



QUANTITATIVE ASPECT OF PHOSPHORYLASE (AB AND A) IN ASCARIDIA GALL/COLLECTED FROM THE AVIAN HOST GALLUS GALLUS DOMESTICUS

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Abstract

The phosphorylase (ab and a) metabolism in nematodes parasites shifts according to the availability of oxygen. Therefore, the study of phosphorylase (ab and a) metabolism is quite interesting not only from biochemical point of view but also from the point of view of chemotherapy as the number of metabolic steps increase, this may lead to an increase in the number of vulnerable points.

In present communication the quantitative estimation of phosphorylase (ab and a) was done to understand the rate of glycogenolysis in *Ascardia galli (schrank, 1788*) Freeborn 1923 collected from the avian host *Gallus gallus domesticus* from Nanded region (M.S.) India.

Keywords: A. galli, Gallus gallus domesticus, Nanded region, Phosphorslase (ab and a)

Introduction

Phosphorylase catalyzes the initial step in glycogenolysis. It catalyzes the breakdown of glycogen, a polymer of glucose to yield Glucose-1-phosphate.

(Glucose)n +Pi →	$(Glucose)_{n-1} +$	Glucose-1-
Glycogen	Shortened glycogen	Phosphate
molecule		

In above reaction the terminal (1+n) glycosidic linkage at the non-reducing end of the glycogen siderchain undergoes phosphorlysis. The cleavage of the terminal glycosidic bond results in the removal of the terminal glucose as glucose-1-phosphate leaving behind a glycogen chain with one less glucose unit.

Present study indicate that the activity of the enzyme was assayed towards the synthetic side. It exists in two forms 1) the active form phosphorylase 'a' and 2) the inactive form phosphorylase 'b'. The phosphorylase 'a' which consist of four identical subunits with each subunit containing a phospho serine residue that is essential for catalytic activity, can be converted to phosphorylase 'b' by the action of the hydrolytic enzymes phosphorylase phosphatases which removes the phosphate groups from the serine phosphate residues (Kellar and Cori, 1953). Again phosphorylase 'b' can be coverted back to active form of phosphorylase 'a' by the intervention of a different enzyme phosphorylase kinase where four molecules of ATP act on two molecules of the 'b' form (Fischer and Kreb's, 1955).

Its activity in few nematodes has been assayed by Rathbone and Rees, (1954), Saz and Hubbard, (1957), Bueding and Saz, (1958), Cavier and Savel, (1959), Srivastava, *et al*, (1968), Srivastava, *et al*, (1970) and Srivastava and Ghatak, (1971).

They focused on the experimental infection of nematode parasites *Ascaridia galli* therefore, this is an attempt to understand the activity levels within the naturally infected domestic fowl.

Materials and Methods

The phosphorylase (1,4 ,glucan : orthophosphate glycosyltransferase, EC 2.4.1.1) activity was determined by the method of Cori *et al.*, (1965).

5% homogenates of male and female worms were prepared in EDTA (0.037M) and NaF (0.IM) buffer. The homogenates were diluted four times in cysteine-βglycerophosphate buffer (0.03M cystein hydrochloride and 0.015M sodium- β -glycerophosphate). To 0.4 ml of each of the above diluted homogenates, 0.2 ml of 2% alvcogen was added and kept for incubation for 20 minutes. The samples, as above, were maintained separately for total (ab) and active (a) phosphorylases. After incubation, 0.2 ml of glucose-1-phosphate (0.016M) was added to the samples of active phosphorylase and 0.2 ml of glucose-l-phosphate and AMP solution (0.016M glucose-1-phosphate and 0.004 M AMP, in equal proportions) was added for phosphorylase ab and incubated for half an hour and 15 minutes, respectively. The reaction was stopped in both the samples by adding 1 ml of TCA. To the controls the TCA was added before adding glucose-lphosphate. The activity of phosphorylase (ab) and (a)

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was estimated and expressed in terms of Pi liberated/hour/100 mg tissue by the method of Fiske and Subba Row (1925).

