OCCURRENCE OF A HIGH AMOUNT OF GLYCERYLPHOSPHORYLCHOLINE IN *ASCARIS LUMBRICOIDES*

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1. Introduction

In the body-wall muscle of *Ascaris lumbricoides* an acid-soluble phosphorus compound was found which did not liberate inorganic phosphate after prolonged acid hydrolysis; its amount is up to 20 μmoles/g fresh weight [1]. The present paper gives evidence that this compound is glycerylphosphorylcholine.

2. Methods

Fresh body walls of *Ascaris lumbricoides* were frozen in liquid nitrogen, pulverized and extracted with trichloroacetic acid. After removing the protein precipitate, the solution was extracted repeatedly with ether to remove trichloroacetic acid and other organic acids. After precipitation with barium acetate at pH 8.3 in 82% ethyl alcohol [2] the supernatant was concentrated by vacuum evaporation, barium ions were removed by Dowex 50 and acetic acid by repeated ether extraction.

High voltage paper ionophoresis was performed in salicylate buffer pH 3.9 [3] and in borate buffer, pH 7.2. For paper chromatography, solvent systems propanol-ammonia-water [4], phenol-acetic acid-water [5] and phenol-formic acid-water [5] were used. Spots of phosphorus compounds were detected by spraying with ferric chloride and sulphosalicylic acid [6]. Enzymic test was made with glyceryl-1-phosphate dehydrogenase (Boehringer & Soehne, Mannheim). Glycerylphosphorylcholine was prepared from pure lecithin [5].

3. Results

In paper ionophoresis with both buffer systems all phosphorus from the supernatant after barium-alcohol precipitation concentrated in a single zone near the start which did not give a white spot by the detection method used. After acid hydrolysis all phosphorus appeared in a readily detectable spot with a mobility of glycerophosphate (0.83 with respect to orthophosphate). The identity of glycerophosphate was confirmed by the enzymic test [7].

In paper chromatography, the compound did not give positive ninhydrine reaction and could be detected by ferric chloride-sulphosalicylic acid only after treatment of the developed chromatogram with

Table 1
R_f values of the glycerophosphate derivative from *Ascaris* muscle compared with glycerylphosphorylcholine.

Chromatography system	R_f values			
	E	GPCH	E + GPCH	E – GP
1	0.97	0.97	0.98	2.82
2	0.51	0.51	0.51	1.33±0.04
3	0.79	0.80	–	1.04

Chromatography systems: 1) phenol-acetic acid-ethanol [5]; 2) propanol-ammonia-water [4]; 3) formic acid-methanol-water [5]. Despite the high R_f values in the system 1 the shape of the spots indicated that the extracted compound (E) did not migrate with the solvent front. The highest separation was revealed in system 2 in which α- and β-isomers of glycerophosphate (GP) separate readily; no sign of separation of glycerylphosphorylcholine (GPCH) and E could be observed in long runs not allowing determination of true R_f values.

hydrochloric acid and heating. In all three systems used it had R_f values identical with glycerylphosphorylcholine; mixtures of both compounds wandered as a single spot (table 1).

The compound was not adsorbed on charcoal, Dowex 50 in H^+ cycle, or Amberlite in OH^- cycle [5], and had no characterisitc UV absorption.

All these properties suggest with a high probability that the compound from Ascaris body-wall muscle is glycerylphosphorylcholine. A substance with identical properties (solubility, ionophoretic and chromatographic behaviour, enzymic test) was also found in other worms: in the liver fluke *Fasciola hepatica* in an amount comparable to that of Ascaris, in the free-living flatworms *Planaria lugubris* and *Planaria gonocephala* in concentrations 1–2 μmoles/g fresh weight.

References

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