Changes in free amino acids and peptides in the haemolymph of *Glossina austeni* during the reproductive cycle

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Summary. The amino acids in the deproteinized haemolymph of *Glossina austeni* females, before and after hydrolysis, have been determined at 12 different times during the 1st 2 pregnancy cycles. Hydrolysis resulted in a large increase in the concentrations of tyrosine, phenylalanine, aspartate and lysine, indicating that these amino acids are present mainly as peptides in the haemolymph. The rate of transfer of the aromatic amino acids to the offspring, based upon the amino acid content of the larval gut, must be exceptionally high and has been estimated to be at least 7.9 µg/h.

Tsetse flies (*Glossina*) reproduce by adenotrophic viviparity[2] and as such, convert and transfer large amounts of nutrients derived from vertebrate blood meals to the developing offspring. Because the solutes of vertebrate blood consist largely of proteins and amino acids, and because the contents of the gut of 3rd instar larva of *Glossina* contain 49% (dry weight) protein, peptide and amino acid[3], there must be a significant transfer of these nutrients from the gut of the mother, through the haemolymph and into the milk gland[4], which provides the secretion upon which the developing larva feeds. In fact, when the transfer of radiolabelled amino acids from mother to offspring is followed by autoradiography or by liquid scintillation spectrometry during a typical pregnancy cycle, the movement of a variety of amino acids is observed to occur with unusual rapidity[5-8]. This implies that there is a rapid flux of amino acids through the haemolymph of the mother to the feeding larva. However, it is not known if amino acids and peptides from the blood meals are taken up from the haemolymph by the milk gland as soon as they enter the hemolymph, or if they are initially stored in some form in other tissues including the haemolymph. In the case of the aromatic amino acids, it appears that there must be some storage of these compounds (and perhaps other amino acids) between blood meals because a) the milk gland is actively secreting only during 5 days of the 9 day pregnancy cycle[2,4] and b) there is insufficient tyrosine and phenylalanine in a blood meal to account for the amount found in the offspring[2,3]. This suggests that there is storage of at least certain amino acids in the mother and although it has been previously demonstrated that some storage occurs in the oenocytes[4-6], this may not represent the major storage site. The haemolymph proteins do not appear to represent a significant storage form for amino acids[6,9] and it has been suggested that peptides in the haemolymph may represent an important storage form for the amino acids which will be transferred to the developing larva[2,6]. Such a storage form for amino acids has been demonstrated in other flies[10,11]. For this reason, we have analyzed the amino acids of the deproteinized haemolymph, both before and after hydrolysis, of the tsetse fly, *Glossina austeni* at 12 selected times during the 1st 2 pregnancy cycles, as well as those of the 3rd instar larval gut. We have found that large quantities of several amino acids, including the aromatics, appear after hydrolysis, suggesting that peptides are indeed the major storage form for amino acids released after digestion and absorption from the gut.

Haemolymph samples at 12 selected times during the 1st 2 pregnancy cylces were obtained by the centrifugation method[12]. At each age, haemolymph from 20-30 flies was pooled (Range: 8-40 µl). Precipitation of haemolymph protein was accomplished by addition of an equal volume of 0.4 M perchloric acid, as described previously[9]. The supernatant was neutralized by addition of 50% potassium hydroxide and the potassium perchlorate removed by centrifugation[13]. Samples of milk secretion were obtained by previously described methods[9] and were extracted as above. An aliquot of each of the supernatants was hydrolyzed in 6 M hydrochloric acid at 110 °C for 18 h under nitrogen[9] and the acid removed under vacuum in the presence of sodium hydroxide. The remaining supernatants were lyophilized and all samples were stored at −60 °C. Known

Table 1. Concentration of amino acids (before and after hydrolysis) in the deproteinized haemolymph of female *Glossina austeni* at selected times during the 1st 2 pregnancy cycles

