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The role of hemolymph proline as a nitrogen sink during blood meal digestion by the Mosquito *Aedes aegypti*

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Abstract

Mosquitoes utilize the amino acids derived from blood meal protein to produce egg proteins. But the amino acids can also be used to produce egg lipid or can be oxidized for energy production. These latter two processes result in the release of nitrogen as toxic ammonia. Therefore, amino acids must be processed in such a way that amino acid nitrogen can be incorporated into non-toxic waste products. Proline is the predominant amino acid in the hemolymph of the adult female mosquito *Aedes aegypti*. After feeding on albumin, hemolymph proline levels increased five-fold over unfed levels, reached maximal levels in the first hours after feeding and remained high through oviposition. Hemolymph proline levels increased as the concentration of protein in the meal increased. When starved of sugar for 24 h prior to feeding on an albumin meal, hemolymph proline levels increased four-fold over the proline levels of non-starved mosquitoes. Proline levels after feeding on a protein deficient in essential amino acids, pike parvalbumin, increased to twice the levels of albumin fed mosquitoes. Based on these observations, we propose that mosquitoes utilize proline as a temporary nitrogen sink to store ammonia arising from deamination of blood meal amino acid.

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1. Introduction

Adult female *Aedes aegypti* mosquitoes can feed on nectar (sugar) or take a blood meal as sources of nutrition. *Ae. aegypti* larvae do not acquire the metabolic resources necessary to produce mature eggs and nectar meals are insufficient to promote egg maturation as well. Therefore, female mosquitoes need to take the blood meal in order to complete oogenesis. Vertebrate blood is deficient in lipid and carbohydrate, but is rich in protein (approximately 20% of the wet weight of blood) (Burke, 1976; Grimes, 1980). Due to the nutritionally skewed nature of this food source, mosquitoes must use amino acids derived from blood meal protein to produce lipid and carbohydrate, as well as to provide energy through

the oxidation of amino acid carbon skeletons, as evidenced by the amount of blood meal nitrogen excreted as waste and the amount of protein used to synthesize egg lipid (Briegel, 1990; von Dungern and Briegel, 2001). During these processes, amino acids must be deaminated, resulting in the release of ammonia, a compound toxic to mosquitoes. Ammonia can be excreted from mosquitoes as uric acid, urea, or as some free ammonia. The importance of the use of amino carbon in pathways other than egg protein biosynthesis is shown by the fact that approximately 80% of blood meal amino acid nitrogen is excreted as nitrogenous waste in *Anopheles* mosquitoes (Briegel, 1990) and at least 57% in *Ae. aegypti* (von Dungern and Briegel, 2001).

Another problem that mosquitoes encounter during blood meal digestion is the accumulation of high amino acid levels in the hemolymph as they are transported from the midgut to the fat body for egg protein synthesis, lipogenesis or gluconeogenesis. This can affect some properties of the hemolymph such as ionic strength and

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pH, both of which can adversely affect the viability of mosquitoes. Insects have developed mechanisms to accommodate high hemolymph concentrations of specific amino acids. Proline in particular has been found to occur in higher levels than other amino acids in hemolymph, as in the cases of the New Zealand alpine weta *Hemideina maori* (Neufeld and Leader, 1998), the kissing bug *Rhodnius prolixus* (Barrett, 1974), the cockroach *Blaberus discoidalis* (Sowa and Keeley, 1996), and the mosquitoes *Culex pipens pallens* (Uchida et al., 1990) and *Anopheles stephensi* (Henn et al., 1998) for examples.

Several roles for high hemolymph proline levels have been presented. Proline has been shown as a suitable source of energy in several insects (see Gäde and Auerwald, 2002, for a recent review). Proline is thought to operate as a compatible osmolyte in hypersaline conditions to offset osmotic shock. This mechanism has been proposed to occur in the brackish water mosquito, *Culex tarsalis* (Patrick and Bradley, 2000a; Patrick and Bradley, 2000b), because hemolymph proline levels increased dramatically when fresh water acclimated larvae were introduced into a highly saline environment. In addition, the alpine weta *H. maori* responds to cold weather conditions by increasing the hemolymph concentration of proline between 4 to 7-fold over warm weather conditions (Neufeld and Leader, 1998).

