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Biochimica. — *Distribution of free amino acids across the midgut of lepidopteran larvae* in vivo (*). Nota di PAOLO PARENTI, FRANCA V. SACCHI, BARBARA GIORDANA e GIORGIO M. HANOZET, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — È stata determinata la concentrazione degli aminoacidi liberi nel contenuto intestinale, negli enterociti e nell'emolinfa delle larve mature dei Lepidotteri *Philosamia cynthia* e *Bombyx mori*. Gli aminoacidi neutri e basici sono più concentrati nell'emolinfa che nel lume intestinale, il che suggerisce un ruolo attivo dell'intestino nel loro assorbimento. Inoltre, dai rapporti tra le concentrazioni cellula/lume e emolinfa/cellula, si può ipotizzare che a livello della membrana luminale sia localizzato un meccanismo attivo di trasporto per gli aminoacidi neutri, e che a livello della membrana basolaterale sia localizzato un meccanismo attivo di trasporto per gli aminoacidi basici.

The midgut of lepidopteran larvae is a model substrate for the study of K-transporting epithelia. The 100 mV, lumen positive trans-epithelial electrical potential difference of this tissue is generated by an electrogenic, luminally directed potassium pump, localized in the apical membrane of specialized cells, the goblet cells [1, 2]. The electrical profile of midgut columnar cells is of the well type, with a trans-apical and trans-basal electrical potential difference of 130 and 30 mV respectively, interior to the cell negative [3, 4]. In addition the midgut separates two environments with a ΔpH of about 3 units: pH 10-11 in the lumen, pH 6.8 in the hemolymph [5]. The ability of the midgut to absorb actively amino acids is well documented [6, 7]. The absorption is due to a secondary active transport mechanism [8], which occurs by means of a K^+-amino acid co-transport system [9], located in the luminal membrane of columnar cells. Some of the amino acids taken up by the midgut enterocytes are also the main source of metabolic energy for the intestinal cell [10]. The ratio between the concentration of individual amino acids in the hemolymph and in the lumen is indicative of the involvement of the intestinal barrier in the active and selective absorption of each amino acid. Therefore, the analysis of the free amino acid composition of the intestinal content, intestinal cells and hemolymph, providing that the electrical profile of the enterocytes and pH values of the three compartments are known, can give indications on the physiological activity of the intestinal wall. Hemolymph composition has been determined

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in different lepidopteran larvae, whereas no reports are available about the free amino acid composition of the intestinal cells and of lumen content. As for the hemolymph, the composition of this compartment is almost constant since lepidopteran larvae are strictly monophagous.

In the present paper, the free amino acid concentrations in the intestinal content, midgut cells and hemolymph of *Philosamia cynthia* and *Bombyx mori* larvae are reported and their relation to intestinal amino acid transport is discussed.

Materials and methods

Larvae in the fifth instar of *P. cynthia* (*Saturnidae*), fed on *Ailanthus glandulosa* leaves, and of *B. mori* (*Bombicidae*), fed on *Morus alba* leaves, were used. The midguts were dissected, deprived of malpighian tubules and intestinal content, rinsed with cold 100 mM mannitol, 10 mM HEPES (N–2–hydroxyethyl piperazine–N'2–ethansulfonic acid)–Tris, pH 7.4 and rapidly homogenized in cold 0.6 M perchloric acid (4 ml/g fresh weight) with a glass teflon Thomas homogenizer, 9 strokes at 3000 rev/min. The homogenate was kept in ice bath for 10 min and then centrifuged at 3000 × g for 15 min at 4 °C. The pH of the supernatant was adjusted to 7.0 by the addition of 2.5 M K_2CO_3. After 15 min at 0 °C, the sample was centrifuged as before and the supernatant was collected. Intestinal content, free from leaf fragments, and *P. cynthia* hemolymph were treated with prechloric acid and processed as above described. Aliquots of the supernatants were used for glutamine assay according to Lund [11]. For *P. cynthia*, the remaining supernatants were diluted 1 : 3 with 0.1% trifluoroacetic acid: methanol 70 : 30 and passed through SEP–Pak C_{18} cartridges (Waters Associated, Millford, MA). The amino acid analysis of the eluate was performed by HPLC, using the apparatus supplied by Waters, and according to the procedure described in the Waters Technical Bulletin n. 3357a. The method employed a strong cation exchange (25 × 0.46 cm) and gradient elution system formed by Na citrate–HNO_3, pH 3.1 (Buffer A) and borate–$NaNO_3$–NaOH, pH 9.6 (Buffer B). The gradient elution was run through a Waters Automated System Controller mod. 720. Detection af amino acids was carried out fluorimetrically by the post–derivatization method with *o*–phtalaldehyde as reagent. Integration of peak area was performed the Waters Data Module mod. 730. For *B. mori*, the free amino acid analysis was performed by means of an Auto Analyzer Carlo Erba model 3A27, after hydrolysis of the samples in 6N HCl at 105 °C for 24 h under vacuum. Glutamic and aspartic acid concentrations were corrected for the values of glutamine and asparagine measured enzymatically [11, 12].