Results and Discussion

Sex	Activity	Male to female ratio ($3/2$)	Percentage difference	
	Mean ± S.D.		(%)	
Male (08) (♂)	188 ± 62	0.302	69.77	
Female (08) (♀)	622 ± 133			

Table 01: Phosphorylase (ab) activity in male and female nematode parasites of Ascaridia galli

• Values puted in parentheses showing number of samples estimated

• Values expressed in µgm of pi/hour/100mg tissue

Graph 01: Phosphorylase (ab) activity in male and female nematode parasites of Ascaridia galli

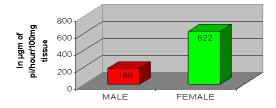


Table 02: Phosphorylase (a) activity in male and female nematode parasites of Ascaridia galli

Sex	Activity	Male to female ratio (♂/♀)	Percentage difference (%)
	Mean ± S.D.		
Male (08) (♂)	170 ± 78.00	0.412	58.73
Female (08) (♀)	412 ± 183	0.412	30.73

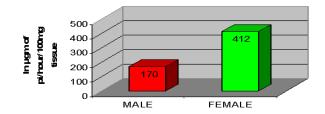
· Values puted in parentheses showing number of samples estimated

• Values expressed in µgm of pi/hour/100mg tissue

Ratio between phosphorylase (ab)/(a)

Parameter details	Male (♂)	Female (♀)
Phosphorylase (ab) / Phosphorylase (a)	1.105	1.509

Graph 02: Phosphorylase (a) activity in male and female nematode parasites of Ascaridia galli



The experimental values are tabulated in observation table no. 01 and 02 and are represented in graph no. 01 and 02. The activity of the present study indicate that the total phosphorylase (ab) activity in males and females is 188 ± 62 and $622 \pm 133 \mu gm/pi/hour/100 mg$ tissue, respectively. The activity is more in females by 69.77% as indicated by the male to female ratio of 0.302 $\mu gm/pi/hour/100 mg$ tissue.

The phosphorylase 'a' activity in males and females is 170 ± 78.00 and $412 \pm 183 \ \mu gm$ of pi/hour/100 mg tissue, respectively. The activity is more in females by 58.73% as indicated by the male to female ratio of 0.412 $\mu gm/pi/hour/100$ mg tissue, the content of phosphorylase activity was in conformity with the result obtained by the other investigators.

The ratio between phosphorylase (ab) and phosphorylase (a) is in males about 1.105 and in females about 1.509.

References

- Bueding, E., and Saz, H.J. (1958): Pyruvate kinase and PEP carboxy kinase activities of *Ascaris* musle, *H. diminuta* and *S. mansoni*. Comp. Biochem. Physiol. 24, 511-518.
- Cavier, R., and Savel, J. (1959): Les reactions de phosphorylation liees qu metabolism glucidique chez *A. lumbricoides* Linne, 1758. Int. congress of Zoology (15th) London. July, 16-23, 1958. Proc. 937-938.
- Cori, G.J., Illingworth, B., and Keller, P.J. (1965): In methods in enzymology. Edscolowick, S.P. and Kaplan, N.O. Academic Press, New York, Vol.1, 200.

- Fischer, E.H., and Krebs, E.G. (1955): Conversion of phosphorylase to phosphorylase a in muscle extracts. J. Biol. Chem. 216, 121.
- Fiske, CH., and Subbarow, Y. (1925): The colorimetric determination of phosphorus. J. Biol. Chem. 66, 375-400.
- Freeborn, S.B. (1923): Nicotine as a poultry vermifuge Science. 57, 692-693.
- Keller, P.J., and Cori, G.T. (1953): Enzymic conversion of phosphorylase a to phosphorylase b. Biochem. Biophys. Acta. 12, 235.
- Rathbone, L., and Rees, K.R. (1954): Glycolysis in *Ascaris lumbricoides* from the pig. Biochem. Biophys. Acta. 15, 126-133.
- Saz, H.J., and Hubbard, J.A. (1957): The oxidative decarboxylation of malate by *Ascaris lumbricoides*. J. Biol. Chem. 225, 921-933.
- Schrank, F. (1788): Verzeichniss der bisher hinanglich bekannten Eingeweide wurmernebst einer. Abhandlung uber irhre Anverwantschaften. 1, 116.
- Srivastava, V.M.L., and Ghatak, S. (1971): Indian J. Biochem. Biophys. 8, 108-111. (Quoted by Von Brand 1973. Biochem. Gtry of Parasites. A.P. New York).
- Srivastava, V.M.L., Ghatak, S., and Krishna Murthy, C.R. (1968): *Chandlerella hawkingi*: Glucose utilization and glycolytic enzymes. Exp. Parasitol. 23, 339-346.
- Srivastava, V.M.L., Ghatak, S., and Krishna Murthy, C.R. (1970): *A. galli* lactic acid production, glycogen content glycolytic enzymes and properties of purified aldolase enolase and G-6-PD.Parasitology.60,157-180.