In *Ae. aegypti* larvae, high proline levels were found to occur in hemolymph relative to other amino acids, and proline levels increased in response to exposure to *Bacillus thuringiensis* toxin (Bounias et al., 1989). The authors of this study also proposed that proline is used as an energy source. Whole body homogenate amino acid levels have been determined for *Ae. aegypti* in two studies (Stidham and Liles, 1969; Thayer and Terzian, 1970). Both studies found alanine to be the predominant free amino acid, but Thayer and Terzian found proline in higher levels than Stidham and Liles. In addition, Thayer and Terzian found that proline levels in adult female whole body homogenates increase with the age of the mosquito.

In this paper, we report the hemolymph concentration of proline in adult female *Ae. aegypti* following a protein meal and under other conditions in which amino acid utilization for energy production would be favored. The very high concentration of proline found in the hemolymph is consistent with the suggestion that proline serves as an ammonia sink.

2. Materials and methods

2.1. Insects

Aedes aegypti (NIH-Rockefeller) were maintained at 28 °C at 70–80% humidity with a light: dark cycle of

16h: 8h. Larvae were maintained on a diet consisting of equal proportions of rat chow (Sunburst Pet Foods, Phoenix, AZ), lactalbumin hydrolysate (USB, Cleveland, OH) and yeast hydrolysate (USB, Cleveland, OH). Adults were kept at 28 °C, 80% RH, and a photoperiod of 16:8 (L: D) h and allowed ad libitum access to pads soaked in 3% sucrose in water.

2.2. Reagents

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless stated otherwise.

2.3. Hemolymph isolation

A small incision was made in the last abdominal segment of the mosquito and the mosquito was placed in a 0.7ml micro-centrifuge tube with a small hole in the bottom. This tube was placed in a 1.7 ml tube and hemolymph was collected by centrifugation at $500 \times g$ for 10 min. The supernatant in the 1.7 ml tube was collected and the hemocyte-containing pellet was discarded.

2.4. Preparation of hemolymph for amino acid analysis

An equal volume of 5% Sulfosalicylic acid in water was added to the hemolymph. Samples were mixed and subjected to centrifugation at $7500 \times g$ for 5 min. The supernatant was used for amino acid analysis.

2.5. Amino acid analysis

Amino acids were analyzed by post-column ninhydrin chemistry on a Beckman 7300 amino acid analyzer. Analyses were performed at The University of Arizona Division of Biotechnology Proteomics Core Facility. Because mosquitoes from different developmental stages were used in these studies, we report amino acid content as nmol/animal, rather than as concentration in hemolymph. This was necessary because we did not know the hemolymph volume in most cases.

2.6. Feeding

All protein meals fed to mosquitoes were prepared in 100 mM NaHCO₃, pH 7.0, containing 150 mM NaCl and 5 mM ATP. Mosquitoes were fed through a parafilm membrane on a “bell feeder” containing the protein meal. The meal was kept at 37 °C by water circulating through the feeder. In some experiments, mosquitoes were weighed before and immediately after feeding in order to determine the size of the protein meal taken.

2.7. Purification of Northern Pike parvalbumin

Parvalbumin from Northern Pike was purified using a modified procedure of Bugajska-Schretter et al. (2000). Frozen Northern Pike muscle was homogenized under liquid nitrogen with a mortar and pestle. The powder was dissolved in 20 mM sodium phosphate, pH 7.2, 150 mM NaCl and 1mM phenylmethylsulfonyl fluoride, and then boiled for 30 min. The boiled homogenate was centrifuged at $5000 \times g$ for 10 min. Ammonium sulfate (ICN Biomedicals, Costa Mesa, CA) was added to the supernatant to a final concentration of 60% (w/v), and mixed for 15 min. The solution was centrifuged at $5000 \times g$ for 15 min and the supernatant retained. The ammonium sulfate was removed from the supernatant by dialysis against water using tubing with a molecular weight cut-off of 3500 Da. The solution was lyophilized and resuspended in 10 mM Tris pH 7.5. Parvalbumin was further purified by anion-exchange column chromatography on Tris-Acryl M resin. Protein was eluted with a gradient of 0–500 mM NaCl in 10 mM Tris pH 7.5 at a flow rate of 4ml/min. Those fractions containing purified parvalbumin isoforms were determined by SDS-PAGE.

2.8. Amino acid analysis of parvalbumin

Purified Northern Pike parvalbumin was subjected to total amino acid analysis on an Applied Biosystems 420A/H analyzer at The University of Arizona Laboratory for Protein Sequence Analysis.

