KETOALDEHYDE LEVELS IN THE LIFE CYCLE OF *PANAGRELLUS SILUSIAE*

Caroline Gardiner and Edward E. Palincsar
Department of Biological Sciences
Loyola University of Chicago
Chicago, Illinois

ABSTRACT

Ketoaldehyde concentrations were measured in the eutelic nematode *Panagrellus silusiae* and were found to be about 16 times higher in the juvenile (L2) than in the adult stage. The high ketoaldehyde concentration in the L2 stage is correlated with the lack of cellular proliferation that is characteristic of the eutelic condition. In later stages there is an increase in cell division accompanying the development of the gonads. Ketaldehyde levels were found to decrease during this period of maturation. The role that ketoaldehydes play in the nematode eutelic system and the clear biochemical delineation between the L2-L3 (eutelic) and the L4-adult (gametic) phases of the life cycle are discussed.

INTRODUCTION

Two decades of work by numerous researchers have shown that methyl glyoxal and related ketoaldehydes effectively inhibit the multiplication of cultured prokaryotic and eukaryotic cells (Egyud, MacLaughlin and Szent-Gyorgyi, 1967; Egyud and Szent-Gyorgyi, 1966a, 1966b; French and Freelander, 1958; Gregg, 1968). Szent-Gyorgyi (1967) has suggested that the glyoxalase system (glyoxalase I and II, glutathinone, glyoxals, and their corresponding hydroxy acids) is intimately involved in the process of cell division, and that a balance of related cellular ketoaldehydes is essential for the maintenance of normal growth patterns.

Eutely is the state of having a fixed number of somatic nuclei throughout the life of the individual. The nematode *Panagrellus silusiae* is an ideal organism for the study of compounds involved in cell division since, in this eutelic system, there is very little somatic cell division after embryological differentiation is complete. We started with the assumption that ketoaldehyde levels would be uniquely predictable during a given stage in the life history in a eutelic system.

Panagrellus (Anguillula) silusiae (de Man, 1913; Goodey, 1945) is a freeliving ovoviviparous nematode. The first of four molts during larval developement occurs within the egg, so that the first free-swimming stage is the second larval stage (L2) which averages 350 ± 50 microns in length (Samoiloff and Pasternak, 1969). We have named the second and third larval stages (L2 and L3) the *eutelic period*, during which there is relative somatic nuclear - and therefore cell number - con-

stancy. The last larval stage (L4) and the adult stage comprise the *gametic period* during which rapid gametogenesis occurs. We wish to show that ketoaldehyde levels are high during the eutelic period and low during the gametic period.

MATERIALS AND METHODS

Cultures of *P. silusiae* (MacMillan Science Company) were maintained in plastic refrigerator boxes at 21 to 23°C on a medium of 1 part Gerber Mixed Cereal to 1 part tap water. Worms migrated up the sides of the containers and were scraped from the sides relatively free of adhering medium, and in large numbers. Worms were added to cold 0.25 M sucrose in graduated Pyrex centrifuge tubes until they settled to a volume of 2 to 3 ml and they were washed 6 times with centrifugation at 2500 x g for 3 to 4 minutes. The worms were then quickly rinsed three times in distilled deionized water to remove the sucrose, and were separated by size as outlined by Samoiloff and Pasternak (1969). Equal volumes of 0.500-0.420 mm and 0.297-0.250 mm spherical glass beads (Microbeads Cataphote Division, Jackson, Miss.) were mixed and placed in a 125 ml cylindrical separatory funnel to a depth of 5 cm. The worms and 100 ml distilled deionized water were added slowly and allowed to drip out at a rate of 1 drop per second. The L2 worms readily passed through the beads while the passage of the larger worms was inhibited. The first 50 ml of effluent, containing numerous L2 worms and no larger ones, was collected and centrifuged at 2500 x g. Adult and late larval worms were collected form the top of the funnel after removal of the L2 stage; about 70% of the worms collected in this manner were adult or late larval.

