DETECTION METHOD FOR IRRADIATED ORIENTAL FRUIT FLY (*BACTROCERA PHILIPPINENSIS*) FOR QUARANTINE PURPOSES

ABSTRACT The presence of a biochemical marker for irreversible radiation injury in an insect pest was identified in pupae of irradiated larvae of *Bactrocera philippinensis* A radiation sensitive marker protein (designated Gs-protein) for radiation injury in the Oriental fruit fly, *B. philippinensis,* 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. Preliminary TXRF (total reflection x-ray fluorescence) spectrometric analysis indicates that Gs-protein is a copper-containing enzyme. The optical absorbance of the soluble fraction from unirradiated pupal total homogenate measured at 360 nm was found to increase with time. The presence of highly absorbing chromophore(s) in the visible range in both samples may indicate that a certain type of tyrosinase activity (other than melanin formation) may be present and may be responsible for color formation in insects. From the results of the studies, the apparent loss of Gs-protein in irradiated larvae is the likely result of loss of melanization capability in irradiated larvae which, in turn, is linked to the absence of a certain type of tyrosinase activity. The data presented indicates the practical role of Gs-protein as a biomarker for gamma irradiation-induced arrest of pupal development and as a convenient indicator of the efffectiveness of gamma irradiation as a quarantine treatment.

OBJECTIVES

1. To isolate a radiosensitive gene product in pupae of irradiated larvae of *Bactrocera philippinensis* using SDS-PAGE.
2. To identify this radiosensitive gene product based on its physico-chemical characteristics.
3. To use this radiosensitive gene product as a marker for radiation injury in irradiated fruit fly.

METHODOLOGY

1. Irradiation

   B. philippinensis (5 to 6 day-old third instar larvae) were irradiated in a Cobalt-60 gamma cell irradiator (301.5075 Gy/hr) at the Cobalt-60 Facility of the Institute. Dose was at

100 Gy. After irradiation, larvae were allowed to continue their life cycle at room temperature. Mature larvae and pupae of *B philippinensis* 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 g to remove the insoluble fraction. A continuous buffer system of 0.025 M Tris, 0.19M glycine and 0.1% 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. Gels were stained with Coomassie Brilliant Blue (Sigma) and destained in acetic acid-methanol solution.

High molecular weight protein markers (Sigma) (-macroglobulin: 230 kDa, -galactosidase: 113 kDa, bovine serum albumin: 66 kDa, and egg albumin: 45 kDa) were used to determine the approximate molecular weight of the Gs-protein.

3. Measurement of protein concentration
Concentration of Gs-protein was measured by the Bradford method (2). Gs-protein isolated from SDS-PAGE gel and eluted with water, was mixed with Coomassie Brilliant Blue dye following the Bradford method of protein assay. Bovine serum albumin (Sigma) served as standard protein.

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

Tyrosinase activity assay was performed, firstly, by oxygenating tyrosine (Sigma) in phosphate buffer (pH 6.5) for 5 min and the subsequent addition of 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.

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

   Absorption spectra in the visible optical range were measured spectrophotometrically for soluble fractions of pupal homogenates from both unirradiated and irradiated larvae.

6. Total reflection x-ray fluorescence (TXRF) spectroscopy
   SDS-PAGE-isolated Gs-protein was isolated following the protocol mentioned above with the exception that all reagents were prepared with triple distilled copper-free water. Gs-protein was analyzed semi-quantitatively for the presence of bound copper using TXRF spectrometry (courtesy of the PNRI Applied Physics Research Section).

RESULTS AND DISCUSSION

A biochemical marker, Gs-protein, for radiation injury, designated as Gs-protein, in the fruit fly *Bactrocera philippinensis* was observed, at the earliest stage, in the SDS-PAGE protein profile of 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 expressed 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 the SDS-PAGE pattern of pupae from irradiated mature larvae . The presumptive Gs-protein gene locus seemed to have sustained at least one mutational lesion which resulted in the loss of Gs-protein activity. 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 for the transformation of pupae into adult flies.

An apparent molecular weight for Gs-protein was calculated at 109 kDa. Since this protein is indeed found only at the pupal and adult stages of the *B. philippinensis* life cycle, it appears to be a constitutive protein in the maturation process of the insect.

Approximately 8 μg of Gs-protein was determined from each pupa based on the Bradford method of protein analysis.

Gs-protein isolated from SDS-PAGE gel was transferred onto nitrocellulose filter, eluted out of the filter with water and tested for tyrosinase activity. The enzymatic activity of pupal homogenate soluble fraction , SDS-PAGE-isolated Gs-protein and mushroom tyrosinase as activity standard , are presented. The irradiated sample exhibited no tyrosinase activity; in contrast, tyrosinase activity is observed in unirradiated samples for both total homogenate soluble fraction and SDS-PAGE-isolated Gs-protein. A standard curve for mushroon tyrosinase is also shown for comparison.

Preliminary semi-quantitative total reflection x-ray fluorescence (TXRF) spectrometry of isolated Gs-protein indicates the presence of bound copper. It is known that molecules of tyrosinase enzyme from mushroom are tetramers with four gram atoms of bound copper per mole. The presence of bound copper as evidenced by semi-quantitative TXRF spectral analysis and a tyrosinase enzyme activity strongly indicates that the Gs-protein is a polyphenol oxidase. A fully quantitative TXRF spectral analysis of Gs-protein from *B. philippinensis* will be presented in a later communication.

The optical absorbance of the soluble fraction from unirradiated pupal homogenate measured at 360 nm was found to increase with time. This result is not seen in irradiated sample. The absorption spectrum of pupal homogenate soluble fraction from unirradiated larvae showed an absorption peak at 364 nm. A small but measurable decrease in the absorption peak at 364 nm was observed for irradiated material. It appears that a decrease in the optical absopbance at 364 nm correlates with the loss of Gs-protein as a result of gamma irradiation, and the consequent loss of a crucial tyrosinase enzyme as evidenced by the loss of tyrosinase activity observed. It has been shown in the case of the Carribean fruit fly that in irradiated larvae, the insect partially loses the capability to undergo the "blackening" process. The loss of the melanization process seems to be related to the loss of tyrosinase activity in irradiated total soluble protein homogenate in both the Carribean and Mediterranean. The presence of highly absorbing chromophore(s) in the visible optical range in both our samples

seems to indicate that tyrosinase may not be the only enzyme responsible for color formation in insects.

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 constitutive tyrosinase activity as a result of somatic 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 evidenced by present data, it has been established that the Gs gene product in *B. philippinensis* pupae can be used as a convenient biomarker for gamma irradiation-induced arrest of pupal development and hence as a convenient indicator of lethal radiation injury in this species. Furthermore, our data is sufficient for the design and construction of more than one type (depending on substrate) of portable and disposable chromogenic test kits that can be custom tailored to suit specific quarantine or other needs. This is the thrust of our further research on the Gs-protein from *B. philippinensis*.

PROJECT PERSONNEL

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