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Free and Protein Amino Acids in *Lytocestus indicus*, *Introvertus raipurensis* and *Lucknowia indica* Parasitizing *Clarias batrachus* (Linn). (Cestoda: Caryophyllidae)

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Introduction

Amino acid pool and/or protein hydrolyzates of *Hymenolepis diminuta* (Aldrich *et al.*, 1954; Goodchild *et al.*, 1957; Foster *et al.*, 1959; Campbell, 1963; Hopkins, 1964; Graff *et al.*, 1964 and 1965; Kilejian, 1966 a,b; Chappel *et al.*, 1973; Defraites *et al.*, 1976; Lussier *et al.*, 1978 and 1980), *Hymenolepis microstoma* (Litchfort, 1970), *Moniezia expansa* (Campbell, 1960; Goodchild *et al.*, 1966), *Thysanosoma actinoides* and *Cittotaenia perplexa* (Campbell, 1960), larval and adult *Hydatigera taeniaformis* (Goodchild *et al.*, 1966; Gaur *et al.*, 1981), *Rallietina cesticillus* (Foster *et al.*, 1959; Goodchild *et al.*, 1966), *Taenia pisiformis*, *Taeniarhynchus saginatum*, *Dipylidium caninum* (Goodchild and Dennis, 1966) and of some cestodes from elasmobranch fishes (Simmons, 1960) have been studied so far.

Clarias batrachus harbours several species of Caryphyllidae, namely, *Lytocestus indicus* Moghe, 1925, *Pseudocaryophyllaeus indica* Gupta 1961, *Djombangia indica* Satpute and Agarwal, 1974, *Introvertus raipurensis*, Satpute and Agarwal, 1980, and *Lucknowia indica* (Niyogi *et al.*, 1982).

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Satpute and Agarwal (1980) found one or more of these species of Caryphyllidae in 50 to 60% of *C. batrachus* by the monthly survey on about 20 fishes per month, for two years in 6 different tanks of Raipur.

The present authors undertook to study free amino acids and protein hydrolyzates of *L. indicus* (from male and female hosts separately) and *I. raipurensis* (both parasitizing duodenum) and *L. indica* (parasitizing intestine) with a view to assess whether the different species of Caryophyllidae in the same habitat or in different habitats show biochemical individuality.

Materials and Methods

Freshly recovered living worms were washed thoroughly in glass distilled water, blotted dry on a filter paper and later dried in an oven at 80 C. Extraction was done by the method of Taylor and Haynes (1966).

(i) Free Amino Acids-Dried worms homogenised in 70% ethanol, kept overnight for thorough extraction, centrifuged and supernatant used for chromatography.

(ii) Protein hydrolyzates- Dried worms homogenized in Hanks' saline. An equal volume of 14% TCA added to precipitate the protein and the whole centrifuged. The precipitate was then purified as

Table 1 Mean percentages of free and protein amino acids of 3 species of Caryophyllidae

