Free Amino Acids and Osmoregulation in Penaeid Shrimp

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Shrimp, *Penaeus stylirostris* Stimpson, were subjected to increasing and decreasing salinity, ranging from 10 to 50‰. Glycine, proline, and alanine levels increased significantly with increasing salinity. Only glycine and proline levels decreased significantly when the salinity was reduced. Glycine levels at 36‰ were similar in both *P. stylirostris* and *P. aztecus*. Regression equations (mmol 100 g^{-1}) for glycine, glycine plus proline, proline, and total amino acids were $10.156 + 0.151x$, $11.426 + 0.233x$, $2.016 + 0.065x$, and $21.097 + 0.249x$ (decreasing salinity), respectively. The regression equation (mmol 100 g^{-1}) for alanine was $0.685 + 0.070x$ (increasing salinity).

Amino acids have been shown to be involved in osmoregulation in crustaceans by several investigators. These studies have been reviewed by Schoffeniels and Gilles (1970). Although total amino acid levels have been shown to participate in osmoregulation in different species of crustaceans, the role of specific amino acids has not been identified. In a recent study Cobb and Vanderzant (1974) indicated that glycine might be the osmoregulator in white shrimp, *Penaeus setiferus*.

In addition to their role as osmoregulators, free amino acids have been implicated as being responsible for much of the flavor in shrimp. Glycine probably causes the sweet taste in freshly caught shrimp (Hashimoto, 1965; Rajendranathan Nair and Bose, 1965). If the levels of certain amino acids increase with increases in salinity, the production of more tasty, cultured shrimp might be possible by manipulating the salinity of the water.

In this study the effect of salinity in the range of 10–50‰ on amino acid levels in the tails (abdomen) of *Penaeus stylirostris* was investigated. Amino acid levels in brown shrimp (*Penaeus aztecus*) from waters of constant salinity were measured to determine the possible applicability of the results to other species.

EXPERIMENTAL SECTION

Materials. Shrimp, *Penaeus stylirostris* Stimpson, used in the experiment were reared in ponds of the Texas Agricultural Extension Service's mariculture facilities in Corpus Christi, Tex. Twelve fiberglass tanks (89 × 52 × 55 cm) were equipped with substrate filters, each with two 1.27-cm PVX airlifts. The filters were covered by a 5-cm layer of sand and shell. Circulation and aeration were maintained by a Conde Dry-Air Blower. Each tank was covered by a lid with an internal black polyethylene lining to prevent water evaporation. Salinity was measured with an A.O. Goldberg refractometer. Water pH was measured with an Amstro pH meter.

Water at 46‰ was pumped directly from the Laguna Madre through 5-μ filters into each tank and maintained at a depth of 30 cm. Shrimp were seined from the production ponds (46‰) and five shrimp, averaging 110 mm and 10 g, were placed in each tank. The salinity was adjusted to 50‰ with seasalts and the shrimp were allowed to acclimate for 7 days. Tanks containing shrimp were designated by random selection, with two replicates of each of the following: 50‰ control (shrimp sampled at end of experiment), 50‰ test (shrimp sampled at beginning of experiment), 40‰ test, 30‰ test, 20‰ test, and 10‰ test. Shrimp were sampled after 4–7 days acclimation to these salinities. Salinities in the test tanks were adjusted with a distilled water drip system at the rate of 5‰ per day until the desired salinity was reached. Water temperature was maintained at 28–32°. Water pH fluctuated between 8.1 and 8.5.

A second test was established to determine the effect of increasing salinities. Seven tanks were set up as previously described with four tanks designated as a control and three tanks designated as tests. Shrimp were seined from the ponds (34‰) and three shrimp averaging 110 mm and 10 g were placed in each tank (34‰). After 7 days acclimation the salinity in the test tanks was adjusted to 10‰ at a rate of 5‰ per day. The shrimp were allowed to acclimate for 3 days at which time one shrimp was removed from each test and control tank for analysis. The salinity in the test tanks was then increased with seasalts at 5‰ per day until a salinity of 34‰ was reached. After 4 days acclimation one shrimp was removed from each tank. The salinity was increased to 50‰ and after 4 days acclimation the remaining shrimp in both test and control tanks were removed for analysis. Water temperature was maintained between 23 and 27°. Water pH fluctuated between 7.2 and 8.1.