3. Results

The amino acid composition of hemolymph from developing mosquitoes is shown in Table 1. These results are presented as nmol/animal. For comparison purposes, we can assume a hemolymph volume of 50 nl for unfed adults (Shapiro et al., 1986). Using this conversion, we obtained hemolymph concentrations comparable to those reported for adult *Culex sp.* (Uchida et al., 1990; Su and Mulla, 1997). During larval development, Glu and Ala were present at high levels, which would be consistent with their role of transporting amino groups to various tissues for the synthesis of nonessential amino acids. In fourth instar larvae and pupae, Tyr is present at high levels, which might be related to the synthesis of the cuticle. Interestingly, His and Arg are present at high levels in larvae and pupae, but drop to low levels in adults. It is not clear what the significance of this is, but Briegel (1990) noted high concentrations of these amino acids in feces of adult females following a blood meal. Because they contain more than one nitrogen atom, they may serve as a means to excrete excess nitrogen. Of particular interest is the fact that proline became

the predominate amino acid in adult hemolymph 72 h post eclosion.

The potential importance of hemolymph proline was emphasized by the comparison of hemolymph amino acids from unfed mosquitoes and mosquitoes 24 h after feeding an artificial blood meal (Kogan, 1990). When fed on Kogan's artificial blood meal, hemolymph proline levels increase approximately five-fold over unfed values (Fig. 1). When the meal contained soybean trypsin inhibitor (2 mg/ml), which inhibits digestion of the protein meal (Barillas-Mury et al., 1995), this increase did not occur (Fig. 1). This shows that the increase in hemolymph proline levels is linked to blood meal protein digestion. In addition, when mosquitoes were fed saline (150 mM NaCl, 5 mM ATP, 100 mM NaHCO₃, pH 7.0) or 3% sucrose in saline there was no increase in proline levels (results not shown). Since both of these meals are directed to the midgut rather than the crop in the presence of ATP as verified by dissection, these results show that feeding per se does not lead to increased hemolymph proline levels.

Mosquitoes were then fed several different concentrations of a porcine fraction V meal (crude albumin) to determine if hemolymph proline levels increase with dietary protein content in a dose dependant manner. Compared to unfed levels, hemolymph proline levels increased significantly after feeding all meals, but there was not a significant difference between meals containing 10% protein or higher (Fig. 2).

When hemolymph free amino acid levels were determined at various times after feeding a 15% albumin meal, proline levels increased within 30 min of feeding, reached peak levels by 3 h and remained high through oviposition (Fig. 3A). Alanine levels followed a similar pattern, although the concentration of alanine was lower than that of proline (Fig. 3B). Glutamate reached its highest level by 3 h, although less than proline, and remained higher than unfed levels through 48 h (Fig. 3C). Glutamine levels increased 30 min after feeding and remained higher than unfed values through oviposition (Fig. 3D). These four amino acids were the predominant amino acids in hemolymph, and although there were changes in the other 16 amino acids, the data are not shown because they accounted for only a small of the total amino acids in hemolymph (see for example Fig. 4).

In order to determine how energy reserves present in the mosquito at the time of protein meal feeding affect hemolymph amino acid levels after protein feeding, one group of mosquitoes was placed in cages with only water available, no sugar, for 24 h prior to feeding on a protein meal, while another group had access to pads saturated with 3% sucrose. Sugar-starved or sugar-fed mosquitoes were fed on 10% albumin, weighed after feeding to determine the protein meal sizes, and 24 h later hemolymph was extracted and subjected to free amino acid

