Essential Amino Acids of the Prawn*¹Akio KANAZAWA*² and Shin-ichi TESHIMA*²

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The essential amino acids of the prawn *Penaeus japonicus* were investigated by a radio-isotopic method using [³H] acetate. After injection of [³H] acetate, the incorporation of radioactivity into the constituent amino acids of protein fraction was examined by column chromatography on Amberlite IR-120, followed by the radioactivity measurement. Radioactivity was unambiguously incorporated into aspartic acid, serine, glutamic acid, proline, glycine, alanine, and cysteic acid. These amino acids were suspected to be unnecessary for the prawn. On the other hand, little or no radioactivity was incorporated into valine, methionine, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, threonine, and tryptophan. We inferred that these 10 amino acids are not synthesized *de novo* and are probably essential for the growth of the prawn. Tyrosine was thought to be formed from the ingested phenylalanine.

As a part of our long term-project of the nutritional studies on the prawn *Penaeus japonicus* the requirements of essential amino acids (EAA) were investigated in the present study. The requirements of EAA are generally estimated on the basis of the following parameters: (1) The weight gain in the feeding experiments using the test diets (amino acid-diets) whose protein sources were replaced entirely or partially by the mixture of amino acids;¹⁻³⁾ or (2) the daily increase of each EAA in the fish bodies when they were fed on the diets containing proteins with high biological values.⁴⁾

In the case of the prawn,⁵⁾ however, the amino acid-diets are known to attain only poor growth as also observed in the carp.⁶⁾ This makes it difficult for us to estimate the EAA requirements of prawn *P. japonicus* by the feeding trials. In the present study, hence, we intend to define the amino acids essential for the growth of the prawn by using radio-isotopic methods.^{7,8)}

Materials and Methods

Injection of [³H] Acetate to the Prawn

Two specimens of the prawn *P. japonicus*, about 6.0 g in total body weight, were each injected with 15 μ Ci of [³H] sodium acetate (specific activity, 200 mCi/mmol; Radiochemical Centre, Amersham England) dissolved in 1.6 μ l of aqueous solution

containing carrier sodium acetate (0.7%). The radioactive acetate was injected into the muscle between the carapace and the second abdominal segment, and then the prawns were maintained on the fresh meat of short-necked clam in the aquarium (30 l) at 25°C.

Fractionation of the Prawns

Six days after injection of [³H] acetate, the whole body of prawns was fractionated into 4 fractions by the method of SCHNEIDER;⁹⁾ that is, the prawns were minced, homogenized in an ice-cold trichloroacetic acid (TCA), and separated into the TCA-soluble compound fraction and the residue. The residue was then extracted with organic solvents such as ethanol-water (4:1), ethanol, and ethanol-ether (3:1) to give the lipid fraction. The defatted residue was further separated into the protein and nucleic acid fractions by using a hot TCA solution.

Separation and Quantification of Individual Amino Acids

The protein fraction was oxidized with performic acid as described by HIRS¹⁰⁾ and then hydrolyzed in a sealed test tube with 5.7 N hydrochloric acid by the method of MOORE and STEIN.¹¹⁾ The performic acid oxidation converts cysteine and cystine to cysteic acid and methionine to the corresponding sulphone. The amino acids of protein hydrolysate were separated and quantified by the method of

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MOORE *et al.*¹²⁾ using column chromatography on Amberlite IR-120 (type II, Rohm and Haas Co.) with sodium citrate buffers. Tryptophan was also quantified and separated in the same manner after hydrolyzing the protein fraction in 5 N sodium hydroxide solution as mentioned by OELSHLEGEL *et al.*¹³⁾

Measurements of Radioactivity

The radioactivity of lipid, TCA-soluble compound, and nucleic acid fractions from the prawns and individual amino acids from the protein fraction was measured with a Beckman Liquid Scintillation Counter LS-230 using a dioxane solution of PPO (0.6%) and naphthalene (11.2%) as a scintillation cocktail. The radioactivity of protein fraction was determined in the similar manner after hydrolysis with hydrochloric acid.

