DETECTION METHOD FOR IRRADIATED ORIENTAL FRUIT FLY (*Dacus Dorsalis*) FOR QUARANTINE PURPOSES

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1994

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Radiation is one of the techniques used to effectively rid fresh produce of insect pests and efficacy of radiation dose on food is measured by a probit 9 (99.9968% mortality) quarantine security. Presence of suitable biochemical markers for irreversible radiation injury in insect pests could be used as convincing proofs of the efficacy of radiation dose. A biochemical marker (designated Gs-protein) for radiation injury in Oriental fruit fly, *Dacus dorsalis*, was detected in the SDS-PAGE profile of two-day old pupae and adult insect stage. Gs-protein is not observed in larvae and eggs. An apparent molecular weight of 109 kDa was calculated. A tyrosinase enzyme activity was observed in the soluble fraction of pupal total homogenate and SDS-PAGE-isolated Gs-protein; however, no tyrosinase activity was measured in irradiated sample. The optical absorbance of the soluble fraction from unirradiated pupal total homogenate measured at 360 nm was found to increase with time. From the results of the studies, the apparent loss of Gs-protein in irradiated larvae is likely the result of loss of melanization capability in irradiated larvae which is linked to the absence of tyrosinase enzyme. The data presented seems to establish the role of Gs-protein as a biomarker for gamma-irradiation induced deactivation of pupal development and as a convenient indicator of the effectiveness of gamma radiation as a quarantine treatment.

INTRODUCTION

Strict quarantine regulations imposed by importing countries on fresh produce necessitates thorough inspection to ensure products are free of live insect pests prior to shipment. Radiation is one of the techniques used to effectively rid produce of insect pests and efficacy of radiation dose on food is measured by a probit 9 (99.9968% mortality) quarantine security. Presence of suitable biochemical markers for irreversible radiation injury in insect pests would be convincing proofs of the efficacy of radiation dose. This paper demonstrates that a radiation sensitive gene product of *D. dorsalis* could be used as a biomarker for the efficacy of radiation as a quarantine treatment.

A biochemical marker for radiation injury in Oriental fruit fly (*Dacus dorsalis*) pupae was detected in SDS-PAGE gel. Cobalt-60 irradiation of third instar larvae at 25 Gy and higher doses caused the loss of a pupal protein band from the electrophoretic profile of soluble fraction of the pupal total homogenate (1). We have ascribed this specific protein band to a gene product, Gs-protein, of a putative radiation-sensitive marker gene. The Gs-protein band was observed only after the irradiated larvae had reached the two-day old pupal stage and older.

METHODOLOGY

1. *Irradiation*

Dacus dorsalis (5 to 6 day-old third instar larvae) were irradiated in a Cobalt-60 gamma cell irradiator at the Cobalt-60 Facility of the Institute. Dose ranges from 25 to 100 Gy. After irradiation, larvae were allowed to continue their life cycle at room temperature. Mature larvae and pupae (both from irradiated and non-irradiated) Larvae of *D. dorsalis* were obtained from the entomology laboratory of Dr. Eugenia C. Manoto.

2. *Polyacrylamide gel electrophoresis*

Pupae were homogenized in sodium phosphate buffer pH 7.6 and centrifuged at 1500 x g to remove the insoluble fraction. A continuous buffer system of 0.1 M Tris, 10% glycine and 3.3% SDS was used to separate soluble pupal proteins. The Laemmli method for slab gel electrophoresis was followed with modifications. A 10-15 µl sample was applied to each electrophoretic well. Electrophoresis was performed at 25 mA for 1.5 h. Gel was stained in Coomassie Brilliant blue and destained in acetic acid-methanol solution.

High molecular weight protein markers (alpha-macroglubulins - 230 kDa, beta-galatosidase - 113 kDa, bovine serum albumin - 66 kDa, and egg albumin - 45 kDa) were used to determine the approximate molecular weight of Gs-protein.

3. *Tyrosinase: Enzymatic assay*

Gs-protein was isolated from unstained SDS-PAGE-gel, centrifuged and lyophilized prior to enzymatic assay.

Tyrosinase activity assay was performed by oxygenating tyrosine in phosphate buffer pH 6.5 for 5 min and subsequently adding 50 µl of Gs-protein solution to 1.45 ml of oxygenated tyrosine solution. Rate of reaction was recorded at 280 nm for 10 min. Purified mushroom tyrosinase (SIGMA) was used as activity standard. The protocol for tyrosinase activity assay that was followed can be found in the Worthington Manual for Enzyme and Related Biochemicals (2).

4. *Chromophore Quantitation*

Optical absorbance, in the visible spectrum, of pupal total homogenate soluble fraction was recorded with a spectrophotometer. The optical absorbance of an aliquot of the soluble fraction in phosphate buffer (pH 7.5) was measured spectrophotometrically at 360 nm. Such measurements were recorded for up to 5 h.