Table 1
Levels of amino acids in hemolymph during mosquito development^a

| Amino acid | Developmental stage | | | | |
|------------|---------------------|-------------------|-------------|---------------------|------------------------------|
| | 3rd instar larvae | 4th instar larvae | Pupae | Adult newly eclosed | Adult 72 hours post eclosion |
| Asp | 0.170±0.067 | 0.408±0.085 | 0.154±0.008 | 0.100±0.036 | 0.009±0.004 |
| Thr | 0.361±0.178 | 1.593±0.399 | 1.063±0.225 | 0.460±0.196 | 0.098±0.048 |
| Ser | 0.578±0.272 | 2.035±0.487 | 0.622±0.207 | 0.248±0.084 | 0.048±0.009 |
| Asn | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.156±0.065 | 0.000±0.000 |
| Glu | 1.145±0.637 | 2.992±0.700 | 1.679±0.219 | 0.300±0.197 | 0.153±0.062 |
| Gln | 0.779±0.335 | 1.782±0.427 | 1.455±0.210 | 0.435±0.167 | 0.036±0.029 |
| Gly | 0.565±0.289 | 2.532±0.526 | 0.556±0.112 | 0.139±0.059 | 0.023±0.004 |
| Ala | 1.435±0.671 | 7.880±2.419 | 2.694±0.469 | 0.565±0.219 | 0.287±0.058 |
| Val | 0.269±0.104 | 1.304±0.271 | 0.902±0.222 | 0.216±0.077 | 0.012±0.010 |
| Cys | 0.031±0.022 | 0.142±0.047 | 0.046±0.020 | 0.003±0.006 | 0.000±0.000 |
| Met | 0.085±0.036 | 0.360±0.067 | 0.134±0.057 | 0.038±0.018 | 0.006±0.003 |
| Ile | 0.190±0.081 | 0.857±0.163 | 0.564±0.091 | 0.092±0.038 | 0.007±0.004 |
| Leu | 0.303±0.134 | 1.434±0.252 | 0.692±0.111 | 0.131±0.062 | 0.006±0.003 |
| Tyr | 0.531±0.362 | 3.554±1.773 | 5.001±1.087 | 0.940±0.328 | 0.008±0.005 |
| Phe | 0.201±0.082 | 0.731±0.139 | 0.231±0.062 | 0.151±0.064 | 0.005±0.003 |
| Trp | 0.006±0.010 | 0.141±0.085 | 0.247±0.059 | 0.050±0.053 | 0.000±0.000 |
| Lys | 0.374±0.151 | 1.350±0.288 | 0.963±0.029 | 0.237±0.084 | 0.015±0.004 |
| His | 0.736±0.397 | 2.758±0.723 | 0.712±0.113 | 0.219±0.070 | 0.038±0.025 |
| Arg | 1.120±0.564 | 4.495±0.973 | 1.517±0.188 | 0.513±0.267 | 0.049±0.023 |
| Pro | 0.483±0.222 | 2.411±0.607 | 1.662±0.352 | 1.288±0.540 | 0.926±0.185 |

^a Values are expressed as average nmol amino acid/animal in hemolymph ± standard deviation as determined from three samples of pooled hemolymph from ten mosquitoes per sample.

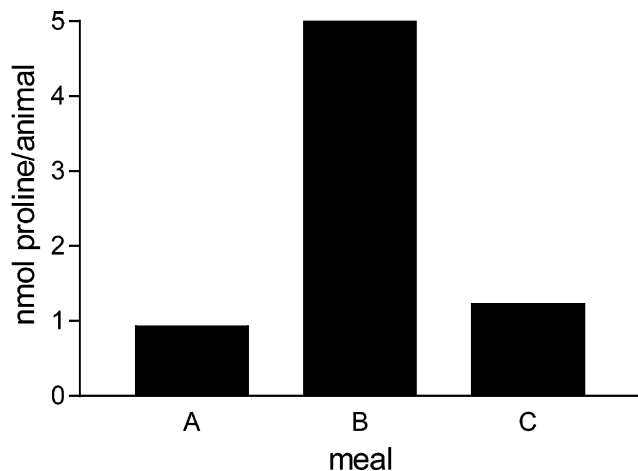


Fig. 1. Levels of proline in the hemolymph of mosquitoes 24 h after feeding various meals. Ten mosquitoes were used for each experiment. A: unfed; B: fed Kogan's artificial blood meal; C: fed Kogan's artificial blood meal + 2 mg/ml of soybean trypsin inhibitor. Data represent the proline levels per mosquito from a single determination of pooled hemolymph from 20 mosquitoes.

analysis. There was no significant difference found in the size of the meal taken between the two groups. Hemolymph free proline levels in the sugar-starved mosquitoes was approximately three fold higher than the control group (Fig. 4). Alanine levels were also significantly higher in the sugar starved mosquitoes, but the level was much lower than the proline level. All other

