

The M-N stretching band for these uranyl complexes have been found to occur in the region 350–380 cm^{-1} and 500–540 cm^{-1} and show medium to weak intensities⁴.

The above discussion on the properties of these complexes show that uranyl *bis*-phenyl acetate forms mixed ligand complexes with the amines undertaken which are apparently seven or eight coordinated.

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HOST PARASITE RELATIONSHIPS IN *CHANNA PUNCTATUS* AND *EUCLINOSTOMUM HETEROSTOMUM*

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ABSTRACT

Free and protein amino acids of cyst wall and metacercaria of *E. heterostomum* and infected and uninfected host liver have been qualitatively and quantitatively studied. Methionine, lysine, serine and cystine are found in worms but absent in cyst wall (lysine and cystine are even absent in host tissue). Obviously the worms have the enzymatic ability to form these amino acids by interconversions.

INTRODUCTION

EUCLINOSTOMUM heterostomum occurs as cysts in the liver of *Channa punctatus*. When fed to herons, they excyst and attain sexual maturity in 6 days in the mouth cavity of herons^{1,2}. During the encystment phase (the metacercaria being progenetic, the encystment phase is almost six months), nourishment is from host liver through cyst wall. An elementary report on electrophoretic behaviour of infected liver of *C. punctatus*, cyst wall and worms was presented³. The present authors have studied free and protein amino acids of host liver, cyst wall and worms to assess correlation between host parasite physiology.

MATERIALS AND METHODS

Cysts of *E. heterostomum* were recovered from *C. punctatus* just killed, washed with distilled water, dried on filter paper and later teased. Excysted worms, cyst wall, infected and uninfected liver were washed several times in double distilled water; blotted dry on

Whatman filter paper and dried at 80° C for 10 hr. Equal weights of worms, cyst wall, infected and uninfected liver tissues were taken and the free amino acids extracted in 70% ethanol.

For protein amino acids, protein was precipitated in 14% TCA; acetone, followed by ether, used to remove fats; phospholipids were removed with a mixture of methanol: chloroform (1:1 v/v) at 55° C; nucleic acids removed by using 7% TCA; the protein was then dried and hydrolysis carried out in 6 N HCl at 115° C for 6 hr. HCl was later evaporated in vacuum and the hydrolysates diluted appropriately with 70% ethanol. Amino acids were detected by uni- and two-dimensional paper and thin layer ascending chromatography using *n*-butanol: acetic acid: water (4:1:1.6 v/v) and *n*-butanol: pyridine: water (1:1:1 v/v) as solvent systems and 0.2% ninhydrin in acetone as locating agent. Identification was by comparing R_f values with standards developed under identical conditions. They were eluted in 70% methanol and quantitative analysis done colorimetrically at 570 nm using

TABLE I

Free and protein amino acids of E. heterostomum (metacercariae), cyst wall, uninfected and infected liver.

Amino acids	E. heterostomum		Cyst wall		Uninfected liver		Infected liver	
	FAA	PH	FAA	PH	FAA	PH	FAA	PH
Leucine	+	+	+	+	+	+	+	+
Isoleucine	+	+	+	+	+	+	+	+
Phenylalanine	+	+	+	+	+	+	+	+
Valine	+	+	+	+	+	+	+	+
Methionine	+	+	—	+	+	+	+	+
Tyrosine	+	+	+	+	+	+	+	+
α -amino- <i>n</i> -butyric acid	—	—	—	+	—	+	—	+
Proline	+	+	+	+	+	+	+	+
Alanine	+	+	+	+	+	+	+	+
Threonine	+	+	+	+	+	+	+	+
Glutamic acid	+	+	+	+	+	—	+	+
Glycine	+	+	+	+	+	+	+	+
Arginine	+	+	+	+	+	—	+	—
Aspartic acid	+	+	+	+	+	+	+	+
Serine	+	+	—	—	—	+	+	+
Histidine	+	+	+	+	+	+	+	+
Ornithine	+	+	+	+	+	+	+	+
Lysine	+	+	—	—	—	—	—	—
Cystine	—	+	—	—	+	—	—	—
Tryptophan	—	—	—	—	—	—	—	—

FAA = Free amino acid, PH = protein hydrolysates, + = Present, — Not detected.

leucine as standard. Proline was not quantified. Data are mean values of 3 observations.

RESULTS

Free and protein amino acids of worms and cyst wall (table 1) reveal that serine, lysine (in FAA and PH) and cystine (in PH) are present in worms and absent in cyst wall; methionine (present in worm in both pools) is absent in the free pool of cyst wall; and further, α -amino *n*-butyric acid (absent in worms) is present in cyst protein. Comparing host tissue with cyst wall and worm, lysine is absent in either pool in host tissue (like cyst wall but unlike worms); cystine is found only in free pool in uninfected liver (unlike cyst wall and worm), while, serine is found both in free pool and protein (unlike cyst wall but like worm);

methionine is present both in free and protein pools (unlike cyst wall but like worm) and α -amino *n*-butyric acid is present only in protein pool (like cyst wall but unlike worm); and further glutamic acid and arginine are absent in protein of host tissue (unlike cyst wall and worm).

Quantitative analysis of free and protein amino acids in worms (table 2) reveals a near correspondence in the free and protein pools, except that alanine and glycine are in higher percentage in free pool, while glutamic acid, aspartic acid, arginine, leucine and isoleucine are relatively more in protein.

DISCUSSION

Encysted metacercaria of *E. heterostomum* in the liver of *C. punctatus*, obtaining nourishment from host tissue through cyst wall, provides an interesting model to study the host parasite interactions.

TABLE 2

Quantitative analysis of free and protein amino acids in *E. heterostomum* (metacercaria).

value in %

Amino acids	Free amino acids	Protein hydrolysates
Leucine + Isoleucine	12.38	21.34
Phenylalanine	2.04	3.24
Valine	7.47	7.55
Methionine	1.30	2.35
Tyrosine	2.73	1.92
α -amino-butyric acid	—	—
Proline	Not quantified	Not quantified
Alanine	27.09	10.25
Threonine	3.22	4.52
Glutamic acid	11.55	13.43
Glycine	13.32	3.97
Arginine	3.16	9.50
Aspartic acid	3.37	6.08
Serine	0.96	1.06
Histidine	4.02	5.40
Ornithine	5.29	7.89
Lysine	2.08	0.81
Cystine	—	0.68

Amino acids when classified according to their charge:-

1. Non polar (Hydrophobic)	50.28	44.73
2. Polar (Neutral)	20.23	12.19
3. Polar (Positively charged)	14.55	23.60
4. Polar (Negatively charged)	14.92	19.51

Methionine, cystine, serine and lysine, found in both free and protein pools of worms, are not found in the free pool of cyst wall (cystine and lysine are even absent in host tissue). Obviously, these are formed by interconversions and the worms have the enzymatic capability to do so. Earlier, Gaur and Agarwal⁴ reported the presence of asparagine in the free pool of metacystode of *Hydatigera taeniaeformis* and its absence from the free pools of cyst fluid and host tissue (rat). Arme and Whyte⁵ found proline constituting 70% of free pool (as against 6% in host blood and mucus) and only 5% of protein pool of *Dichidophora merlangi*. Bologun and Braide⁶ too found levels of amino acids in free pool of *Fasciola gigantica* very different from the host tissue. Thus, free and protein amino acids of species of worms are not entirely host dependent and that species of worms can synthesise amino acids by transamination and interconversion. von Brand⁷ considered α -ketoglutaric-glutamic acid and pyruvic-alanine system of transamination widely occurring in parasites.

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