

The ability of the mitochondria isolated from nuclear (p), cytoplasmic (q^-) and double (pq^-) respiratory-deficient yeast mutants to incorporate ^3H -UTP into trichloroacetic acid-insoluble fraction shows that, at least in the strains tested, mitochondrial protein synthesis is blocked at the level of translation¹⁷.

Zusammenfassung. Aus verschiedenen cytoplasmatischen (q^-) und nuklearen (p) atmungsdefekten Hefemutanten isolierte Mitochondrien weisen eine Aktinomycin-empfindliche Inkorporation von ^3H -UTP in die TCS-unlösliche Fraktion auf. Die Absenz der mitochon-

drialen Proteosynthese dieser Mutanten könnte daher auf einen Hemmer im Translationsmechanismus zurückgeführt werden.

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Different ATPase Systems in Glycophytic and Halophytic Plant Species

In a recent review on the halophyte problem, JENNINGS¹ concluded that with regard to their response to sodium, halophytes differ from mesophytes only quantitatively and not qualitatively. Increased light, aridity and high sodium concentrations had a similar effect on plant cells of various ecological characteristics, namely a change in the ATP metabolism.

In general, there is little information on the influence of saline media on enzymatic activity in animal^{2,3} and plant tissues^{4,5}. Part of the information regarding ATPase activity concerns its role in ion transport⁶⁻⁹. However, relatively little is known of the presence of various ATPase systems in plants of different ecological groups¹⁰, and an investigation into this problem seemed worthwhile.

Two glycophytic species, i.e. bean (*Phaseolus vulgaris* L. c.v. *Brittle wax*) and corn (*Zea Mays* L. c.v. *White horse tooth*), and 2 halophytic species, i.e. *Suaeda monoica* Forsk. and *Atriplex halimus* L. were used for the following investigation. Plants were grown in an aerated Hoagland's nutrient solution for 10 days (bean and corn) and 35 days (*Suaeda* and *Atriplex*) respectively. By that time, the plants were at a more or less equal phase of growth. Sodium chloride was then added to the growth media of half of the plants, so as to give a final concentration of 30 mM. After 3 days the roots were harvested. Tissue fractionation and ATPase activity determinations were performed according to GRUENER and NEUMANN and NEUMANN and GRUENER^{11,12}. Homogenization of the

roots was performed by grinding the tissue in a cold mortar in a medium containing *Tris* 0.04 M-sucrose 0.5 M. The homogenate was filtered through a sheet of gauze and centrifuged for 5 min at $200 \times g$ to remove unground cells and wall debris. The mitochondria were separated by centrifugation for 20 min at $20,000 \times g$ and the microsomes by centrifugation for 60 min at $120,000 \times g$. The fractions were dialyzed for 30 h against *Tris* 3×10^{-3} M-EDTA 5×10^{-4} M, ptt. 7.7, at 5°C. The solution was

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The effect of NaCl in the growth media and in the reaction mixture on the ATPase activity of various plants

| Plant species | Reaction mixture | | Growth media | | | | |
|---------------------------|------------------|---|--------------|-----|------------|-----|-----|
| | | | — NaCl (A) | | + NaCl (B) | | |
| | | | % from a | | % from a | | |
| <i>Phaseolus vulgaris</i> | — NaCl | a | 4.78 | | 7.00 | | 146 |
| | + NaCl | b | 5.06 | 106 | 8.04 | 115 | 159 |
| <i>Zea Mays</i> | — NaCl | a | 2.67 | | 4.85 | | 182 |
| | + NaCl | b | 5.42 | 203 | 11.30 | 233 | 208 |
| <i>Suaeda monoica</i> | — NaCl | a | 4.04 | | 1.87 | | 46 |
| | + NaCl | b | 2.53 | 63 | 1.20 | 64 | 47 |
| <i>Atriplex Halimus</i> | — NaCl | a | 7.02 | | 4.33 | | 62 |
| | + NaCl | b | 4.76 | 68 | 2.60 | 60 | 55 |

Data denote $\mu\text{moles Pi}/30 \text{ min}/\text{mg protein}$.

changed 3 times during this period. Inorganic phosphorus was measured according to TAUSSKI and SHORR¹³, proteins were determined by measuring absorbancy at 260 and 280 nm. The 120,000 $\times g$ supernatant, where most of the enzymatic activity reside in the soluble fraction, was used for the experiments. The reaction mixture contained: 25 μ moles Tris at pH 8.8; 2.5 μ moles ATP; 2 μ moles CaCl_2 ; 100 μ moles NaCl; 0.2 ml dialyzed enzyme fraction. Volume was brought to a total of 1.0 ml with double distilled water. The reaction mixture was incubated for 30 min at 30°C and the reaction stopped by addition of 0.2 ml cold TCA 50%. Experiments were conducted in 3–4 replications and repeated at least 3 times.

