SPECIAL PUBLICATION

DEDICATED TO

Dr. N. K. PANIKKAR

MARINE BIOLOGICAL ASSOCIATION OF INDIA

The Investigator

MAY 1973

BIOCHEMICAL STUDIES ON MARINE ZOOPLANKTON THE AMINO ACID COMPOSITION OF SOME LOCAL SPECIES.

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ABSTRACT

Further analyses of the amino acid composition of protein hydrolysates of *Neomysis integer* has confirmed the importance of glutamic, aspartic, lysine, leucine and arginine (in that descending order) in both ovigerous females and less mature animals. *Mesopodopsis slabberi* closely resembles *Neomysis* in its amino acid composition. *Sagitta setosa* shows considerable similarity to the mysids in its protein amino, acids but alanine replaces leucine in importance. There is a somewhat larger concentration of glycine (~ 7%) and of histidine (~ 5%) among the less important amino acids. Despite the low level of protein in *Pleurobrachia pileus*, the pattern of amino acids shows considerable resemblance to the other planktonic species but glycine occurs in greater concentration than leucine or arginine.

In both mysid species glycine, taurine and arginine are the dominant free amino acids. No taurine is found in *Sagitta setosa;* glutamic is the major amino acid followed by aspartic, lysine, glycine and arginine.

INTRODUCTION

Relatively few studies have been made on the amino acid composition of species of marine zooplankton. Cowey and Corner (1963) investigated the amino acid composition, including the free amino acids, of *Calanus finmarchicus*. Jeffries (1969) dealt with the free amino acids in some zooplankton, especially *Acartia* species, though these were not "pure" samples. Several authors, Suyama, Nakajima and Nonaka (1965); Sidhu *et al.* (1970); Burkholder *et al.* (1967); Moiseev (1970) and Srinivasagam *et al.* (1971) have examined the composition of the body protein in *Euphausia superba.* The latter authors also compared the composition of *E. superba* with that of the euphausiid, *Meganyctiphanes norvegica,* and included an analysis for a marine mysid, *Neomysis integer*. In the present work the amino acid composition of *Neomysis* has been re-investigated using ovigerous females as well as less mature specimens. The composition of this mysid has also been compared with that of three other local zooplankton species. Of these species *Neomysis* occurs highest up the estuary in fluctuating but generally rather low salinity (mean~ 9%o). The other species rarely encounter salinities less than 30%o.

We are grateful to Miss Janet Redmond for assistance with the analyses. This work was supported by a grant from the Natural Envirnoment Research Council.

Material and Methods

Two different groups of animals were compared in the case of *Neomysis*. One set consisted of large ovigerous females which were analysed fresh immediately after capture in the field. The other group consisted of more or less full grown individuals of both sexes, but the marsupium in the females was little developed. These animals also differed in that they had been maintained in the laboratory before analysis for $\sim$10 days in 9‰ seawater. This approximates to the average salinity in their natural habitat.

Both the *Sagitta setosa* material and that of *Mesopodopsis slabberi* after sorting was deep frozen in the laboratory (−20°C approximately) before analysis. As a test of the possible use of freeze dried material for analysis, some *Mesopodopsis* taken from the same haul were freeze dried in the laboratory and used subsequently. The results reported concern only the deep frozen material, but tests showed that there was exceptionally good agreement between the results for amino acid composition for protein hydrolysates of the deep frozen and freeze dried material. Some discrepancies were noted in the analysis of free amino acids from the freeze dried material; the concentrations of many of the amino acids were considerably reduced especially those occurring in smaller quantities. This has been encountered before in unpublished work on freeze dried material of *Sagitta enflata* and *Calanus plumchrus*.

Pleurobrachia pileus material was also freeze dried. As there seemed to be some doubt about the validity of concentrations of free amino acids in freeze dried material, only the protein hydrolysate analysis has been reported for *Pleurobrachia*.