RESULTS AND DISCUSSION

A biochemical marker, Gs-protein, for radiation injury in fruit fly *Dacus dorsalis* was observed, at the earliest stage, in the SDS-PAGE protein profile of a two-day old pupae and was also observed in the adult insect stage. The Gs-protein band is not apparent in the SDS PAGE profiles of eggs nor of larvae. This suggests that the gene coding for Gs-protein is

turned on for synthesis only at the pupal stage. Cobalt-60 gamma irradiation (doses of 25 Gy and higher) of mature larvae resulted in the loss of the Gs-protein band from SDS-PAGE pattern of pupae from irradiated mature larvae (1). The presumptive Gs-protein gene locus seemed to have sustained at least one mutational event which resulted in the loss of Gs-protein. The putative gene susceptible to such damage (and coding for the Gs-protein) may be responsible for the synthesis of a critical primary or secondary gene product necessary to transform pupae into adult flies.

An apparent molecular weight for Gs-protein was calculated at 109 kDa (Fig.1). Since this protein is indeed found only at the pupal and adult stages of the *D. dorsalis* life cycle, it appears to be an obligatory protein in the maturation process of the insect.

Gs-protein isolated from SDS-PAGE gel was transferred to nitrocellulose filter. Gs-protein was eluted out of the filter with water and tested for tyrosinase activity. The enzymatic activity of total protein homogenate soluble fraction, SDS-PAGE-isolated Gs-protein and as well as mushroom tyrosinase (taken as activity standard), are shown in Fig.2 B, C,and A respectively. A comparison between irradiated and unirradiated samples is also presented. As seen in Fig.2 B, the irradiated sample has no tyrosinase activity. A tyrosinase activity is observed in unirradiated samples for both total homogenate soluble fraction and SDS-PAGE-isolated Gs-protein (Fig.2 B and C respectively). Fig.2 A represents the standard curve for mushroon tyrosinase.

The optical absorbance of soluble fraction from unirradiated pupal total homogenate measured at 360 nm was found to increase with time. This is not seen in irradiated sample (Fig.3). It has been observed that in irradiated larvae, the insect lost the capability to undergo the "browning process" (3). Melanization occurs in insects and the loss of this process seems to be related to the loss of tyrosinase activity in irradiated total protein homogenate. From the results of our studies on the apparent loss of Gs-protein in pupae of irradiated larvae, it strongly appears that the loss of melanization capability of pupae of irradiated larvae is due to the loss of a tyrosinase activity as a result of mutational events at the Gs-protein gene locus--- implying the strong likelihood that the Gs-protein is an enzyme with critical, stage-dependent, tyrosinase activity.

As evidence from our data, it seems fairly well established that the Gs gene product in *D. dorsalis* pupae can be used as a convenient biomarker for gamma-irradiation induced deactivation of pupal development and hence as a convenient indicator of the effectiveness of gamma radiation as a quarantine treatment. The wider monitoring application of the present methodology will result in significant reductions in the risk of rejection of export shipments of fresh fruits that have undergone irradiation in compliance with foreign quarantine regulations. The design and quarantine use of a field test kit based on the results presented in this report should therefore be actively pursued.

REFERENCES

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Fig. 1. Gs-Protein Molecular Weight Determination

