DETERMINATION AND in vitro INHIBITION OF α-AMYLASE AND PROTEASE ACTIVITY IN Spodoptera frugiperda

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Introduction

Proteinaceous inhibitors of digestive enzymes from insects might play an important role in plant protection (1). Different inhibitor preparations from wheat and barley endosperms inhibited α-amylases from 23 agricultural insect pests (2), or trypsin activities (3). We determined the main digestive activity of the armyworm *Spodeptera frugiperda*, and evaluated its inhibition by dimeric, monomeric and tetrameric α-amylases inhibitors from wheat and a trypsin inhibitor (CMe) from barley cv. Bomi.

Materials and Methods

Insects
Larvae of *S. frugiperda* were grown on diets as (4).

Insect extracts
Extracts from various instars *S. frugiperda* larvae were made as (1) with Tris and Glycine buffer to elucidate α-amylase activity during larval cycle. Extracts for trypsin inhibition was prepared in HCl.

Inhibition tests
α-amylase activity and *in vitro* inhibition was made essentially as (5). L-BAPA was used for trypsin activity of extracts and *in vitro* tests were made according to (6).

Inhibitors
Monomeric, dimeric, and tetrameric inhibitor fractions from wheat were purified as (7), and CMe proteins were prepared following reported protocols (8).

Results and Discussion

S. frugiperda α-amylase showed maximal activity at pH 8.5-9.5 in Tris buffer. Figure 1 shows maximal α-amylase activity during 11 and 12 days of larvae life, coinciding with the highest feeding activity of the insect for life stage change. *S. frugiperda* α-amylase activity in non-denaturing PAGE was observed during larval life, and no difference among the 5 different isoenzymes was detected. The tetrameric inhibitor inhibits 25 and 60 % respectively of the total α-amylase activity at 7 and 11 days of larval life cycle, whereas inhibition with dimeric and monomeric inhibitors was not significant.

Figure 1 shows also α-amylase inhibition with 5 µg of wheat tetrameric inhibitor. This was minimal between 7-10 days of larvae life cycle, probably because of the high quantity of insect pigment compounds interfering the assay. Figure 2 shows *in vitro* α-amylase inhibition.

Protease activity of the trypsin type in the larvae midgut was detected and it was inhibited at 64 % by 5 µg of CMe proteins from barley cv. Bomi. We concluded that the tetrameric and CMe trypsin inhibitors could be good candidates for protecting plants against *S. frugiperda* attack by genetic engineering.

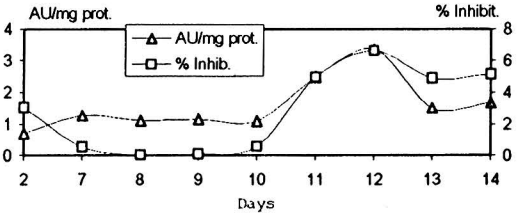

Figure 1. *Alpha-amylase activity and inhibition.*

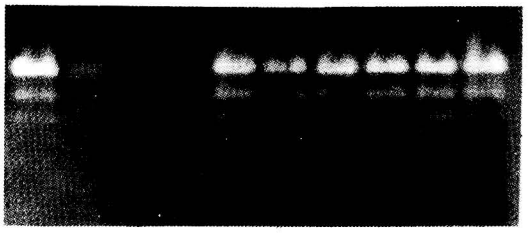

Figure 2. *In vitro α-amylase inhibition; a- S. frugiperda α-amylase activity; b-g, S. frugiperda α-amylase activity inhibition by: b-d, 1, 3, 5 µg wheat tetrameric inhibitor; e-g, 1, 3, 5 µg wheat dimeric inhibitor; h-j, 1, 3, 5 µg wheat mono-meric inhibitor.*

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