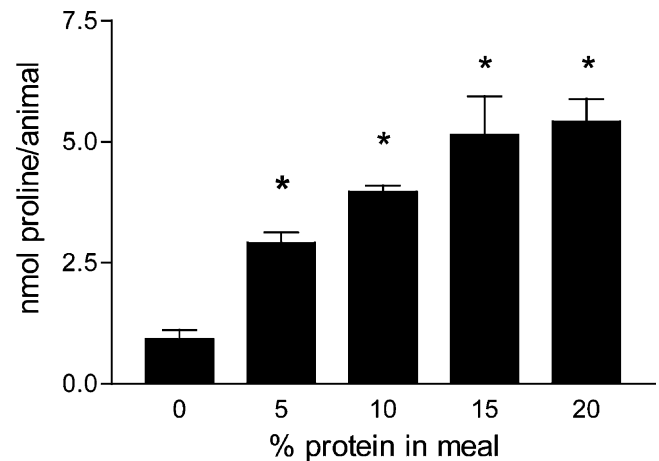


Fig. 2. Proline levels in hemolymph 24 h after feeding mosquitoes albumin meals containing different levels of protein. For 5, 10 and 15% meals ten mosquitoes were used, while six mosquitoes were used with 20% albumin and five mosquitoes were used for the unfed sample (0%). Values represent the mean ± standard deviation of proline levels per mosquito as determined from three samples of pooled hemolymph from ten mosquitoes per sample. *: Samples that were significantly greater than unfed values, $P < 0.05$ as determined by t -test.

amino acid levels were not significantly different between the two groups.

Parvalbumin from Northern Pike was purified according to the procedure of Bugajska-Schretter et al. (2000). Total amino acid analysis performed on the purified protein showed no detectable amounts of the essential

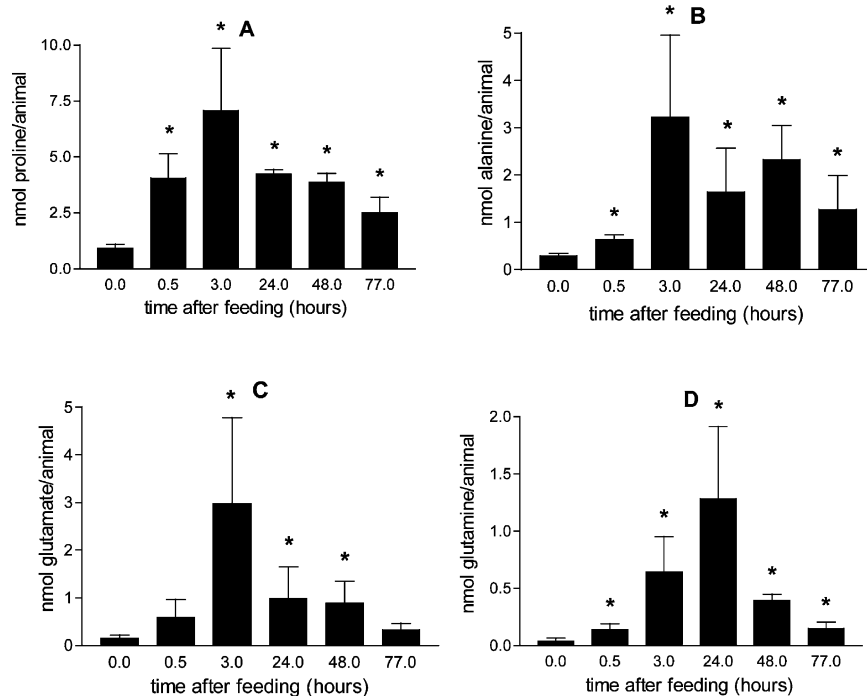


Fig. 3. Hemolymph proline (A), alanine (B), glutamate (C) and glutamine (D) levels at various times after feeding on a 15% albumin meal. Data represent mean \pm standard deviation of amino acid levels per mosquito as determined from three samples of pooled hemolymph from ten mosquitoes per sample. *: Samples where the proline content of hemolymph is significantly greater than unfed mosquitoes, $P < 0.05$ as determined by t -test.

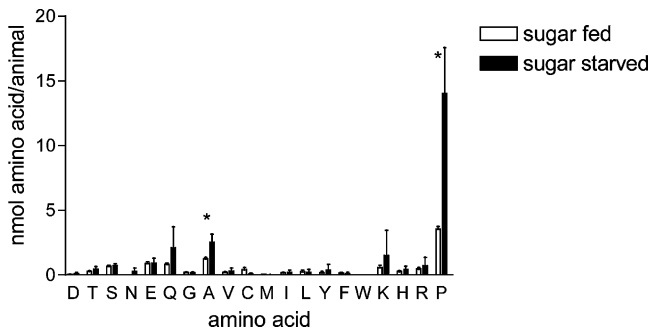


Fig. 4. Comparison of hemolymph amino acid levels from females 24 h after feeding on 10% albumin meal to mosquitoes that were fed 3% sucrose or starved for sucrose for 24 h prior to feeding. Data represent mean \pm standard deviation of amino acid levels per mosquito as determined from three samples of pooled hemolymph from five mosquitoes per sample. *: Samples where the hemolymph protein content was significantly greater in non-sugar fed mosquitoes than in sugar-fed mosquitoes, $P < 0.05$ as determined by t -test.

amino acids (Clements, 1992) His and Trp (data not shown). Mosquitoes were fed a 5% parvalbumin meal and a 5% albumin meal. Five percent meal concentrations were chosen due to the limited availability of purified parvalbumin. The average size of the protein meals taken was 3.01 μ l/animal. After 24 h, hemolymph free amino acid levels were determined. Proline levels in the parvalbumin fed mosquitoes were approximately two fold greater than in the albumin fed controls (Fig.