For all analyses, the lipid was first removed from the tissue according to the method of Folch *et al.* (1957), except that in the case of freeze dried material a small quantity of distilled water was first added to the dried tissue so that the material was allowed partly to hydrate. It was inconvenient, however, to hydrate the tissues completely; this was particularly true of *Pleurobrachia*. Following the lipid removal, the free amino acids were extracted from the lipid free tissues with hot distilled water. Proteins were then precipitated with hot trichloroacetic acid and the hydrolysates prepared by refluxing with 6N hydrochloric acid.

Standard methods were used for obtaining 21 hour chromatograms of both free amino acids and protein hydrolysates, employing a Technicon auto analyser and using external norleucine standards at the beginning and end of the chromatogram run. An internal norleucine standard (0.1 μmols) was used in both sample and the standard amino acid solutions.

Results and Discussion

Neomysis integer (Leach)

Although the precise values of the individual protein amino acids reckoned as mg/gram dry weight are different in the ovigerous females and the less mature animals (Table 1), when reckoned as a percentage of the total amino acids, there is good agreement between the two groups of mysids (Table 2). The pattern of relative importance of the amino acids is generally similar in the two groups, and the percentage of individual amino acids never disagrees by more than 0.3%, except for methionine and cystine. These amino acids occurred in both groups in very small amounts and were the lowest in order of concentra tion (Table 1). They were however slightly higher in concentration in the large ovigerous females than in the less mature specimens. There is also very good agreement between these analyses and the data on amino acid composition of protein hydrolysates of *Neomysis* reported earlier by Srinivasagam *et al.* (1971). This good agreement suggests as might be expected that only relatively very minor changes occur in the composition of the protein of the body with age and maturity.

TABLE 1. *The amino acid composition of protein hydrolysates in the four species (Values as mg/g dry wt.)*

Amino Acids		*Neomysis integer* Ovigerous females	*Neomysis integer* Less mature	*Mesopodopsis slabberi*	*Sagitta setosa*	*Pleurobrachia pileus*
Aspartic acid	..	51.9	54.1	60.4	40.1	4.6
Threonine	..	19.1	19.8	24.4	16.0	2.1
Serine	..	17.5	18.1	25.2	18.1	2.2
Glutamic acid	..	79.9	97.5	81.2	54.6	4.8
Proline	..	13.9	12.4	17.4	16.2	2.0
Glycine	..	16.8	20.9	22.0	30.9	3.0
Alanine	..	24.3	27.7	31.9	38.6	2.3
Valine	..	24.2	27.7	30.8	26.8	2.2
Cystine	..	5.0	3.7	7.5	8.3	0.2
Methionine	..	10.7	8.7	12.9	12.4	0.2
Isoleucine	..	24.5	27.2	29.2	24.6	1.9
Leucine	..	36.0	42.6	42.6	27.1	2.9
Tyrosine	..	18.8	19.9	23.7	14.8	1.1
Phenylalanine	..	23.5	25.3	25.1	21.2	1.7
Lysine	..	47.5	51.8	56.8	42.6	3.4
Histidine	..	16.2	12.2	19.6	22.7	1.3
Arginine	..	34.0	39.6	36.5	38.3	2.8
Taurine	..	—	—	—	—	—
Ornithine	..	1.1	0.9	1.2	1.4	—
Total	..	464.9	510.1	548.4	454.7	39.4

TABLE 2. *The amino acid composition of protein hydrolysates in the four species expressed as percentages*