Results of one of the experiments are presented in the Table. As can be seen, the additions of NaCl to the growth medium resulted in the stimulation of soluble ATPase activity of bean and corn roots, but induced an inhibition of this activity in *Suaeda* and *Atriplex* roots.

The addition of 100 μ moles of sodium chloride to the enzymatic reaction mixture revealed that the activity of the ATPase in the supernatant of bean and corn roots was stimulated (Na^+ activated), while the activity of this enzyme in the supernatant of *Suaeda* and *Atriplex* roots was inhibited.

Evidently, the results of these experiments suggest that a basic qualitative difference in the responses to Na

exists between the ATPase systems in the 2 glycophytic and 2 halophytic species, a difference that might be of a general nature.

The effects of other cations on the ATPase activity of plants with different salt tolerances, are now being investigated¹⁴.

Zusammenfassung. Die Wurzeln zweier Glycophyten (*Phaseolus vulgaris*, *Zea Mays*) und zweier Halophyten (*Suaeda monoica*, *Atriplex Halimus*), angezogen auf NaCl-freier und NaCl-haltiger Nährlösung, wurden auf ihre ATPase-Aktivität untersucht. Die Anwesenheit von NaCl in der Nährlösung sowie im Reaktionsgemisch aus aufbereitetem Wurzelhomogenat und Cofaktoren bewirkt in Glycophyten eine Förderung und in Halophyten eine Hemmung der ATPase-Aktivität.

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An Elastase Inhibitor from Canine Mandibular Gland

Pancreatic elastase (endopeptidase EC 3.4.4.7) is unique among proteolytic enzymes of animal tissue in that it hydrolyzes elastin very quickly, liberating approximately 130 moles of NH_2 -terminal residues per 100,000 g of substrate¹.

The destruction of intrapancreatic vessels observed in experimental^{2–4} as well as in human⁵ acute pancreatitis includes the dissolution of elastic tissue by pancreatic elastase. The equivocal results obtained with the protease inhibitor from bovine lung⁶ in the treatment of acute pancreatitis may be explained, at least partially, by the fact that this polypeptide inhibits elastolytic activity only slightly. A 2500-fold excess (weight for weight) of this inhibitor is needed to reduce elastase activity by 50%. The preparation of a potent elastase inhibitor and its use in acute pancreatitis, alone or in combination with the beef lung polypeptide, might prove of therapeutic benefit. In the course of previous studies⁷ it was noted that canine mandibular tissue extracts exhibited significant inhibitory activity on pancreatic elastase in vitro. The present study was undertaken for the isolation and characterization of the mandibular elastase inhibitor.

Extraction and purification. Freshly dissected dog submandibular glands were homogenized for 3 min at 4°C with distilled water and the homogenate was centrifuged at 44,000 g. The clear supernatant was subjected to ultrafiltration in a 1/4 inch Visking tubing at 4°C, and the ultrafiltrate was concentrated to a small volume. The concentrate was applied to a Sephadex G-50 (fine) column and the column was developed with distilled water. The effluent was tested for inhibitory activity and the active fractions were pooled and concentrated. Rechromatography of a small amount of concentrate on a Sephadex G-50 column which had been calibrated with

NADP, oxytocin, trasyolol, ribonuclease and trypsin indicated a molecular weight of 12,000 for the submandibular inhibitor. 100 μ l portions of inhibitor concentrate were applied to strips of Whatman No. 1 paper and subjected to electrophoresis in 0.05 M formic acid at 2.5 mA/strip for 2.5 h.

Inhibitor activity was detected in a band about 4.5 cm on the cathodic side, which gave a peptide stain with chloroimide reagent and a pink color with Sakaguchi reagent. The eluates (2% v/v acetic acid) of the inhibitor fraction from 19 electrophoresis paper strips were concentrated to a small volume. A 15 μ l portion of this concentrate was applied to a cellulose acetate strip and subjected to electrophoresis for 30 min at 3.7 mA/strip using a formic acid solution, 0.05 M, pH 3.6, as before. 2 closely associated but distinct bands appeared when the strip was stained with Amido Black 10B.

Extracts from submandibular glands of different species. Extracts of submandibular glands from man, beef and pig were prepared in an analogous fashion. The ultracentrifugates and ultrafiltrates were tested for inhibitory activity against elastase (10 μ g), trypsin (5 μ g), and chymotrypsin (10 μ g). For purposes of comparison an

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