Amino Acid	Physiological age[a]									
	6		0		3-4		8		1-2	
	F	H	F	H	F	H	F	H	F	H
	µmole/ml haemolymph									
Taurine	5.71	Trace	6.24	6.51	4.88	5.18	5.88	6.04	2.74	3.00
Aspartate	0.24	6.66	0.26	14.00	0.25	15.39	Trace	19.45	Trace	18.01
Threonine[b]	0.93	4.21	1.01	6.18	1.13	7.03	1.34	7.65	1.81	7.92
Serine[b]	3.85	6.10	4.17	9.00	4.00	10.01	4.44	11.35	4.19	10.16
Asparagine[c]	1.76	-	1.55	-	1.56	-	3.07	-	1.73	-
Glutamate	2.86	20.44	5.74	18.98	7.37	33.04	6.08	41.98	3.06	30.80
Glutamine[d]	8.92	-	5.80	-	5.81	-	11.34	-	7.38	-
Proline	45.77	46.08	56.71	70.16	63.60	64.76	70.52	74.04	89.68	86.43
Glycine	3.68	10.55	2.34	12.12	1.47	12.11	2.75	15.18	4.65	16.69
Alanine	33.59	37.18	28.33	34.91	40.64	52.34	40.48	51.66	30.43	39.86
Valine	7.39	9.97	4.69	10.28	5.91	12.22	5.05	14.71	5.45	13.43
Methionine	-	-	Trace	-	0.32	Trace	Trace	Trace	0.42	-
Isoleucine	-	2.40	Trace	4.21	Trace	5.15	Trace	6.21	Trace	5.47
Leucine	3.93	6.46	2.46	9.99	3.22	13.08	2.61	15.10	2.92	13.76
Tyrosine[b]	Trace	0.94	Trace	2.68	0.36	3.65	Trace	3.79	0.28	3.70
Phenylalanine	0.21	3.24	0.52	7.70	0.58	8.84	0.58	10.85	0.87	10.17
Lysine	1.75	7.42	1.67	15.02	1.28	15.67	0.78	19.44	0.85	17.32
Histidine	Trace	3.25	1.01	4.93	0.84	5.34	1.26	7.17	1.48	5.93
Arginine	2.47	3.68	2.16	4.75	1.61	4.10	2.33	5.07	1.86	4.81

F, free amino acids before hydrolysis; H, after hydrolysis of deproteinized haemolymph. [a] 6, 6 days before ovulation; 0, ovulation (9-10 days after emergence); 3-4, 3-4 days before the 1st larviposition; 8, 8 days before the 2nd larviposition; 1-2, 1-2 days before the 2nd larviposition. [b] Acid hydrolysis may cause partial destruction of serine, threonine and tyrosine. [c] Asparagine yields aspartate upon acid hydrolysis. [d] Glutamine yields glutamate upon acid hydrolysis.

amounts of nor-leucine were added to all samples prior to extraction to serve as an internal standard.

The samples were dissolved in lithium citrate buffer (pH 2.20) and aliquots analyzed on an automatic amino acid analyzer (Beckman, Model 116), using a single column (UR 30), 2-buffer, lithium citrate system (pH 2.64, 39 °C; pH 4.00, 60 °C) for 'physiological runs' (modified from Beckman bulletin 116-TB-003, January 1968).

Table 1 shows the concentration of amino acids in the haemolymph before and after hydrolysis, at 5 of the 12 selected times during the 1st 2 pregnancy cycles. With few exceptions, there was little variation in free amino acid concentrations in haemolymph samples from flies of different ages and therefore, only these 5 representative times are shown in the table. It is apparent from table 1 that: a) there was a significant increase (greater than 10fold) in the concentration of aspartate, tyrosine, phenylalanine and lysine following hydrolysis b) proline and alanine were present in the highest concentrations in free form whereas after hydrolysis, glutamate plus glutamine also were present in high concentrations. There was little change in the concentration of proline and alanine following hydrolysis.

As has been demonstrated in other species of *Glossina*[14,15], the major amino acids in the haemolymph of *G. austeni* are proline and alanine. The present study reveals that these were present almost exclusively in their free form. The fact that proline and alanine as well as taurine did not increase significantly after hydrolysis suggests that there was little contamination of the samples with haemolymph proteins or polypeptides, since hydrolysis of these proteins would result in an apparent increase in the concentration of these amino acids (both proline and alanine are present in significant amounts in haemolymph protein)[9].