Amino Acids		*Neomysis integer* Ovigerous females	Less mature	*Mesopodopsis slabberi*	*Sagitta setosa*	*Pleurobrachia pileus*
Aspartic acid	..	11.2	10.6	11.0	8.8	11.7
Threonine	..	4.1	3.9	4.4	3.5	5.3
Serine	..	3.8	3.5	4.6	4.5	5.6
Glutamic acid	..	17.2	19.1	14.8	12.0	12.2
Proline	..	3.0	2.4	3.2	3.6	5.0
Glycine	..	3.6	4.1	4.0	6.8	7.6
Alanine	..	5.2	5.4	5.8	8.4	5.8
Valine	..	5.2	5.4	5.6	5.9	5.6
Cystine	..	1.1	0.7	1.4	1.8	0.5
Methionine	..	2.3	1.7	2.4	2.7	0.5
Isoleucine	..	5.3	5.3	5.3	5.4	4.8
Leucine	..	7.7	8.4	7.8	6.0	7.4
Tyrosine	..	4.0	3.9	4.3	3.2	2.8
Phenylalanine	..	5.1	5.0	4.6	4.7	4.3
Lysine	..	10.2	10.2	10.4	9.4	8.6
Histidine	..	3.5	2.4	3.6	5.0	3.3
Arginine	..	7.3	7.8	6.7	8.4	7.1
Taurine	..	—	—	—	—	—
Ornithine	..	0.2	0.2	0.2	0.3	—

If the amino acids of protein hydrolysates reckoned on a dry weight basis are totalled, the large ovigerous females show a lower amount (465 mg/g dry wt.) as compared with the less mature animals (510 mg/g dry wt). This may reflect a total lower protein content for the large ovigerous females and this may be associated with an increased lipid content, as suggested by Linford (1965). But it will be remembered that the less mature animals had been maintained in the laboratory in constant diluted seawater; their total amino acids (510 mg/g) show some decrease when compared with the fresh non-ovigerous females, previously analysed by Srinivasagam *et al.* (1971), where the total was 585 mg/g. However, if the free and protein hydrolysate amino acids are totalled together, the animals kept in diluted seawater in the laboratory show relatively little difference from the total amino acids for fresh mysids (655 and 681 mg/g respectively). The reduction in protein amino acids and the raised concentration of free amino acids in those mysids maintained in 9‰ seawater may be associated with osmotic regulation in a constant salinity. In their natural environment the mysids are subject to salinities varying with the tide. It is perhaps significant that the total of protein and free amino acids approximates reasonably well to that of animals obtained fresh from the natural habitat.

Since the percentage composition of amino acids in protein hydrolysates is so similar for ovigerous females and the less mature animals, the results may be reported together. Glutamic acid is overwhelmingly predominant of the amino acids; then in descending order of importance follow aspartic, lysine, leucine and arginine, these five amino acids together equalling 54% and 56% respectively for the two groups. In the earlier work reported by Srinivasagam *et al.* (1971) these five amino acids totalled 54%. Moreover, in all three crustaceans previously analysed (*Neomysis, Euphausia* and *Meganyctiphanes*), the five dominant protein amino acids were identical and occurred in the same order of importance.

Cowey and Corner (1963) quote results for the amino acid composition of *Calanus finmarchicus.* On the basis of g. amino acid N/100 g total amino N, the order in descending importance is arginine, lysine, glycine, glutamic, alanine, aspartic and leucine. For the closely related species, *C. helgolandicus,* Corner and Cowey (1964) list arginine, glycine, lysine, alanine, glutamic, aspartic and leucine. While the data are not strictly comparable with values quoted as percentages, the same important amino acids appear as in other planktonic crustaceans, but the order is not identical and glycine occurs in larger concentrations in *Calanus.*

In *Neomysis,* following the five major amino acids, a group occurs with only relatively minor differences: valine, alanine, isoleucine and phenylalanine, each amounting to approximately 5% of the total (Table 2.) This result shows again very good agreement with our earlier findings.

