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Enzymatic Evidence That Leucine From Tentoxin Is Levorotatory

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Introduction

Tentoxin is a highly specific, biologically active peptide from **Alternaria tenuis** Auct., a common seed and soil inhabiting fungus. Grossly, its biological activity consists solely of irreversibly blocking the development of chlorophyll in cotyledons of certain plants when applied to seeds during imbibition or to young seedlings prior to their emergence from the soil. Most dicots tested, with the exception of tomato and members of the cruciferae, have been found to be sensitive while most monocots tested were resistant with the exceptions of sorghum and crabgrass (1, 2, 6, 7).

Halloin and associates (4) have shown that tentoxin disrupts chloroplast development in sensitive species without noticeably affecting the ultrastructure of other organelles. They also demonstrated that it does not block the conversion of protochlorophyll to chlorophyll in either sensitive or insensitive species. In fact, they showed a slight but consistent increase in chlorophyll in resistant species.

The peptide was purified by Grable (3) from culture filtrates or mycelial mats of **Alternaria tenuis** grown on Richards solution supplemented with V-8 juice. Structural determinations (7) have revealed it to be a cyclic tetrapeptide consisting of leucine, N-methylalanine, glycine and N-methyldehydrophenylalanine. The sequence of these amino acids and the stereoisomerism of all but glycine must be established to complete the determination of the primary structure of this peptide.

This study was undertaken to determine which optical antipode of leucine is present in tentoxin.

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Materials and Methods

Hydrolysis of the peptide

The crystalline toxin was hydrolyzed in 6 normal HC1 at 110°C for 24 hours according to the procedure of Spackman (5). After removal of the HC1 in vacuo the hydrolyzate was taken up in 10% 2-propanol for immediate spotting of preparative thin layer plates.

Separation of Leucine from the Hydrolyzate.

Leucine was separated from the hydrolyzate by preparative thin layer chromatography on 2mm thick plates of Silica gel PF (Merck) with a propanol: water (80:36) solvent in one dimension. Leucine was located on the developed chromatogram by reacting one edge of the plate with ninhydrin. The surface of the plate containing leucine (unreacted) was scraped into a Millipore filter and the leucine was leached from it with distilled water. The concentration of leucine recovered was determined on an aliquot from this solution using a standard colorimetric procedure.

Enzymatic Oxidation of Leucine

Snake venom L amino acid oxidase was used in a Warburg manometric procedure as outlined by Wellner and Meister (9) to determine the susceptibility of tentoxin-leucine to this enzyme. The concentration of tentoxin-leucine was 12 millimoles per ml. The enzyme was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio and was used at a concentration of 100 mg/ml. At this enzyme level oxygen uptake with L leucine was 3.6 microliters per minute. Commercially prepared D and L leucine were used as controls. Three replications were employed.

Results

L amino acid oxidase oxidized leucine from tentoxin

at a rate comparable to that of known L leucine (Figure 1). The D leucine control was not oxidized.

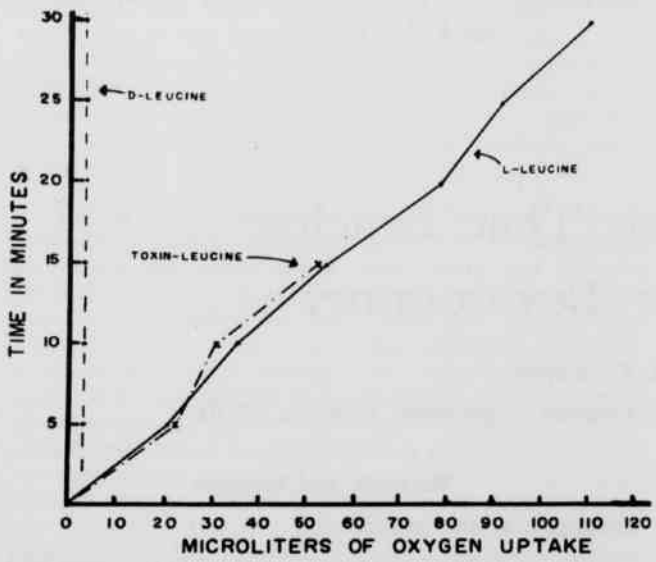

Figure 1. Enzymatic Oxidation of Leucine from Tentoxin.

Summary and Conclusion

These data indicate that the leucine in tentoxin is levorotatory. Supporting data with higher concentration of this antipode subjected to D amino acid oxidase would be highly desirable but is currently considered too extravagant with the limited supply of peptide available.

These data further suggest that the optical isomerism of leucine is not the basis for tentoxins inhibition of chlorophyll or plastid formation in sensitive plant species.

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