The concentration of both tyrosine and phenylalanine in free form in the haemolymph is very low (table 1) and this may in part be a function of the low solubility of these compounds in aqueous solution[5]. However, a large increase in tyrosine and phenylalanine concentrations following hydrolysis was observed suggesting that the bulk of these amino acids were present in peptides in the haemolymph at all stages in the pregnancy cycle. The composition of these peptides is unknown but in other flies, the dipeptides glutamyl-phenylalanine and β-alanyl-tyrosine are present in large quantities in the haemolymph[11]. The large increases in glutamate, aspartate, and lysine after hydrolysis might indicate that these are also important constituents of the peptides. Rapid uptake of radiolabelled tyrosine by the milk gland and subsequent transfer to the larva has been demonstrated in *G. austeni*[5] and although a portion of the tyrosine may be taken up directly as free amino acid by the milk gland, it has been suggested that some of the injected tyrosine is incorporated into peptide and released several hours later[6]. In view of the large quantity of tyrosine transferred to the larva, the rate of turnover of the aromatic-containing peptides must be extremely rapid. It is not known if the milk gland takes up only free amino acids or if peptides also enter the gland directly.

The amino acid composition of the larval gut contents (presumed to be equivalent to the milk secretion) of *G. austeni* is shown in table 2. The predominant free amino acids were tyrosine, phenylalanine and lysine with the other amino acids present in only trace quantities. Following hydrolysis of the deproteinized gut contents, the other amino acids underwent dramatic increases in concentration, indicating that they were present in the larval gut (and presumably in the milk secretion as well) as peptides. Tyrosine, glutamate, phenylalanine and alanine were present in the highest concentrations. It is significant that the concentration of tyrosine does not increase appreciably after hydrolysis - thus it is present mainly in free form and far in excess of its solubility in aqueous solution. Cmelik et al.[3] observed that the larval gut contents of *G. morsitans* also contained large quantities of free tyrosine (1.4 g/100 ml) and in *G. austeni*, assuming that the volume of the gut contents is 15 µl[6], the quantity of free tyrosine is even higher (4.5 g/100 ml). Phenylalanine is also present in large quantities in its free form. Thus the milk secretion of *G. austeni* contains aromatic amino acids in exceptionally high quantities.

Assuming the haemolymph volume of adult female *G. austeni* is 6 µl[12], the total quantity of tyrosine plus phenylalanine (hydrolyzed values from table 1) in the haemolymph is 11.7 µg whereas the total quantity of tyrosine plus phenylalanine in the larval gut contents is 1323 µg. Assuming the secretion is elaborated over a period of 7 days[4,5], 7.9 µg of tyrosine plus phenylalanine is transferred to the larva each hour. Thus more than 50% of the aromatics in the haemolymph are transferred to the larva every hour. This gives some indication of the extremely high turnover rate of the aromatic peptides and suggests that the tissues of the parent must store significant quantities of the aromatics. The results presented in this paper further reveal the high degree of efficiency with which the tsetse nutrient transfer system operates.

Table 2. Amino acid composition of deproteinized gut contents of 3rd instar larvae of *G. austeni*

Amino acid	Free µmole/animal	Hydrolyzed
Taurine	*	*
Aspartate	*	1.84
Threonine	*	1.89
Serine	*	0.94
Asparagine	*	-
Glutamate	*	3.28
Glutamine	*	-
Proline	-	1.78
Glycine	-	0.96
Alanine	*	2.07
Valine	-	1.31
Methionine	*	0.39
Isoleucine	*	0.76
Leucine	*	1.06
Tyrosine	3.74	4.84
Phenylalanine	1.18	2.70
Lysine	0.28	1.85
Histidine	*	0.73
Arginine	*	0.73

*Trace.

1 Acknowledgments. I am grateful to Dr P.A. Langley for providing *G. austeni* females and to Mr L. Gringorten for amino acid analyses, for useful discussions and for a critical reading of the manuscript.

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