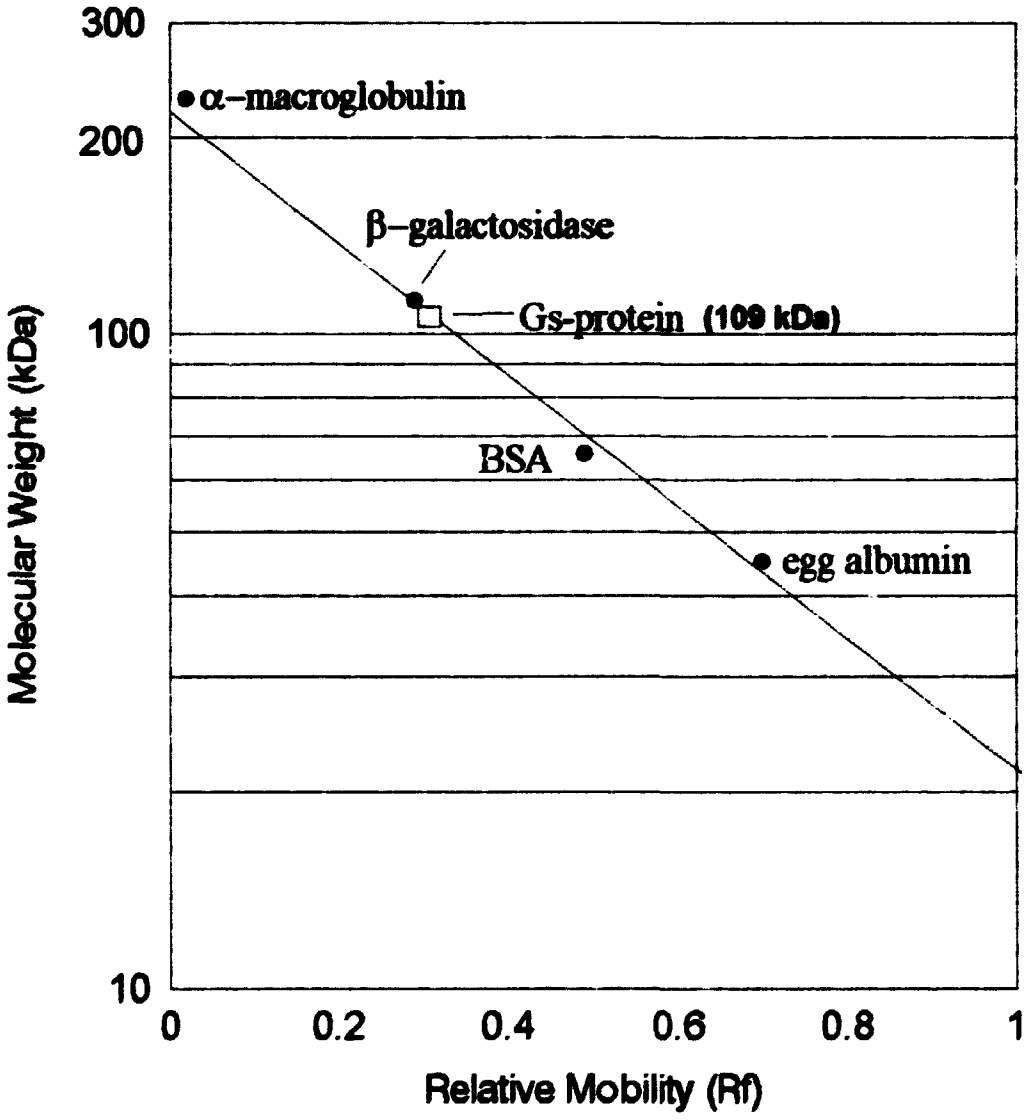
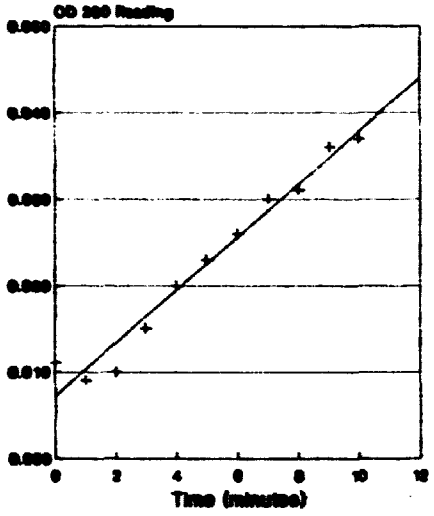


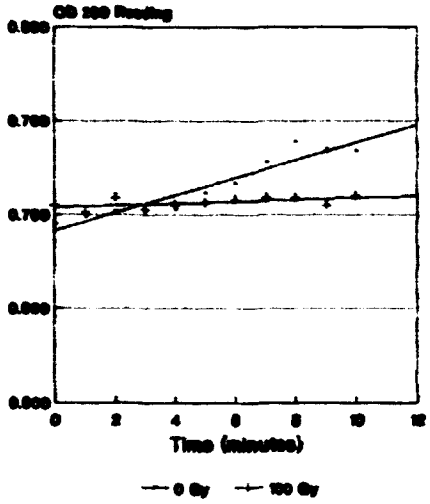
Fig. 2. Tyrosinase Activity

A



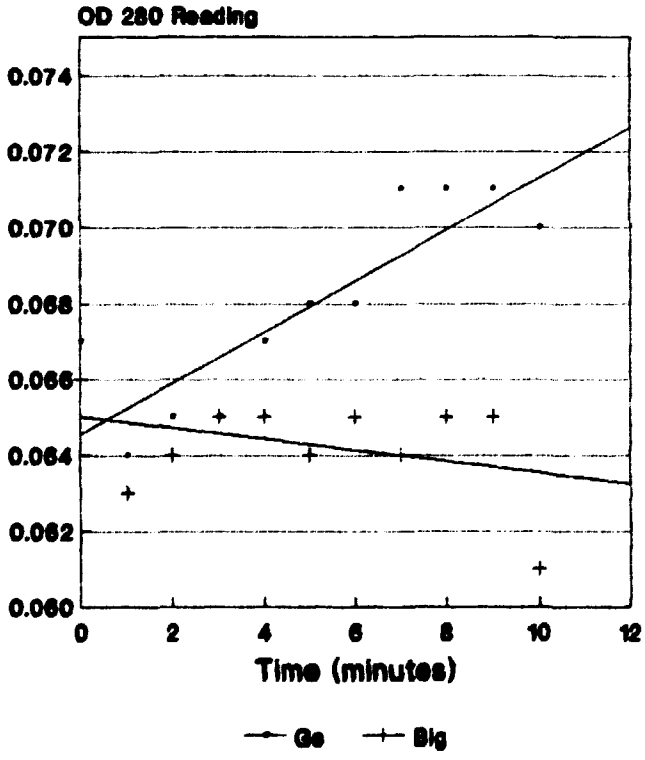
Mushroom Tyrosinase (Standard)

B



Homogenate (Soluble Fraction)

C



Gs-protein

Fig. 3. Optical Absorbance of Chromophore from Pupae of *Dacus dorsalis*