactivant l'IAA. Le pH optimum de ce complexe enzymatique est de 6.0 et sa température optimale de réaction de 32 °C. Cette induction est inhibée par des analogues de l'IAA (acides β-indolyl carboxylique et β-indolyl propionique; DL-tryptophane). L'apparition de cette «IAA-oxydase» permet de rendre compte, dans une large mesure, des effets de l'IAA sur la croissance des microorganismes testés.

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Kinin-Releasing Activity of Pronase

REIS et al.[1] observed that after fractionation of pronase by paper electrophoresis at pH 8.6, a cathodic fraction was separated which when eluted and incubated with heat denatured plasma-preparation released bradykinin. Pronase, a commercial preparation from *Streptomyces griseus*, contains several peptidases (carboxy- and aminopeptidases) as well as proteinases active towards casein, of which one possesses in addition esterolytic activity towards N-benzoyl-L-arginine ethyl ester (BAEE)[2–4]. The specifity, inhibition and homology about the disulfide bridges of the latter enzyme suggest a close similarity with tryspin[5,6]. The experiments here reported were made in order to investigate which enzyme of pronase is responsible for the kinin-releasing activity.

Pronase P (Serva, Heidelberg, Germany) was separated into several fractions by column-chromatography on CM-Sephadex C-25 using a linear buffer gradient (Figure 1). 3 of the fractions obtained showed proteolytic activity

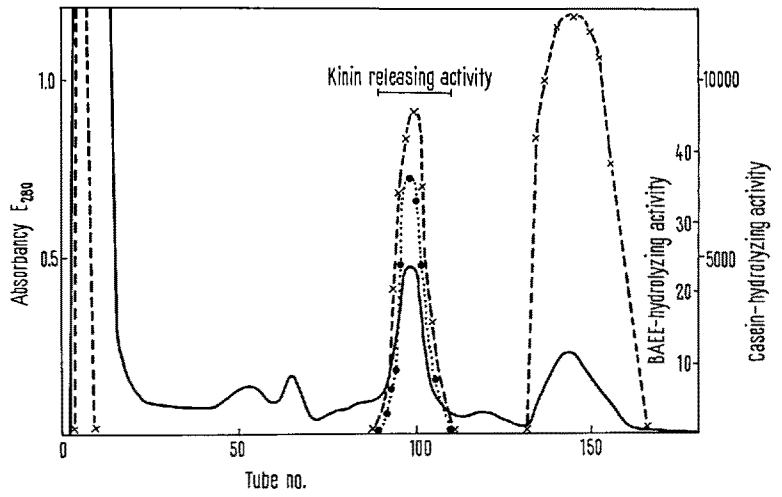

Fig. 1. Chromatography of pronase (300 mg) on CM-Sephadex C-25 (column 1.5 × 22 cm) equilibrated with 0.02 *M* ammonium acetate buffer pH 5.0. Elution was carried out using a linear buffer gradient (0.02–0.2 *M* ammonium acetate buffer pH 5.0) at a flow rate of 30 ml/h (5 ml per tube). BAEE hydrolyzing activity is expressed in µmoles BAEE hydrolyzed per min/ml, casein hydrolysis in units/ml.

towards casein (KUNITZ-test[7]: 1 unit is defined as the amount of enzyme, which, after incubation for 20 min at 37 °C, caused an increase in absorbancy of the trichloro-acetic acid-filtrate of 0.001); moreover, the second one hydrolyzed BAEE also, measured according to the spectrophotometric method of SCHWERT and TAKENAKA[8]. For testing the kinin-releasing activity 0.5 ml of 3% globulin solution (bovine plasma fraction, precipitated between 0.3 and 0.5 fold ammonium sulphate saturation) in 0.06*M* phosphate buffer pH 8.0 were incubated with 0.1 ml of enzyme fraction for 3 min at 37 °C and an aliquot amount tested on the isolated guinea-pig ileum preparation (in 10 ml aerated Tyrode-solution with 1 mg atropin and 0.1 mg Avil (Farbwerke Höchst AG, Germany) per litre at 37 °C); synthetic bradykinin (Sandoz AG, Basel, Switzerland) was used as standard. Only the BAEE-hydrolyzing fraction showed kinin-releasing activity. Casein- and BAEE-hydrolysis of this enzyme, as well as its release of kinin from plasmaglobulins, is completely inhibited (enzyme-inhibitor ration 1:5, w/w, 30 min preincubation) by soybean- (Serva) and ovomucoid-trypsin inhibitor (Calbiochem), Trasylol (Bayer AG, Germany), N-tosyl-L-lysyl chloromethane (Calbiochem) and phenylmethylsulfonyl fluorid (Serva, specific for serine-enzymes), but not by N-tosyl-L-phenylalanyl chloromethane (Calbiochem, specific for chymotrypsin). Preincubation of the enzyme with different amounts of BAEE resulted in inhibition of the kinin-release, probably due to competitive inhibition (Figure 2). In contrast to the observations of REIS et al.[1], none of the enzyme fractions possessed hydrolytic activity towards N-acetyl-tyrosine ethyl ester (ATEE).