5). Feeding on the parvalbumin meal did not result in egg maturation or oviposition as feeding on the albumin meal did.

4. Discussion

The purpose of these experiments was to understand the significance of the very high levels of proline in the hemolymph of female *Ae. aegypti* mosquitoes, which can approach 100 mM after a blood meal. In unfed

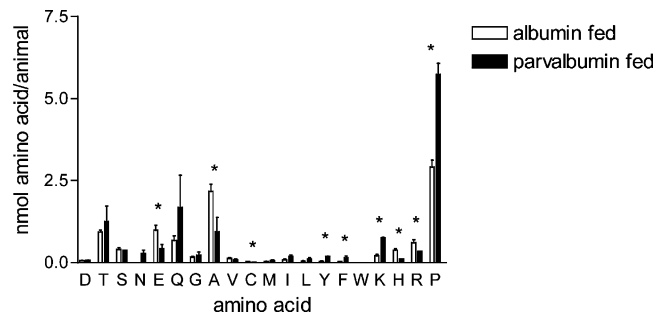


Fig. 5. Hemolymph amino acid levels 24 h after feeding on a 5% albumin or 5% parvalbumin meal. Data represent mean \pm standard deviation of amino acid levels per mosquito as determined from three samples of pooled hemolymph from five mosquitoes per sample. *: Samples where the hemolymph protein content was greater in parvalbumin fed mosquitoes, than in albumin fed mosquitoes, $P < 0.05$ as determined by t -test.

females proline is the major amino acid in hemolymph and increases in concentration significantly after ingesting a protein meal. The increase in proline content requires digestion of the protein meal, because it was not observed when soybean trypsin inhibitor is added to the meal, which prevents digestion of meal proteins (Barillas-Mury et al., 1995). In addition, feeding a meal with 10% protein caused a greater increase in hemolymph proline than did feeding a meal containing 5% protein. This data are all consistent with the hypothesis that hemolymph proline is somehow derived from dietary amino acids that are released during protein meal digestion.

Another factor controlling the level of hemolymph proline was the energy status of the female at the moment of protein meal feeding. When mosquitoes were starved for sucrose for the 24 h prior to feeding on a protein meal, they accumulated almost two-fold more hemolymph proline than those mosquitoes fed after being maintained on sucrose. This result cannot be an artifact based the size of the protein meal, as there was no significant difference in the size of the meal taken between the two groups. The sugar-starved mosquitoes would be expected to have lower energy reserves (glycogen, lipid and free carbohydrates, Briegel, 1990) than the fed mosquitoes. Mosquitoes with lower energy reserves excrete a larger proportion of the blood meal nitrogen as waste (Briegel, 1990; von Dungern and Briegel, 2001), presumably because the amino acid carbon backbone is used for energy production. This result suggests that the production of proline is necessary in order to temporarily store the toxic ammonia released during amino acid deamination until it can be converted to urea or uric acid for excretion.

This suggested role of proline is strengthened by the observation that when parvalbumin, which is deficient in the essential amino acids his and trp, was fed, the level of hemolymph pro was very high. In the absence of the essential amino acids, protein synthesis cannot occur, and this is confirmed by the fact that this meal does not support egg maturation and oviposition. Since the protein is not utilized for egg maturation, dietary amino acids are degraded, producing additional ammonia, which is stored as proline.

