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Research Article

Nature of Wriggling Action of the Filarial Parasite *Setatia digitata*

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Abstract

Setaria digitata, a cattle filarial parasite, is always in constant motion called the wriggling movement. It is known to take up oxygen even in presence of cyanide. One of the partial reduction products of oxygen formed in the parasite is H_20_2, generated by the parasite specific quinol oxidase called alternative oxidase. The wriggling movement as well as the activity of the parasite specific quinol oxidase and formation of H_20_2 was found to be SHAM sensitive. The wriggling movement was also found to be inhibited by iodoacetate a specific inhibitor of SH groups, showing the involvement of SH groups movement of the parasite. Under *in vitro* conditions H_20_2 was found to increase the wriggling movement of the parasite and the increase was found to be reversed in presence of added catalase. These findings definitely suggest that the wriggling movement of *S. digitata* is associated with the reduction of oxygen. The process appears to be maintained by the hemoglobin, GSH/GSSG system and the SHAM sensitive quinol oxidase activity.

Keywords: *Setaria digitata*; Wriggling Action; Hydrogen Peroxide; Alternative Oxidase

Abbreviations

SHAM: Salicyl Hydroxamic Acid; H_2O_2: Hydrogen Peroxide; Ubiquinones; Q_0, Q_6 and Q_8.

Introduction

Setaria digitata is located in the peritoneal cavity of the host, where oxygen tensions are low. It is a lymphatic nematode of the cattle *Bos indicus* and is recommended by WHO [1] as one of the model systems for the study of human filariasis. This parasite is devoid of typical cytochromes [2] and possesses the ubiquinone Q_6 and Q_8 respectively showing electron transport [3] and antioxidant properties [4]. It also possesses many other unique features such as rotenone sensitive and insensitive NADH dehydrogenase [5] mitochondrial lactate oxidation, presence of glyoxylate cycle, absence of glucose-6-phosphatase etc. Presence of activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and nitric oxide synthase [6] and presence of a number of nonenzymatic antioxidants such as glutathione, a-tocopherol, ubiquinol Q_8. and carotenoids [7] are also reported. *S. digitata* shows high and continuous wriggling activity *in vitro.* This requires enormous amount of energy production and should be supplied continuously. The functional machinery and mechanism of energy production for this puzzling activity and its relevance in the physiology of the worm is not well understood. The studies carried out using H_2O_2 as modulator may give a possible mechanism and components for generating energy in these parasites.

Parasites in general were once considered to be facultative anaerobes utilizing oxygen when available. Though classes as a facultative anaerobes *S. digitata* takes up oxygen even in presence of cyanide [8], shows parasite specific alternative oxidase activity and H_2O_2 is considered to be a product of this parasite specific oxidase [5]. It also possesses functional hemoglobin like system [9]. The formation of H_2O_2 in living systems as a part of metabolic activity of oxygen. In fact, catalase and other peroxidases utilize H_2O_2 in some cellular oxidation processes leading to several important metabolites [10]. Nematodes including filarial parasites are always in constant motion, known as the wriggling movement. Ubiquinone (Qo) and a known antifilarial drug Diethyl carbamyzine (DEC) have also been shown to inhibit this movement [11,12]. Further study on the wriggling movement of *S. digitata* in response to certain aspects of the metabolism of oxygen forms the subject matter of this paper.

Materials and Methods

Setaria digitata located in the peritoneal cavity of cattle *Bosindicus* were collected in Tyrode medium [13]. From the local abattoir Tyrode medium has a composition of Sodium chloride 0.8% Potassium Chloride 0.02% Calcium Chloride 0.02% Magnesium Chloride 0.01% Sodium Bicarbonate 0.015%, Sodium Dihyrogen Phosphate 0.05% and Glucose 0.5%. The extraneous materials sticking on the surface of the parasite were removed by thorough washing with the medium. The worms were then kept in tyrode medium at 37 ^{0}C until use.

Biochemicals used in the study were purchased from Sigma Chemical Company, USA and all other chemicals used were of high purity.

Measurement of wriggling score

In order to find out the role of quinone in designing antifilarial drug the experiments were carried out by incubating the live worms in presence of H_2O_2, Coenzyme Q, SHAM, and Iodoacetate, glucose oxidase and catalase, were dissolved uniformly in ethanol and water respectively, according to their solubility. Four worms were put in 25. 0 ml of tyrode solution in the presence and absence of inhibitors.