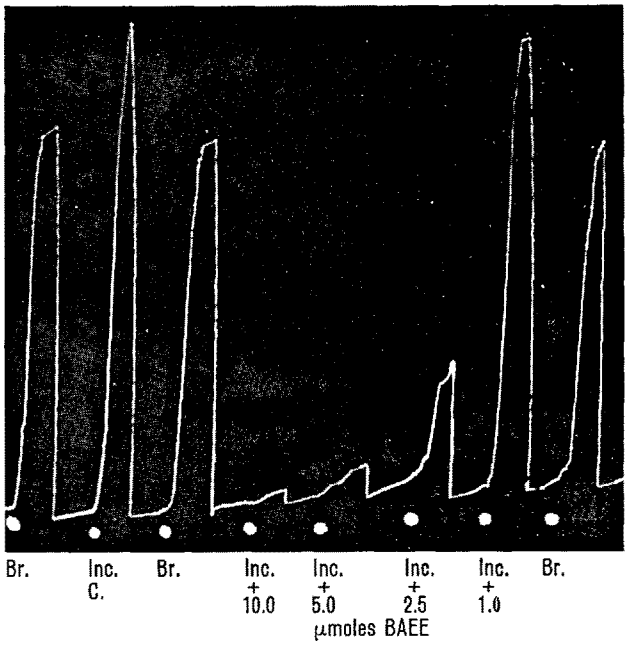

Fig. 2. Inhibitory effect of BAEE on the kinin-releasing activity of the trypsin-like enzyme from pronase, tested on the isolated guinea-pig ileum. Br = synthetic bradykinin 0.02 μg/10 ml Tyrode solution; Inc. = 0.05 ml of the following incubate: 0.1 ml enzyme (30 μg) were preincubated with BAEE for 1 min at 37 °C, than 0.5 ml 3% plasmaglobulin-solution added and incubated for 3 min. C = control, incubate without BAEE.

Our results confirm the suggestion that the kinin-releasing activity of pronase can be referred to a trypsin-like enzyme.

Zusammenfassung. Die Kinin freisetzende Eigenschaft von Pronase, einem Proteasen-Gemisch von *Streptomyces griseus*, ist auf ein Trypsin ähnliches Enzym zurückzuführen.

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Identification of Clones of Mammalian Cells by Isoenzyme Distribution Patterns

In the field of cell culture, a continuing problem is that of identifying the cell line being studied and of knowing if and when changes in that cell line have occurred[1]. Isoenzyme distribution patterns (zymograms) have been used to differentiate between samples of the same tissue from different species[2-6], different tissues from the same species[2,5,7-9] or different cell lines[1,10,11]. The work presented here describes the use of this technique to distinguish among different clones from the same cell line.

Materials and methods. Cell strains and cloning technique. Cultures of the LM cell line and a clone, Cl 1, from this cell line were maintained serum- and antibiotic-free in spinner culture on medium 199 peptone. Clone 1 was recloned by a one-step dilution method: 10-μl samples from the spinner were mixed with 10 ml of 199P containing 20% horse serum, 500 units penicillin, and 500 μg streptomycin. From this pool, 100 μl of the cell suspension were placed in Falcon tissue-culture-grade plastic petri dishes containing 10 ml of the above medium and incubated in a 5% CO_2 atmosphere. At 4 weeks, 15–35 colonies (15–35% plating efficiency) were observed; and from these, clones (Cl 1–1, 1–2, 1–3, 1–9) were picked at random. The clones were isolated with glass tubing (6 mm) wells, aspirated from the surface of the petri dish with fresh cloning medium, and placed in Falcon T-30 flasks. Cells from the monolayer cultures of the individual clones were harvested, resuspended at 1×10^7 cells/ml in fresh medium containing 5% dimethyl sulfoxide, and stored at liquid nitrogen temperatures.

Growth of the cells and preparation of cell-free extracts. Cultures were obtained by placing the contents of a