A known volume of washed and separated worms was homogenized in an equal volume of distilled deionized water in a 3 ml pyrex tissue homogenizer, and the homogenate was separated into 1 ml samples for replicate studies; 0.1 ml of the homogenate was used to determine protein content following the Lowry *et al.* (1951) technique. Ketoaldehyde determination followed the Egyud *et al.* (1967) technique for rat liver ketoaldehyde determinations. The ArO_3 used by Egyud *et al.* was omitted as the amount required was prohibitively small for the sample size involved. The reaction mixture contained 1 ml each of worm homogenate with a known protein content, glacial acetic acid and EDAM reagent (10 ml ethylene diamine in 75 ml methanol) plus 2 ml of 50% methanol. This mixture was placed in a 15 ml screwtop test tube in boiling water for 10 minutes. The mixture was cooled and centrifuged at 2500 x g for 10 minutes to give a clear yellow supernatant. The per cent transmission was measured at 372 mμ in a Beckman DB-GT grating spectrophotometer (slit #1) against a standard using known concentrations of methyl gloyoxal. An 0.666 M solution of methyl glyoxal (methyl glyoxal grade II, 40% w/v aqueous solution, Sigma Chemical Co.) was made daily as a stock for the standardization.

RESULTS

Forty-five samples of the unseparated population were measured for ketoaldehyde content. Thirty-three adult and seven L2 samples were measured. The specific activity of ketoaldehydes was calculated on the basis of 100 ug cellular protein per ml. Ketoaldehyde levels of stages of the life cycle in *Panagrellus silusiae* are summarized in the following table (Table 1).

DISCUSSION

Panagrellus silusiae has been shown by Pasternak and Samoiloff (1972) to have an exceedingly low rate of mitosis during the approximately 50 hours of the eutelic period. Pasternak and Haight (1975) have studied embryogenesis in *P. silusiae*, and their published data suggest that the onset of cell division inhibition in the eutelic system of *P. silusiae* is at the ninth round of nuclear division, from the 256 cell stage (L1) to the so-called 512 cell stage (L2) which has about 400 nuclei. The total growth period for *P. silusiae* requires 80 to 90 hours with the second generation larvae first appearing after about 120 hours (Samoiloff and Pasternak, 1968, 1969; Chow and Pasternak, 1969). There is an average of 410 somatic nuclei in the L2 stage and 553 in the adult stage (Sin and Pasternak, 1970), indicating that *P. silusiae* departs from perfect eutely by about 26% during the entire life span, and only by about 18% during the eutelic period. Thus the large increases in size from 350 to over 1000 microns are accomplished primarily by an increase in cell volume. This deviation from perfect eutely may cause an elevation of the ketoaldehyde levels in the adult samples.

Pasternak and Samoiloff (1972) demonstrated a large jump in the rate of postembryonic DNA synthesis as indicated by a continuously increasing rate of ^{3}H-thymidine incorporation. They showed a 22 x increase during the eutelic period followed by only an additional 4 x increase during the gametic period. This early large increase in DNA occurs without a large increase in cell number; the relatively small increase in the number of somatic nuclei (1, 3 x) is limited to intestinal and tissues, while the hypodermis and nerve tissues remain constant. The large increase in DNA synthetic rate (87 x) without a correspondingly large increase in the amount of nuclear division is best explained by the development of polyploidy, although the possibility of polyteny cannot be eliminated. The L2 muscle nuclei show an essentially diploid value, but the muscle nuclei of the L3, L4 and adult stages show a slightly greater than diploid value. In intestinal nuclei, the values jump quickly from the diploid level in the L2 to an average 3.5 x the expected diploid 2C value in L4. This increase fell into three classes reflecting ploidy values of 4C, 8C and 16C (Sin and Pasternak, 1970). Sin and Pasternak (1970) also showed a varying increase in cell volume, 1 to 2 x for nerve, muscle and hypodermis, and as much as 12 x for intestinal cells. This increase in cell volume is also to be expected in tissues that are undergoing polyploidy.