Shrimp were fed 2.1 g of Ralston Purina 25% protein diet at the same time each day. In order to minimize the effect of feeding, shrimp for analyses were removed just prior to the time for feeding.

Brown shrimp (*Penaeus aztecus*) (Ives) were taken by trawl from different locations at the 18 fathom level in the Gulf of Mexico.

Four or five shrimp were pooled for each analysis in the decreasing salinity experiments; one or two shrimp were pooled for each analysis in the increasing salinity experiments. The shrimp head was removed. The tail (abdomen) was then blotted dry and homogenized in 7% trichloroacetic acid solution in a Waring Blendor. The homogenate was slowly stirred at 3° for 2 hr after which it was centrifuged to remove insoluble protein. The resulting extract was diluted 1:20 with 0.1 *M* HCl and analyzed with a fully automated Beckman Spinco Model 120C amino acid analyzer. In order to get good glycine values, samples were also analyzed at 1:50 dilution.

RESULTS AND DISCUSSION

There was a significant relationship ($P < 0.01$) between the free amino acid (FAA) concentration in the abdomen of *Penaeus stylirostris* and the salinity of the water from which the shrimp were taken (Table I). The variation in free amino acid content with salinity was caused mainly by variation in the levels of free glycine and proline (Figures 1–3) with glycine contributing the major portion of the increase. When the shrimp was subjected to increasing salinity, alanine contributed significantly to the increase in free amino acid levels. When the shrimp was subjected to de-

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Table I. Correlation Coefficients and Regression Lines for Free Amino Acids in the Abdomen of *Penaeus stylirostris* Taken from Water with Varying Salt Content

	Increasing salinity		Decreasing salinity	
Amino acid	Corr. coeff.	Regression line	Corr. coeff.	Regression line
Lys-Orn	−0.158	$0.311 - 0.0006x$	N.L.[c]	
His	0.299	$0.072 + 0.0005x$	−0.012	$0.104 + 0.000x$
NH_3	0.315	$1.464 + 0.0051x$	0.374	$0.588 + 0.0027x$
Arg	0.233	$3.352 + 0.0049x$	0.443	$3.000 + 0.0083x$
Tau	−0.247	$1.300 - 0.0026x$	0.195	$0.760 + 0.0012x$
Asp	0.745[a]	$0.005 + 0.0024x$	0.007	$0.063 + 0.001x$
Thr	0.428	$0.152 + 0.0014x$	0.370	$0.256 + 0.002x$
Ser-Gln-Asn	0.316	$0.791 + 0.0046x$	N.L.	
Glu	0.788[a]	$0.205 + 0.0085x$	0.317	$0.539 + 0.002x$
Prl	0.620[b]	$2.330 + 0.063x$	0.877[a]	$2.016 + 0.065x$
Gly	0.818[a]	$9.358 + 0.163x$	0.914[a]	$10.156 + 0.151x$
(Gly + Pro	0.897[a]	$11.689 + 0.226x$	0.943[a]	$11.426 + 0.233x$)
Ala	0.629[b]	$0.685 + 0.070x$	0.456	$1.447 + 0.0131x$
Val	0.585[b]	$0.108 + 0.0023x$	0.024	$0.139 + 0.008x$
Met	−0.293	$0.021 - 0.0005x$	0.014	$0.114 - 0.001x$
Ile	−0.210	$0.257 - 0.0004x$	0.298	$0.140 \mp 0.0004x$
Leu	0.483	$0.299 - 0.0014x$	0.250	$0.210 + 0.0008x$
Tyr	0.238	$0.056 + 0.006x$	N.M.[d]	
Phe	N.M.		N.M.	
Total	0.927[a]	$20.125 + 0.324x$	0.948[a]	$21.097 + 0.249x$

[a] Significant at 0.01 level. [b] Significant at 0.05 level. [c] Nonlinear. [d] Not measurable.