Results and Discussion

Tables 1 and 2 report free amino acid concentrations in the lumen content, intestinal cells and hemolymph of the mature larvae of *P. cynthia* and *B. mori*

TABLE I

Amino acid concentration in the lumen content, midgut cells and hemolymph of P. cynthia *larvae* (*)

Amino acid	Lumen	Cell	Hemolymph
Lysine	0.60 ± 0.01	< 0.01	4.48 ± 0.87
Histidine	1.91 ± 0.22	3.02 ± 1.11	9.17 ± 1.53
Arginine	0.20 ± 0.02	2.39 ± 0.21	1.22 ± 0.15
Aspartic acid	0.77 ± 0.09	0.54 ± 0.14	0.01
Serine	4.98 ± 0.39	19.35 ± 2.75	30.33 ± 4.51
Glutamic acid	1.92 ± 0.38	7.62 ± 0.87	0.14 ± 0.03
Glutamine	1.31 ± 0.31	10.71 ± 2.11	15.24 ± 3.36
Glycine	2.39 ± 0.23	6.54 ± 0.36	10.99 ± 0.68
Alanine	0.35 ± 0.02	11.76 ± 2.21	2.73 ± 0.46
Valine	0.27 ± 0.06	1.46 ± 0.44	1.77 ± 0.19
Metionine	0.11 ± 0.02	0.29 ± 0.09	0.21 ± 0.03
Tyrosine	0.37 ± 0.04	< 0.01	2.44 ± 0.08
Phenylalanine	0.58 ± 0.10	0.71 ± 0.37	0.70 ± 0.09

(*) Lumen and hemolymph concentrations are given as mmol/l fluid. Intracellular concentration is given as mmol/l cell water. Mean ± SE of three determinations.

respectively. In order to calculate intracellular concentrations, tissue values (obtained as mmoles/l tissue water) were corrected for the amino acid concentration in the extracellular luminal and hemolymph space volumes (ECS_l and ECS_h respectively). The midgut extracellular space volumes for both species have been previously reported [13, 14]: ECS_l and ECS_h are 10.9 ± 3.0 and $20.4 \pm 2.1\%$ tissue water (means ± SE, 4 experiments) respectively in *P. cynthia*, and 13.1 ± 1.3 and $31.7 \pm 1.1\%$ tissue water (means ± SE, 6 experiments) respectively in *B. mori*. Moreover, it must be taken into account that the midgut is composed of columnar, goblet, muscle and tracheal cells, of which only columnar ones are absorptive. Nedergaard [6] reports that goblet cells account for one eighth to one tenth of the overall volume of the tissue whereas muscle and tracheal cells can be assumed, from histological observations [15], to be at most one tenth of the entire cellular volume. Therefore, the intracellular amino acid concentrations reported in the tables have been calculated assuming that the total volume of the absorptive cells is 80% of the overall cell volume of the tissue, and that the amino acid concentration is negligible in the non-absorptive cells.

The amino acid pattern of *P. cynthia* hemolymph is in many respects similar to that found in other lepidopteran larvae. Glycine and serine are the most concentrated amino acids together with the basic amino acids histidine and lysine. This feature is shared by the larvae of *B. mori* [16, 17], of *Spodoptera littoralis* [18], of *Erinnys hello* [19] and of other Lepidoptera [20]. The neutral

amino acids, with threonine, proline, aspartate, glutamate and their amides are taken up by the silk glands directly from the hemolymph for the biosynthesis of silk proteins, whereas the basic amino acids are involved in the regulation of the hemolymph osmotic pressure [20]. It is worth noting the high ornitine concentration, also observed in *S. littoralis* [18] and *B. mori* [21]. For amino acid concentration values of *B. mori* hemolymph in *Table II*, references was made to data reported in the literature [17].