The peristaltic type wriggling movement from one side to the other and back on the medium was scored as one and counts were made for one minute on each organism at the periods indicated is recorded.

Results

The normal wriggling score of the worm when kept in Tyrode medium was measured and the values are presented in Table 1. Maximum wiggling movement was obtained in the first hour followed by a gradual decrease in the score during subsequent hours. The effect of H_2O_2, a product considered generally to be an end product of cyanide sensitive parasitic organisms [12] and fond to be produced in this parasite [3,9] and the wiggling score was examined. With increase in concentration of H_2O_2, the score was found to increase initially and then dropped and this is clear from figure 1.

Time (hr)	Wriggling score/min
1	8.3 ± 0.13
2	8.1 ± 0.09
3	7.4 ± 0.13
4	7.1 ± 0.07
5	6.8 ± 0.10
6	5.1 ± 0.08

Table 1: Normal wriggling rate of *S. digitata* in Tyrode medium. Mean ± SE, n = 6.

The effect of added H_2O_2 in the incubation medium on the wriggling action is given in the fig 1.it is clear from the result that H_2O_2 induces the wriggling movement. As increase in the concentration of the H_2O_2 from 0.015 to 0.025 micromoles increased the wriggling score from 8.3/min to about 12/min. However with further increase in the concentration of H_2O_2 the rate of the movement fell rapidly and the worms got paralyzed.

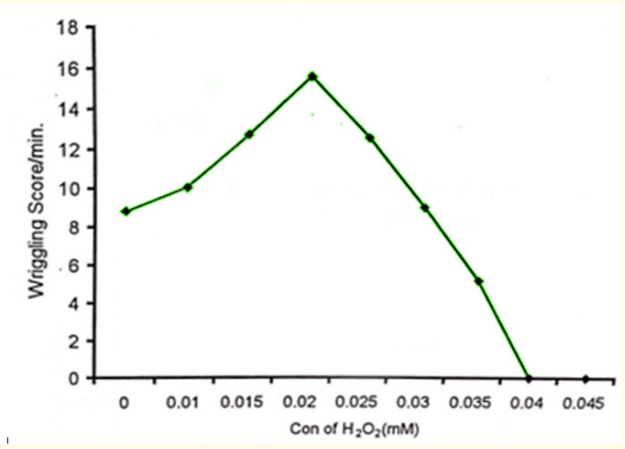

Figure 1: Effect of H_2O_2 on Wriggling Score.

Addition of catalase reversed the effect and the result is shown in figure 3. the paralyzed worm did not show reversal of the effect on addition of catalase. However the enhanced rate of wriggling did come down to normal rate following the addition of catalase in a dose dependent manner.

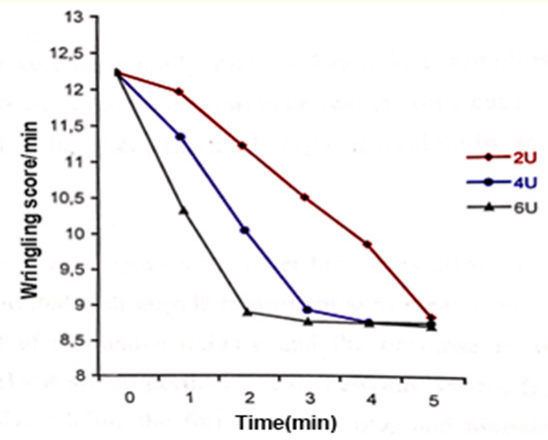

Figure 2: Effect of catalase on H_2O_2 induced Wriggling Score.

System	% of wriggling score at 30 min	Nature of worm
A. Tyrode without glucose +Qo(0.16mm)	0	(+)
A + catase	0	(+)
B. Tyrode without glucose +SHAM	0	(+)
B + catase	0	(+)
C. Tyrode without glucose + Iodoacetate	0	(+)
C + catalase	0	(+)
D. control (system minus Q_0, SHAM, Iodoacetate)	100	(-) Normal score

Table 2: *In vitro* effect of Qo , SHAM , and Iodoacetate in the wriggling score of *S. digitata* in different systems.

N = 6 (+) Total paraysis of worm within 10 min,

Q_o 2,3Dimeithoxy 5 –methyl 1,4 benzoquinone

(-) No paralysis.