As regards the free amino acids, the comparison of the two groups of mysids shows general agreement in the proportions of the major free acids. They also confirm the earlier report that, glycine, taurine, and to a lesser extent arginine, are the chief constituents. Together they account for 68% and 71% respectively for the immature mysids and the large ovigerous females. This may be compared with the earlier finding of 73% for *Neomysis.*

TABLE 3. *A comparison of the major free amino acids in three planktonic species (Each concentration is expressed as a percentage of the total. Note that only eight of the major amino acids are listed)*

Amino Acids	*Neomysis integer* Ovigerous females	*Neomysis integer* Less mature	*Mesopodopsis slabberi*	*Sagitta setosa*
Aspartic	3.1	2.9	4.7	12.0
Glutamic	6.0	5.6	7.9	17.8
Proline	5.9	2.4	—	8.9
Glycine	27.4	29.6	22.5	10.0
Alanine	4.8	5.2	2.9	6.7
Lysine	1.3	2.2	9.0	11.0
Arginine	17.6	12.0	25.5	10.0
Taurine	26.4	26.6	14.7	—

Of the other free amino acids, glutamic, proline, aspartic and alanine are present in relatively significant quantities, together totalling 15% in the immature mysids as compared with 16% in the earlier investigation. Ovigerous females in the present study show slightly higher amounts of these four free amino acids especially proline; together they amount to 20% (Table 3).

Ornithine is omitted from discussion because, though usually present in small quantities, this amino acid may result in part from decomposition of arginine. Two amino acids, both sulphur containing, cystine and methionine, in addition to being invariably present in the lowest concentration of the protein hydrolysates (*vide supra*) are also present in very small amounts among the free amino acids.

Mesopodopsis slabberi (Van Beneden)

Results of analysis of the protein hydrolysates show a remarkable similarity to that of *Neomysis;* glutamic, aspartic, lysine, leucine and arginine are again the most important amino acids in that descending order. Together they account for 51% of the total protein hydrolysates. The same pattern of amino acids, valine, alanine and isoleucine each of the order of 5% of the total, follow as in the case of *Neomysis*. But in *Mesopodopsis* the concentration of phenylalanine approximates more closely to that of serine, threonine and tyrosine, all amounting to approximately 4.5% of the total. Cystine and methionine are again present in the lowest concentrations (Table 2).

Analysis of deep frozen material showed the same three major free amino acids, glycine, taurine and arginine, as in *Neomysis*. Together they reached some 63% of the total free amino acids, a value somewhat lower than for *Neomysis*. But more significantly, arginine is the major free amino acid being slightly greater in amount than glycine (Table 3).

Mesopodopsis lives in relatively more saline conditions and inhabits lower reaches of Southampton water. It occurs in the deeper water layers where fluctuations in salinity with tide are comparatively small. This habitat difference may be associated with the relatively small amounts of glycine and taurine which are recognised as important in osmoregulation. Arginine is well known on the other hand as an energy reserve in crustaceans. Proline is another amino acid often participating in osmoregulatory functions in crustaceans. While occurring in *Neomysis* to a small extent (*circa.* 5%), it has not been recognised in the analysis of free amino acids in *Mesopodopsis*. Apart from proline, as in *Neomysis*, the quantities of glutamic, aspartic and alanine are also significant among the free amino acids of *Mesopodopsis*. Lysine apparently accounted for 9% of the free amino acids (Table 3); there is some suspicion, however, that this may be an artefact.

Earlier investigations by Srinivasagam *et al.* (1971) involved an examination of the free amino acids of two euphausiids, *Euphausia superba* and *Meganyctiphanes norvegica*, as well as of *Neomysis*. Although the concentrations of the free amino acids showed some differences between the three crustaceans, glycine, taurine and arginine were important in all the species; lysine occurred in fair quantity in the two euphausiids, and proline and alanine were found in moderate amounts in *Euphausia* and *Neomysis*. There is, therefore, some degree of agreement among these planktonic crustaceans as regards the more important free amino acids, though the lack of proline in *Mesopodopsis* is outstanding.

Some general agreement also appears from analyses by Jeffries (1969) of another planktonic crustacean group. For the copepods, *Acartia clausi* and *A. tonsa*, glycine is the major free amino acid; proline, taurine, alanine and arginine also occur in relatively large concentrations. Jeffries points out that the first four amino acids are subject to considerable and apparently fairly rapid changes in concentration. He believes that these may reflect the physiological condition and possibly productive capacity of the animals, apart from any osmoregulatory function. Cowey and Corner (1963) also report glycine, taurine, arginine, alanine, glutamic and proline as the most important of the free amino acids in *Calanus*.