It is important to note that the increase in hemolymph proline levels cannot be derived from dietary proline alone. When fed a 5% pike parvalbumin meal, mosquitoes took an average meal size of 3.01 μl based on body masses before and after feeding. This amounts to approximately 150 μg of parvalbumin, or 10.8 nmol based on an apparent molecular weight of 14 kDa as determined by SDS-PAGE. Proline represents 1.39 mol% of parvalbumin as determined by amino acid analysis, indicating that mosquitoes ingested 150 pmol of proline in the parvalbumin meal, far less than the hemolymph proline content displayed in Fig. 5. Thus, it

is apparent that proline must be derived from other dietary amino acids. After transamination or deamination the carbon backbone of many amino acids can enter the Krebs cycle and produce α -ketoglutarate. α -Ketoglutarate is a major acceptor of ammonia in transamination reactions, producing glutamate, which is converted to proline (see Fig. 6). In further support of the transient nitrogen sink role of proline, is the fact that proline is not the predominant amino acid in the hemolymph of the aquatic larval and pupal life stages as seen in Table 1. Rather alanine occurs as the most abundant amino acid, with other amino acids such as glutamate occurring in higher levels than proline. Proline is not needed as a nitrogen sink in aquatic mosquitoes because ammonia can easily be excreted into the water. An important aspect of this system is that the energy cost is neutral to accumulate proline as a nitrogen sink. Proline is made from α -ketoglutarate in a reversible process (Gäde and Auerswald, 2002) and we propose that a proline cycle exists in mosquitoes that serves as a temporary storage form for ammonia produced during amino acid deamination (Fig. 6). When the rate of ammonia production from the degradation of dietary amino acids exceeds the capacity of the mosquito to produce urea and/or uric acid, the excess ammonia can be stored temporarily as proline. Eventually the ammonia is recovered from proline for excretion and the carbon skeleton can be used for energy production or for lipid and carbohydrate synthesis. Such a sink is probably necessary because the existing pathways that produce uric acid and urea, even though they are operating and incorporating ammonia at appreciable rates, may not have the capacity to deal with

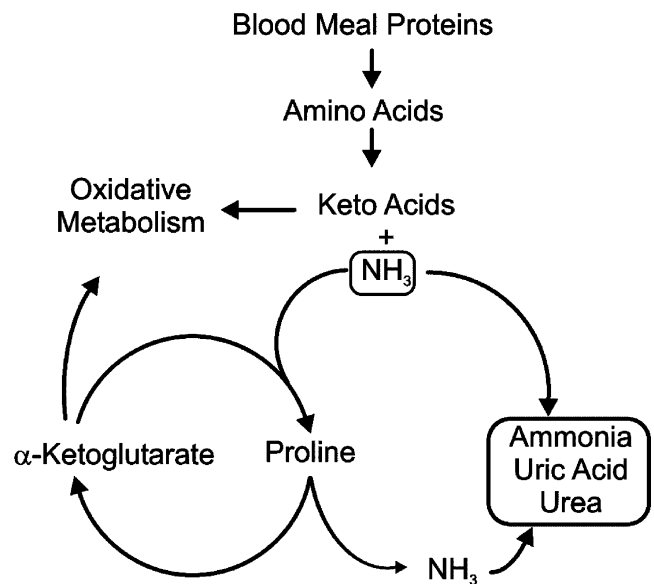


Fig. 6. A proline cycle in mosquitoes that allows temporary storage of ammonia derived from amino acid deamination in a non-toxic form. Proline nitrogen can be incorporated into nitrogenous waste as it becomes appropriate.

the large amounts of ammonia produced during deamination of blood meal amino acids that are rapidly derived from dietary protein digestion. This proposal is supported by the findings of von Dungern and Briegel (2001) who found that larger blood meals led to greater amounts of nitrogen excreted as waste products. Although Xanthanine Dehydrogenase (XDH), the enzyme involved in the synthesis of uric acid, the major nitrogen waste product in *Ae. aegypti*, increases in activity in response to increased protein ingestion, it does not reach peak activity until 18–24 h after feeding, later than with smaller meals (von Dungern and Briegel, 2001). This might indicate that uric acid could not be synthesized rapidly enough to compensate for the increased ammonia load during the initial stages of protein digestion. By temporarily storing nitrogen as proline, any detrimental effects can be averted until uric acid synthesis can incorporate waste nitrogen. Indeed, proline levels are highest (see Fig. 3) before XDH has reached maximal levels and proline levels have fallen at the times of peak XDH activity. Although the difference in experimental procedures might make an interpretation difficult, the higher whole body mosquito free proline levels found by Thayer and Terzian (1970) in aged mosquitoes might be elevated in response to the lower XDH levels found by von Dungern and Briegel (2001) in older mosquitoes.