SI. NO.	Amino Acids.	*Lytocestus indicus*				*Lucknowia indica*		*Introvertus raipurensis*	
		from male host		from female host					
		FAA	PH	FAA	PH	FAA*	PH	FAA	PH
1+2	Leucine+Isoleucine	16.03±0.72	17.31±1.80	14.62±0.02	15.54±3.45	23.22	14.95±1.44	20.72±2.29	16.85±1.27
3	Phenylalanine	1.67±0.28	3.49±1.29	1.11±0.14	4.67±4.18	3.25	3.50±1.26	2.41±0.53	2.13±0.33
4	Valine	6.36±0.14	7.11±0.41	5.39±0.98	6.56±1.07	9.37	5.68±1.33	5.95±0.07	6.92±0.11
5	Methionine	—	0.70±0.12	—	1.60±0.31	—	1.56±0.34	—	1.56±0.40
6	Tyrosine	1.21±0.09	1.30±0.21	1.03±0.24	1.61±0.33	1.42	1.70±0.43	1.69±0.16	1.93±0.27
7	α-amino-n-butyric acid	—	—	—	—	0.40	—	T	—
8	Proline	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
9	Alanine	34.51±5.85	12.02±0.24	35.70±1.26	13.68±1.71	11.61	14.58±1.58	17.02±0.36	11.60±0.41
10	Threonine	—	4.10±0.40	—	5.70±1.51	—	6.31±0.51	—	5.33±0.27
11	Glutamic acid	4.77±0.42	15.80±0.52	2.81±0.04	11.73±1.10	8.55	12.84±0.99	9.55±0.32	13.40±0.64
12	Glycine	6.21±0.43	3.04±0.24	9.00±0.49	5.01±2.67	12.42	5.45±2.49	10.85±3.29	3.90±1.71
13	Aspartic acid	5.92±0.91	9.73±0.18	6.52±0.61	5.92±0.54	7.74	7.63±1.44	7.31±0.71	9.75±1.91
14	Arginine	1.11±0.34	8.65±0.23	0.73±0.18	9.95±2.19	1.22	8.90±2.15	1.05±0.11	9.40±0.40
15	Serine	2.02±0.48	0.60±0.31	2.02±0.57	1.46±0.38	2.23	1.32±0.31	2.36±0.18	1.32±0.36
16	Histidine	2.03±0.60	6.80±0.29	1.30±0.12	6.51±1.49	3.25	6.42±1.68	8.39±0.02	7.06±0.36
17	Ornithine	2.35±0.72	9.44±0.48	1.81±0.59	9.99±1.63	4.88	9.12±1.70	—	8.81±0.45
18	Lysine	1.24±0.70		1.12±0.12		0.40		1.30±0.07	
19	Cystine	1.03±0.51	—	1.12±0.12	—	0.60	T	0.43±0.02	—
20	UI_1	T	—	1.30±0.12	—	1.42	—	1.23±0.17	—
21	UI_2	1.83±0.23	—	1.02±0.02	—	T	—	0.31±0.13	—
22	UI_3	0.83±0.43	—	0.82±0.31	—	1.22	—	1.30±0.07	—
23	UI_4	8.30±2.05	—	8.93±1.13	—	5.49	—	5.95±0.07	—
24	UI_5	2.42±0.64	—	3.52±0.67	—	1.22	—	2.06±0.03	—

FAA: Free Amino Acid, PH: Protein hydrolyzates, NQ: Not Quantified, T: In traces, —: Not present, $UI_{1\sim5}$: Un Identified spots 1 to 5, FAA*: Based on a single observation.

follows: washed twice in TCA, washed in acetone to remove fats, treated with a mixture of 1:1 methanol/chloroform at 55 C to remove phospholipids, washed in ether to remove remaining fats, treated with 7% TCA at 90 C for 20 minutes to remove nucleic acids and dried in acetone, followed by ether. Hydrolysis of this purified protein was then carried out in 6 N HCl at 115 C for 5 hours. The hydrolyzate was evaporated in vaccuo to dryness, redissolved 3 times in a small volume of water to remove all traces of acid and then taken up in 70% ethanol for chromatographic analysis.

Amino acids, both free and in protein, were detected by single and two dimensional paper and thin layer 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. 0.2% Ninhydrin in acetone was used as the locating reagent, followed by drying at room temperature. Amino acids were identified by comparing the Rf values with those of authentic samples developed under identical conditions. Quantitative analysis was done as described by Jayaraman (1981). Stained spots were cut into small strips from the paper chromatogram and eluted in 70% methanol. The colour intensity of the eluate was read photocolorimetrically at 570 nm.

Results

Qualitative picture of amino acids of free pool and protein hydrolyzates of *L. indicus, I. raipurensis* and *L. indica* (Table 1) reveals that threonine and methionine are absent in the free pool but most significantly present, the former about 4 to 6% and the latter about 0.7 to 1.6%, in the protein hydrolyzates of all the three species. Cystine is present in the free pool of all, but absent in the protein hydrolyzates of *L. indicus* and in traces in *I. raipurensis* and *L. indica*; α-amino-n-butryic acid preseent in the free pool of only *L. indica* and absent in the protein hydrolyzates of all species; certain unidentified spots (UI_{1-5}, Table 1), may be, amino-derivatives, while present in all species in free pool were absent in protein hydrolyzates.