Sagitta setosa J. Muller

There are considerable resemblances in the pattern of the dominant protein hydrolysate amino acids to those of the two mysid species. For instance, in *Sagitta* glutamic is the major acid, though it is slightly less prodominant than in the crustaceans. Lysine, aspartic and arginine are also important among the first five amino acids, but alanine has replaced leucine in order of importance (Table 1). The first five amino acids together from approximately 45% of the total.

The next grouping of amino acids shows some similarities to that found in the crustaceans in that valine and isoleucine are present in comparable concentrations. A significant difference is the relatively high amount of glycine (6.8%) (Table 2). Histidine, normally of minor importance in crustaceans is found in a concentration equal to 5% of the total and ranks tenth in descending order of importance. Once again the two sulphur containing amino acids, cystine and methionine, are present in lowest concentrations (Table 2).

Some comparison may be made with several analyses of samples of *Sagitta elegans* obtained earlier from the Millport Marine Station. These analyses are subject to slight suspicion since the material, deep frozen after collection, was maintained in the laboratory for a somewhat prolonged period. Nevertheless, the general order of importance of protein hydrolysate amino acids is very similar to that in the related species, *Sagitta setosa*. Thus glutamic is again dominant; lysine, aspartic, leucine and arginine are next in importance, though in one or two analyses of *S. elegans* alanine replaced arginine in order of ranking. The first five amino acids total 50% as against 47% in *Sagitta setosa*. Valine and isoleucine are again in the next important group of amino acids, and with glycine occur in approximately the same amount (~ 5%).

Cystine is always in lowest concentration in the analyses of *Sagitta elegans*.

It is perhaps of interest that in another more typically oceanic and relatively warm water species, *Sagitta enflata*, preliminary analysis shows the same first six amino acids followed by glycine.

Some difficulty was experienced in estimating the free amino acid concentrations in *sagitta setosa* mainly owing to the relatively small amount of material available. However some differences between *Sagitta* and the two mysids are obvious. Glutamic, aspartic and lysine are the first three amino acids in *Sagitta*, followed fairly closely by glycine and arginine; there is also a fairly substanital amount (~ 9%) of proline. (Table 3). No taurine was recorded and the much decreased importance of glycine and arginine is in marked contrast not only to the present results for *Neomysis* and *Mesopodopsis*, but also to the data of Jeffries (1969) and Cowey and Corner (1963) for copepods. If these results are confirmed, they might suggest different metabolic pathways in Chaetognatha and Crustacea.

Pleurobrachia pileus (O. F. Muller)

Only freeze dried material has been used in these analyses (cf. Methods), largely on account of the very high water content of fresh tissue and owing to the brief seasonal appearance of the animal.

The concentration of total amino acids obtained from protein hydrolysates is relatively low (~ 40 mg/g dry tissue—Table 1). This is in accord with the very low protein and high ash content, reckoned on a dry weight basis, found in earlier proximal analyses of these ctenophores.

The relative importance of the individual amino acids obtained from protein hydrolysates shows, however, some considerable resemblance to that already recorded for other zooplankton species. Thus glutamic is again predominant, though aspartic is only slightly less abundant; leucine and lysine and arginine again are present in very significant amounts. A difference, however, is the relatively high concentration of glycine, which is more abundant than leucine and arginine (Table 1). These six amino acids tobether comprise ~ 55% of the total.

There is some resemblance to other zooplankton in the relative importance of alanine and valine, but isoleucine and phenylalanine are less abundant. Methionine and cystine are again in least concentration and indeed are barely detectable (0.5%—Table 2).

No data are included for free amino acids owing to the extremely small concentrations detected, mostly less than 0.1 mg/g.

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