The induction of wriggling movement observed with H_2O_2could be reversed with the addition of catalase and hence H_20_2, or H_2O_2 generating systems appears to have a modulatory role in the wriggling movement of the parasite. This is further confirmed by *in situ* generation of H_2O_2 by glucose oxidase system where H_20_2 is released according to the reaction.

Glucose +O_2 ---------->Gluconolactone + H_2O_2

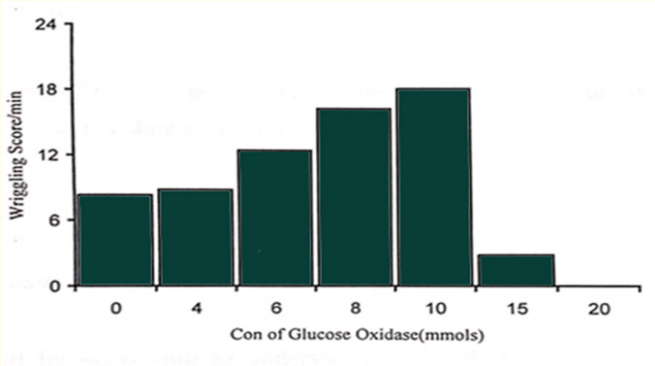

Figure 2: *In situ* Generation of H_2O_2 on the Wriggling movement.

Unlike other intermediates of oxygen, the generation of H_2O_2 cannot be dismissed as a mere undesirable byproduct because of some of these observations made [14]. It was also observed that even at high concentrations, H_20_2 does not induce lipid peroxidation in the parasite [6].

In systems where oxygen uptake is cyanide insensitive, the reduction is carried out by a cyanide insensitive alternative oxidase [15,16] in fact the presence of SHAM sensitive alternative oxidase has been reported in many other cyanide insensitive system particularly in plants. But, unlike *Setaria,* such systems have prominent cytochrome oxidase system. In some it is also a major route of oxidation while in others as in *Setaria* the system is absent or present in significant amounts [15] additionally unlike the plant systems studied, the filarial system reported here is in constant motion and aerobic oxidation takes place via the alternative route [3].

In plant system generally this oxidation is a non-phosphorylating step. However, instances of heat generation is known in some cases and there are recent reports indicating that ROS particularly H_2O_2 is used up by some of the systems [17].

In the *Setaria* system on the other hand it is different and the process is intimately connected with muscle movement as it is clear from SHAM sensitivity. The inhibition of alternative oxidase and the decrease wriggling score in presence of SHAM are proportional. It was already reported from this laboratory that SHAM is also inhibits the formation of H_2O_2 and associated generation of ATP [3]. Lower organisms generally depend on glycolysis for their energy needs and the case of *S. digitata* cannot be different. This is clear from the fact that the major steps in Krebs's citric acid cycle leading to the generation of NADH are missing in *Setaria*. It is true that, though NADH generation is considerably low in *Setaria,* NADPH is relatively high because of the presence of malic enzyme and pentose oxidation [18]. High activities of glutathione peroxidase, glutathione-S-transferase and glutathione reductase indicate a predominant role for the GSH/GSSG system in the metabolism of *S. digitata* [7]. This and the capability of the parasite to synthesize sterols [19] demand a high NADPH level and hence, the latter may not be available for conversion to NADH using the trans-hydrogenases present in the parasite [20]. The presence of hemoglobin system in *Setaria digitata* [9] offers promise for a steady supply of oxygen even though the oxygen tension in the parasite environment is relatively low. Studies with diethyl maleate reveal that depleting glutathione can cause the paralysis of the parasite. The effect of SH-inhibitors on the wriggling movement further confirms the role of thiol compounds in the wriggling movement.

Conclusion

Hence, it is concluded that the wriggling movement of *S. digitata* is associated with aerobic oxidation leading to the generation of H_2O_2.The process appears to be maintained by the hemoglobin, GSH/GSSG system and the SHAM sensitive quinoloxidase activity. The oxidation of a protein sulphide to a disulphide can lead to contraction and reduction of the oxidized form can restore the original form, and these two actions can result in the wriggling motion shown by the parasite. The parasite specific quinol oxidase generally called the alternative oxidase is currently under detailed investigation.

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Conflict of Interest

Authors do not have any conflict of interest for the present investigation.

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