Quantitative analysis shows that alanine constitutes the bulk (about 35%) of the free pool of *L. indicus*, while leucine and isoleucine are predominant in the free pools of *I. raipurensis* (about 21%) and *L. indica* (about 23%). The neutral amino acids (nonpolar and polar) alanine, leucine, isoleucine, valine and glycine constitute about 63 to 65% of the free pool of *L. indicus* and about 55 to 56% of *I. raipurensis* and *L. indica*. In protein hydrolyzates they almost exactly correspond in the three species, viz., 39 and 41% in *L. indicus* about 41% in *L. indica* and about 39% in *I. raipurensis*.

The polar negatively charged amino acids, aspartic acid and glutamic acid respectively are about 6% and 2–4% of the free pool in *L. indica* and *I. raipurensis*, whereas in protein they comprise respectively, about 6–9% & 11/16% in *L. indicus*, 7% & 13% in *L. indica* and 9% & 13% in *I. raipurensis*.

Arginine, histidine, ornithine and lysine, the other polar but positively charged amino acids, are about 4 to 7% of the free pool in *L. indicus* and about 10 to 11% of *I. raipurensis* and *L. indica*, whereas, they are nearly constant in protein in *L. indicus* (about 25, 26%), *I. raipurensis* (about 25%) and *L. indica* (about 24%).

Discussion

Absence of threonine and methionine in free pool and their presence (about 5 to 8%) in protein of all species under study is most revealing. This very clearly suggests that these caryophyllidae have the

enzymatic ability to convert cystine, serine, and/or alanine into threonine and methionine. Further, this also suggests that these worms are lumen feeders; if only they were tissue feeders, their free pool could not have been without these two amino acids.

Further, the proteins in all these caryophyllidae comprise about 40% neutral amino acids (leucine, Isoleucine, alanine, valine and glycine), about 25% polar positively charged amino acids (Arginine, histidine, ornithine and lysine) and about 20 to 25% of polar negatively charged amino acids (aspartic acid and glutamic acid). While leucine, isoleucine, aspartic and glutamic acid are much more in protein of *L. indicus*, recovered from males, all other amino acids are in greater concentration in worms recovered from female *C. batrachus*. Only marginal differences in the percentages of protein amino acids exist in caryophyllidae studied.

Near correspondence in the free and protein amino acid profiles of *L. indicus* and *I. raipurensis*, both from duodenum, and *L. indica*, from intestine, further suggests that possibly there is no duodenum as such in *C. batrachus*, only that the anteriormost portion is a little more distended. Detailed physiology of the regions hitherto considered as duodenum and intestine would be revealing.

Summary

Amino acids in free pool and in protein of *Lytocestus indicus* (from male and female *C. batrachus* separately), *Introvertus raipurensis* and *Lucknowia indica* have been both qualitatively and quantitatively studied. Methionine and threonine, not found in free pool, are significantly present in protein. About 40% non polar neutral amino acids, about 20–25% polar negatively charged amino acids and about 25% polar positively charged amino acids, besides threonine and methionine, constitute protein in all these caryophyllidae. Only marginal differences in the percentages of protein amino acids are observed in the three species under study.

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Caryophyllidae 科の3種条虫 (*Lytocestus indicus, Introvertus raipurensis* および *Lucknowia indica*) の游離アミノ酸と蛋白構成アミノ酸の比較検討

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Caryophyllidae 科の三種条虫 (*Lytocestus indicus*, *Introvertus raipurensis* 及び *Lucknowia indicus*) の游離アミノ酸と乾燥虫体の加水分解により得られた蛋白構成アミノ酸を定量的，且つ定性的に比較検討した．メチオニンとスレオニンは三種条虫に於て游りアミノ酸としては存在しないが，蛋白加水分解産物中には有意に存在する．上記2種アミノ酸に加えて，40%の中性アミノ酸，20～25% の酸性アミノ酸及び 25%の塩基性アミノ配が種条虫の蛋白質を構成している．又上記3種条虫の蛋白構成アミノ酸の含有量には大差は認められなかった．