Results and Discussion

Table 1 shows the distribution of radioactivity in the sea water and the fractions from the prawns 6 days after injection of [³H] acetate. About 90% of the injected radioactivity was recovered in the sea water and the fractions from the prawns; most of the recovered radioactivity was present in the sea water (95.2%) containing presumably excretory products. However, radioactive acetate was incorporated into all the fractions from the prawns such as the TCA-soluble compound, lipid, nucleic acid, and protein fractions, the largest amounts of radioactivity being detected in the lipid fraction.

The protein fraction was hydrolyzed and separated into the constituting amino acids to check the incorporation of radioactivity. As shown in Table 2, the high specific activity (cpm/ μ mol) was observed in aspartic acid, serine, glutamic acid, proline, glycine, alanine, and cysteic acid. These amino acids were conceived to be synthesized *de novo* in the prawn *P. japonicus* and presumably to be essential. However, it should be confirmed by the feeding experiments that the above mentioned amino acids are synthesized at sufficient rate to attain the optimum growth. Even if animals are capable of synthesizing amino acids *de novo*, some of the amino acids such as glycine are known to be dietary essential in rats and chick.^{14,15)}

Contrarily, little or no significant radioactivity was incorporated into the following 11 amino acids; tyrosine, valine, methionine-sulphone, isoleucine, leucine, phenylalanine, lysine, histidine, arginine, threonine, and tryptophan. The results

Table 1. Distribution of radioactivity in the fractions obtained from the prawns 6 days after injection of [³H] acetate (30 μ Ci)

Fraction	Total radioactivity recovered (μ Ci)
TCA-soluble compounds	0.39
Lipids	0.73
Nucleic acids	0.03
Proteins	0.15
Whole body of prawns	1.30
Sea water	25.80
Total radioactivity recovered	27.10

Table 2. Incorporation of radioactivity into the individual amino acids of protein fraction in the prawns 6 days after injection of [³H] acetate

Amino acids	Specific activity (cpm/ μ mol)	Amino acid	Specific activity (cpm/ μ mol)
Aspartic acid	203	Valine	0.1
Serine	102	Methionine* ¹	2.5
Glutamic acid	196	Isoleucine	0.7
Proline	138	Leucine	0.1
Glycine	132	Phenylalanine	0.1
Alanine	187	Lysine	0.2
Cysteic acid	159	Histidine	0.7
Tyrosine	1.2	Arginine	1.2
Threonine	1.9	Tryptophan	0.4

*¹ Determined as the corresponding sulphone.

of the present study on the amino acid-labelling pattern in the prawn *P. japonicus* were almost similar to those reported for the plaice and sole injected [¹⁴C] glucose¹⁶⁾ and also for the shrimp *Palaemon serratus* received [¹⁴C] acetate.⁶⁾ In the present study, tyrosine was not labelled significantly. But, we assume that this amino acid is formed from phenylalanine as pointed out in mammals.

The rationale and reliability of radio-isotopic methods for determination of amino acid requirements have been discussed by KASTING and MCGINNIS.⁷⁾ Later, the radiometric methods have been applied to the studies on amino acid requirements of insects,¹⁷⁾ sole,¹⁸⁾ plaice,¹⁸⁾ abalone,¹⁸⁾ crabs, *Uca pugilator*¹⁹⁾ and *Cancer magister*,²⁰⁾ shrimps, *P. serratus*,²¹⁾ and *Penaeus aztecus*,²¹⁾ freshwater shrimps, *Macrobrachium ohione*,²²⁾ and *M. rosenbergii*,²³⁾ crayfish,²⁴⁾ and lobster.²⁵⁾ In consideration of the data on the above animals, we conclude that EAA for the prawn *P. japonicus* are probably threonine, valine, methionine, isoleucine,

leucine, phenylalanine, lysine, histidine, arginine, and tryptophan. The 10 amino acids were also revealed by the feeding trials to be essential for the sockeye salmon,^{28,27)} rainbow trout,²⁸⁾ channel catfish,^{29,30)} eel,³¹⁾ carp,³²⁾ chinook salmon,³³⁾ and *Tilapia zillii*.³⁴⁾

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