STRUCTURE DETERMINATION OF SYRINGOTOXIN BY 1D AND 2D NMR

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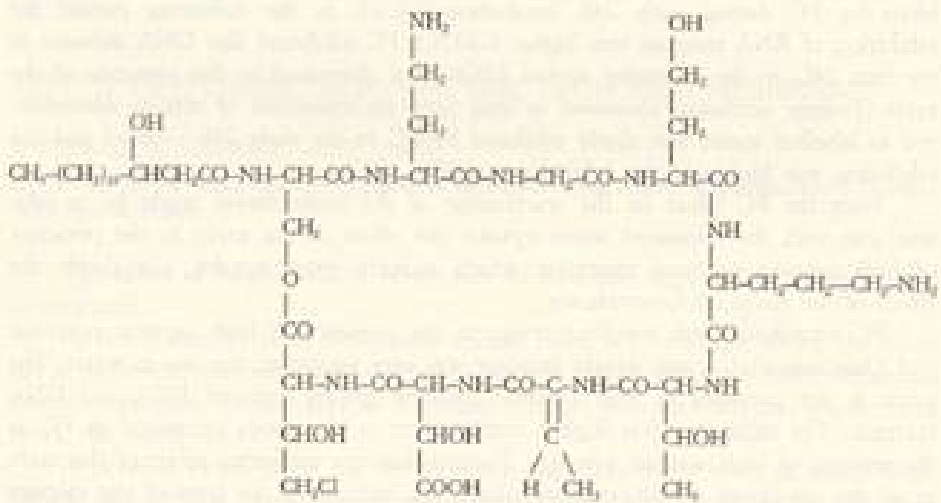
Syringotoxin is a lipodepsipeptide produced by strains of *Pseudomonas syringae* pv. *syringae* pathogenic to citrus plants.

A ^{1}HNMR study by 1D and 2D techniques in different solvents (deuterated water, dimethyl sulphoxide and acetonitrile/water) allowed us to obtain the complete primary structure.

From the COSY type experiments, a full assignment of the spin system led to the aminoacid identification. The fatty acid chain was completely identified by ^{13}CNMR.

The sequential assignment has been obtained from ROESY type experiments with different mixing times.

The following structure is in complete agreement with chemical and mass spectrometry results.



PHYTOTOXIC EFFECTS OF MICROBIAL PECTIC ENZYMES

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Microbial pectic enzymes not only macerate plant tissue but also kill plant cells and elicit synthesis and accumulation of phytoalexins. We have evidence suggesting

that these three physiological activities of pectic enzymes may be regulated by factors such as endo-polygalacturonase inhibiting proteins (PGIP) and pH.

Homogeneous endo-polygalacturonase (PG), purified from *Aspergillus niger*, was applied to potato medullary tissue disks. After 4 hrs of incubation at various pH, maceration of the tissue was measured and viability of cells was assessed by the Evans Blue staining procedure. The optimum pH for the macerating activity was between 5.0 and 5.5, in accordance with the optimum pH at which *A. niger* PG depolymerized polygalacturonic acid (PGA) *in vitro*. The *A. niger* PG also caused cell death of potato cells at pH 5.0. However, in the presence of an excess of PGIP purified from *Phaseolus vulgaris*, the macerating activity of PG was completely inhibited, while the killing activity was maintained. The experiments were repeated using PG purified from *Fusarium moniliforme* and similar results were obtained. In the presence of an excess of PGIP, *F. moniliforme* PG lost its macerating activity and maintained its killing activity.

Experiments performed with a homogeneous endo-pectate lyase (PL) purified from *Erwinia carotovora* gave similar results. PL depolymerized PGA *in vitro* at an optimum pH of 9.0 and had very little activity at pH 5.5. Macerating activity of PL was exhibited at pH 9.0, whereas the enzyme did not discernibly macerate the potato tissue at pH lower than 7.0. Killing activity, on the contrary, was displayed by PL at all the pH values tested, that is from 9.0 through 5.5.

In order to ascertain the role of PGIP in regulating the macerating and killing activity of fungal endopolygalacturonase, we have amplified by polymerase chain reaction (PCR) and cloned a 0.7 kb fragment of *Phaseolus vulgaris* genomic DNA. This fragment corresponded to the N-terminal coding region of the gene for PGIP. We will now screen both a genomic and a cDNA library of *P. vulgaris* in search of full lenght PGIP clones and use these clones to transform tomato plants *via Agrobacterium* Ti plasmid-derived vectors. Transformed plants will be analyzed for susceptibility to the toxic effects of fungal polygalacturonases.

ACTIVATION OF THE PLASMA MEMBRANE H^+-ATPase BY FUSICOCCIN

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The effect of fusicoccin (FC) on the plasma membrane (PM) H^+-ATPase activity has been characterized in a purified PM fraction obtained from radish seedlings by phase partitioning.

FC-induced stimulation of the PM H^+-ATPase is strongly pH dependent: the absolute increase in activity is maximal around pH 7 (thus shifting the pH optimum of the ATPase activity of about 0.3 pH units) and percent stimulation increases with the increase of pH up to 100-130% at pH 7.5.