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References

- Barillas-Mury, C.V., Noriega, F.G., Wells, M.A., 1995. Early trypsin activity is part of the signal transduction system that activates transcription of the late trypsin gene in the midgut of the mosquito, *Aedes aegypti*. *Insect Biochemistry and Molecular Biology* 25, 241–246.
- Barrett, F.M., 1974. Changes in the concentration of free amino acids in the haemolymph of *Rhodnius prolixus* during the fifth instar. *Comparative Biochemistry and Physiology* 48B, 241–250.
- Bounias, M., Vivares, C.P., Nizeyimana, B., 1989. Functional relationships between free amino acids in the hemolymph of fourth instar larvae of the mosquito *Aedes aegypti* (Diptera, Culicidae) as a basis for toxicological studies. *Journal of Invertebrate Pathology* 54, 16–22.
- Briegel, H., 1990. Fecundity, metabolism, and body size in Anopheles (Diptera: Culicidae), vectors of malaria. *Journal of Medical Entomology* 27, 839–850.
- Bugajska-Schretter, A., Grote, M., Vangelista, L., Valent, P., Sperr, W.R., Rumpold, H., Pastore, A., Reichelt, R., Valenta, R., Spitzauer, S., 2000. Purification, biochemical, and immunological characterization of a major food allergen: different immunoglobulin E recognition of the apo- and calcium-bound forms of carp parvalbumin. *Gut* 46, 661–669.
- Burke, S.R., 1976. *The Composition and Function of Body Fluids*. The C.V. Mosby Company, St. Louis, MO.
- Clements, A.N., 1992. *The biology of mosquitoes*, Volume 1, Development, Nutrition and Reproduction. Chapman & Hall, London.
- Gäde, G., Auerswald, L., 2002. Beetle's choice—proline for energy output: control by AKHs. *Comparative Biochemistry and Physiology B* 132, 117–129.
- Grimes, A.J., 1980. *Humand Red Cell Metabolism*. Blackwell Scientific Publications, Oxford, UK.
- Henn, M.W., Schopf, R., Maier, W.A., Seitz, H.M., 1998. The amino acid composition of *Anopheles stephensi* (Diptera: Culicidae) infected with *Nosema algerae* (Microsporidia: Nosematidae). *Journal of Invertebrate Pathology* 71, 42–47.
- Kogan, P.H., 1990. Substitute blood meal for investigating and maintaining *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology* 27, 709–712.
- Neufeld, D.S., Leader, L.P., 1998. Freezing survival by isolated Malpighian tubules of the New Zealand alpine weta *Hemideina maori*. *Journal of Experimental Biology* 201, 227–236.
- Patrick, M.L., Bradley, T.J., 2000a. Regulation of compatible solute accumulation in larvae of the mosquito *Culex tarsalis*: osmolarity versus salinity. *Journal of Experimental Biology* 203, 831–839.
- Patrick, M.L., Bradley, T.J., 2000b. The physiology of salinity tolerance in larvae of two species of Culex mosquitoes: the role of compatible solutes. *Journal of Experimental Biology* 203, 821–830.
- Shapiro, A.B., Wheelock, G.D., Hagedorn, H.H., Baker, F.C., Tsai, L.W., Schooley, D.A., 1986. Juvenile-hormone and juvenile-hormone esterase in adult females of the mosquito *Aedes aegypti*. *Journal of Insect Physiology* 32, 867–877.
- Sowa, S.M., Keeley, L.L., 1996. Free amino acids in the hemolymph of the cockroach, *Blaberus discoidalis*. *Comparative Biochemistry and Physiology A* 113, 131–134.
- Stidham, J.D., Liles, J.N., 1969. Free amino acid composition of the ageing female mosquito *Aedes aegypti* as determined by automatic ion-exchange chromatography. *Journal of Insect Physiology* 15, 1969–1980.
- Su, T., Mulla, M.S., 1997. Quantitative determination of free amino acids in the hemolymph of autogenous and anautogenous strains of *Culex tarsalis* (Diptera: Culicidae). *Journal of Medical Entomology* 34, 729–734.
- Thayer, D.W., Terzian, L.A., 1970. Free amino acids and related compounds in the tissues of the aging female *Aedes aegypti* mosquitoes. *Journal of Insect Physiology* 16, 1–15.
- Uchida, K., Ohmori, D., Yamakura, F., Suzuki, K., 1990. Changes in free amino acid concentration in the hemolymph of the female *Culex pipiens pallens* (Diptera: Culicidae), after a blood meal. *Journal of Medical Entomology* 27, 302–308.
- von Dungern, P., Briegel, H., 2001. Enzymatic analysis of uricotelic protein catabolism in the mosquito *Aedes aegypti*. *Journal of Insect Physiology* 47, 73–82.