All the amino acids found in the hemolymph are also present in the midgut luminal fluid, but the profiles of the two compartements are quite different. Since all but acidic amino acid are more concentrated in the hemolypmh than in the lumen, trans-epithelial active mechanisms for amino acid transport must exist. Furthermore, as already mentioned, the midguts of *P. cynthia* and *B. mori* show a trans-epithelial electrical potential difference of more than 100 mV, lumen positive, and they separate two environments with ΔpH of 3 units. In this condition neutral amino acids bear a negative charge in the lumen, and the midgut polarity would hamper their transfer to the hemolymph: therefore they must be actively absorbed by the midgut, expecially glutamine, alanine, serine, tyrosine, valine and isoleucine, which exhibit the highest hemolymph-to-lumen concentration ratio. Basic amino acids bear almost no charge at luminal pH, and their accumulation in the hemolymph cannot be accounted for by the trans-

TABLE II

Amino acid concentration in the lumen content, midgut cells and hemolymph of B. mori *larvae* (*)

Amino acid	Lumen	Cell	Hemolymph
Lysine	2.17 ± 0.29	< 0.01	10.81
Histidine	0.93 ± 0.13	5.35 ± 0.35	11.67
Arginine	0.81 ± 0.07	4.92 ± 0.35	1.95
Aspartic acid	1.10 ± 0.16	2.01 ± 0.64	0.60
Asparagine	3.54 ± 0.58	8.17 ± 1.85	4.00
Serine	3.57 ± 0.32	2.02 ± 0.21	11.13
Glutamic acid	1.52 ± 0.14	5.94 ± 0.89	0.75
Glutamine	5.55 ± 0,35	16.27 ± 0.90	13.55
Glycine	14.42 ± 0.81	8.59 ± 0.75	7.00
Alanine	3.40 ± 0.20	3.24 ± 0.36	5.95
Valine	2.17 ± 0.20	0.16 ± 0.10	3.76
Metionine	0.72 ± 0.06	1.82 ± 0.09	1.00
Tyrosine	1.24 ± 0.05	< 0.01	2.21
Phenylalanine	1.25 ± 0.09	0.24 ± 0.06	0.79

(*) Lumen and hemolymph concentrations are given as mmol/l fluid. Intracellular concentration is given as mmol/l cell water. Hemolymph values were calculated from data reported by Wyatt *et al.* [16] for larvae in the fourth day of the last instar. Mean ± SE of three determinations.

epithelial electrical potential difference as already observed in *Hyalophora cecropia* midgut [22].

The active step suggested by a hemolymph–to–lumen ratio higher than 1 could occur in principle either at the luminal or at the hemolymph pole of the enterocyte, or at both poles. The intracellular concentration of each amino acid, from which the chemical gradients across the luminal and basolateral membrane can be evaluated, gives an indication about which barrier is involved in the accumulation of the amino acid. The distribution of each amino acid across the three compartments is, with the exception of alanine remarkably similar in the two larvae. The ratio of cell to lumen concentration of neutral amino acids, except phenylalanine, shows that an uphill movement of neutral amino acids should occur at the luminal pole of the enterocyte. This feature agrees with the presence of secondary active, K–dependent, transport mechanisms for neutral amino acids, which has been characterized in brush border membranes from both larvae [8, 9, 23].

Furthermore, a very high hemolymph–to–cell ratio is present for serine, tyrosine and basic amino acids. At the intracellular pH, which is presumably slightly acidic and similar to the hemolymphatic one (pH 6.8), basic amino acids are present as cations and their transport across the basolateral membrane is hampered by the polarity of the trans–membrane electrical potential difference (— 30 mV, cell negative [4]). Therefore, provided that all columnar cells have the same absorptive ability and the same intracellular amino acid concentration, an active transport mechanism should be present at the basolateral membrane for these amino acids as well as for serine and tyrosine. Capraro and co–workers [24, 25, 26] have also proposed that the basolateral membrane plays an active role in the trans–epithelial transport of glucose in vertebrate intestine. Similar involvement has been suggested for rabbit renal epithelium [27].

The data reported in this paper suggest that, in addition to the K–dependent co–transport systems located in the luminal membrane, active transport mechanisms possibly exist also on the basolateral membrane of the enterocyte, which cooperate, in some instances, with the luminal mechanism in the transcellular transport, e.g. of serine in *P. cynthia* and of histidine in *B. mori*. Therefore, a definite insight into the transport mechanisms involved in the absorption of amino acids in lepidopteran midgut is still open to investigation.

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