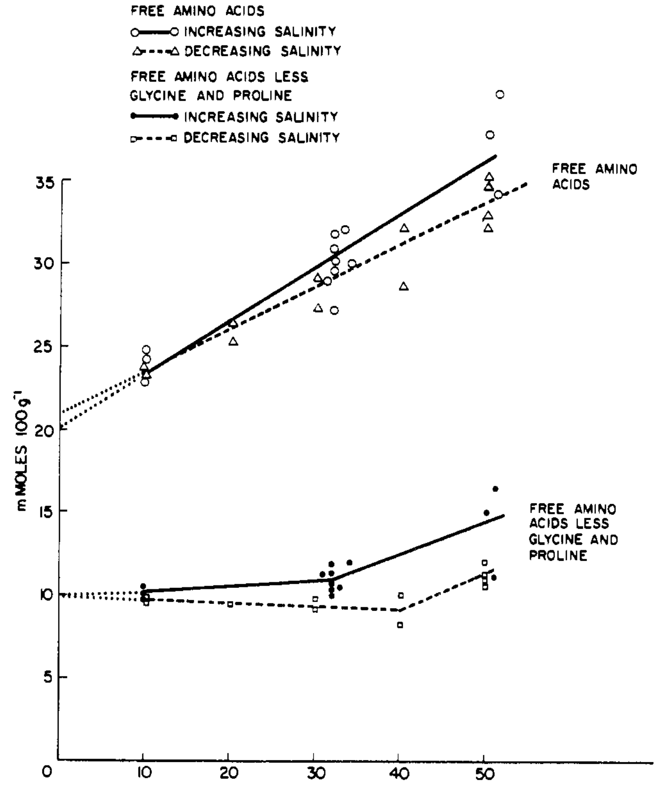

Figure 1. Variation in total free amino acid and free amino acid less glycine and proline content of *P. stylirostris* with salinity.

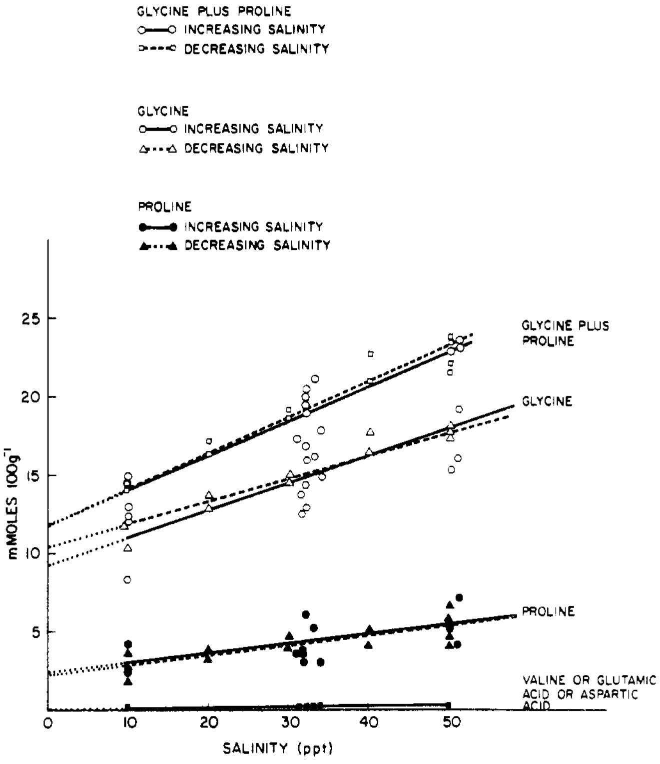

Figure 2. Amino acids whose levels decreased and/or increased significantly in *P. stylirostris* as salinity increased or decreased.

creasing salinity, there was no apparent relationship between the alanine content and salinity. Glutamic acid, aspartic acid, and valine levels, which increased significantly as the salinity of the water increased, did not contribute significantly to the increase in free amino acid levels (Figure 2). The levels of the amino acids, which are not illustrated in Figures 2 and 3, were less than 1 mmol 100 g^{-1} and showed little variation from sample to sample. The levels of free amino acids in the controls maintained at constant salinity did not change significantly during the experiments.

The increase in the free amino acid levels less glycine

Table II. Free Glycine, Proline, Alanine, Taurine, Arginine, and Total Free Amino Acid Levels in Brown Shrimp (*Penaeus aztecus*) Taken from Off-Shore Areas in the Gulf of Mexico

Sampling month	Glycine	Proline	Glycine-Proline	Alanine	Taurine	Arginine	Total amino acids
	mmol 100 g^{-1}						
June[a]	14.87[b]	2.06	16.93	0.659	2.79	4.62	27.64
June[a]	15.51	1.36	16.87	0.795	3.01	4.36	26.79
August	15.08	1.44	16.52	0.640	2.88	4.72	27.49
Mean	15.15	1.62	16.77	0.698	2.89	4.57	27.31

[a] Samples taken in June were from different areas. [b] Five shrimp tails were used for each extract.