Ketoaldehydes inhibit division in prokaryotes (Egyud and Szent-Gyorgyi, 1966b) as well as in eukaryotes (French and Freelander, 1958; Szent-Gyorgyi *et al.*, 1967; Gregg, 1968). If ketoaldehydes are involved in the natural inhibition of cell division in *P. silusiae*, it is expected that the levels of ketoaldehydes would be high during the eutelic period, and would fall during the gametic period to allow gametogenesis. Our data confirm this. We show a high level for the L2 larvae and a low level for the L4 plus adult sample; the levels differ by a factor of 16. As the only discernable difference between the groups was age, we suggest that the levels of ketoaldehydes decrease as a function of larval maturation.

Although ketoaldehydes seem to play a role in the inhibition of cell division during the eutelic period, it is not yet possible, on the basis of existing knowledge, to identify the mechanism of ketoaldehyde action. Spindle formation is probably not involved because the inhibitory effect has been demonstrated in prokaryotes (Egyud and Szent-Gyorgyi, 1966a, 1966b). Inhibition of DNA synthesis has

already been ruled out by studies on mammalian tissues by Szent-Gyorgyi *et al.* (1967) and Gregg (1968), as well as by studies on *P. silusiae*, where Pasternak and Samoiloff (1970) showed a rapid increase in DNA synthesis during the eutelic period. Ketoaldehyde action by RNA inhibition has been ruled out by Pasternak and Samoiloff (1970) who showed that RNA synthesis is linear throughout the growth period, and is not affected by altering ketoaldehyde levels.

While the possibility remains that ketoaldehydes act by inhibition of a specific "mitotic protein" blockage of general protein synthesis, as was suggested by Szent-Gyorgyi *et al.* (1967), this seems unlikely to be active in *P. silusiae*. Normal end-stage development of the gonads in the late larval stages requires normal protein synthesis during the eutelic period (Pasternak and Samoiloff, 1970) when we show a high titer of ketoaldehydes. The studies of Chow and Pasternak (1969) of the enzyme proteins, such as malate dehydrogenase and lactate dehydrogenase, show unique polyacrylamide gel band patterns for each of the larval stages, giving additional evidence of extensive, although differential, protein synthesis during the eutelic period, when ketoaldehyde levels are high.

The inhibitory action of ketoaldehydes probably occurs during the G2 stage of interphase, but the identification of the exact mode of action must wait until detailed determinations can be made of the response of each of the various tissues involved - hypodermis, muscle, nerve, and intestine - to exposure to extraneous ketoaldehydes. Because of the number of tissues involved in the current assay technique, it has not been possible to pin-point ketoaldehydes within the organism. At this point ketoaldehydes cannot be convincingly implicated in the development of probable polyploidy in the intestinal cells in *P. silusiae*, but it does not seem far-fetched to study ketoaldehydes in this context.

In summary, it is probable that endogenous ketoaldehydes act in the process of eutely in the free-living nematode *Panagrellus silusiae* by interfering with some as yet unidentified G2 function, which results in apparent polyploidy. G2 inhibition would permit the continued DNA replication, and the increase in cell volume that is observed, but would prevent cell division itself.

ACKNOWLEDGEMENT

This work was supported by a grant from the ONR under contract NONR 352(3) NR 104-925.

The authors wish to express their appreciation to Dr. Ruth L. Willey for her valuable criticisms during the preparation of this manuscript.

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Table 1. Ketoaldehyde Content in *Panagrellus sillusiae*.

Number of Replicate	Samples	m/Moles/100 ug Protein
Unseparated	45	0.75 ± 0.25 s.d.
Adult (gametic)	33	0.56 ± 0.36 s.d.
L2 (eutelic)	7	9.66 ± 1.11 s.d.