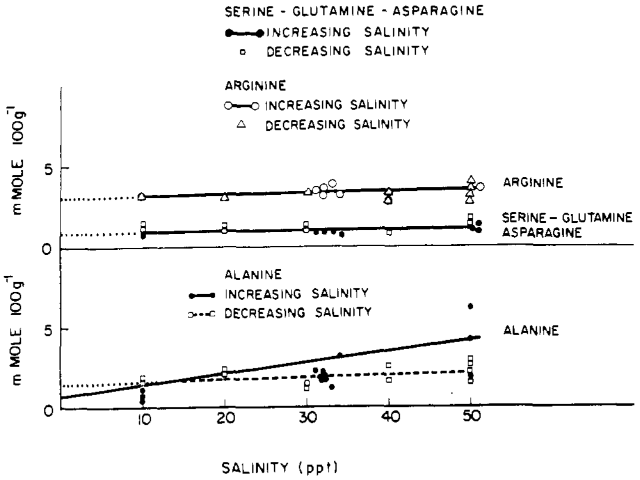

Figure 3. Other amino acids whose levels were sufficiently high to contribute significantly to internal osmotic pressure in *P. stylirostris*.

and proline at 50‰ salinity (Figure 1) suggests that some dehydration of the shrimp could have occurred. However, dehydration was not evident as length–weight measurements did not change significantly.

If glycine and/or glycine plus proline and/or alanine are osmoregulators in other species of penaeid shrimp, we would expect that the levels of these amino acids would be similar in shrimp taken from waters where the salinity is relatively constant. In Table II are listed the free glycine, proline, glycine–proline, alanine, and total free amino acid levels in brown shrimp (*Penaeus aztecus*) taken from the 18–20 fathom level in the Gulf of Mexico. Bottom salinities in this area remain at approximately 36‰ (Collier, 1958). Glycine, glycine plus proline, and total amino acid levels were very similar in all three samples while proline and alanine levels varied considerably. Mean glycine and total amino acid values were 99.97 and 90.8%, respectively, of those predicted for 34‰ salinity from the *P. stylirostris* data (decreasing salinity). Mean proline and glycine plus proline values were 37.2 and 84.6%, respectively, of predicted values. Mean alanine values were only 21.8% of those predicted from the increasing salinity data. The low proline values in *P. aztecus* were off-set by taurine and arginine values which were approximately 2 and 1 mmol 100 g^{-1} higher, respectively, than corresponding values for *P. stylirostris*. Since one group of *P. stylirostris* had been maintained at approximately 34‰ salinity and the second group at 46‰ salinity prior to transfer to the laboratory, these differences may represent species differences.

In order for a substance to be utilized by the shrimp for osmoregulation in a rapidly changing environment, it should: (1) be a readily synthesized metabolite and/or an available substance in the water and/or a substance which can be stored in an "osmotically inactive form," and (2) it should be rapidly synthesized or eliminated when the osmotic environment changes. Very little information is available on the metabolism or elimination of glycine and proline in penaeid shrimp, but both have been shown to be nonessential amino acids (Shewbart et al., 1973). Proline has been shown to be metabolized and alanine synthesized in post-mortem shrimp muscle (Cobb and Vanderzant, 1974). These studies indicated that in penaeid shrimp glycine and proline are the osmoregulators, whether the salinity is increasing or decreasing, but the long equilibrium time used in these experiments eliminated any conclusion as to the rapidity of synthesis or elimination.

In addition to salinity a number of factors could possibly affect the free amino acid content of shrimp. Among these are (1) nutritional state of the shrimp, (2) sampling time after feeding, and (3) status of molting cycle. The first two variables were minimized by feeding the shrimp a good diet daily and by sampling just prior to normal feeding time. Molting was not evident in the shrimp used in this study, suggesting that the molting cycle did not significantly affect the free amino acid levels.

The FAA content found for *P. stylirostris* and *P. setiferus* in seawater agrees with amino acid data presented for *Eriocheir sinensis* by Bricteux-Grégoire et al. (1962) and for *Carcinus maenas* by Duchâteau et al. (1959). Glycine values in penaeid shrimp were considerably higher than those reported for either of these species. However, combined glycine–proline values were similar in *C. maenas* and the penaeid shrimp.

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