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AMINO ACID METABOLISM OF *ASTACUS LEPTODACTYLUS* ESCH.—III. STUDIES ON THE BIOSYNTHESIS OF α- AND β-ALANINE FROM ASPARTATE

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Abstract—1. Six hours after injection of 1- or 4-^{14}C-aspartate into the crayfish *Astacus leptodactylus* almost all radioactivity incorporated was found in the amino acids.

2. From both precursors only the amino acids α-alanine and glutamic acid were labelled. The biosynthesis of α-alanine from aspartate via decarboxylation of aspartate, oxaloacetate or malate is discussed.

3. No synthesis of β-alanine via α-decarboxylation of aspartate had occurred during the incubation period. Its probable formation through the pyrimidine metabolism is discussed.

INTRODUCTION

IN TISSUES of *Astacus leptodactylus* marked variations are observed in the concentration of β-alanine, reaching values up to one-fifth of the total free amino acid pool (Partmann, 1971; van Marrewijk, 1972). Incubation of the crayfish with U-^{14}C-glucose resulted in a high concentration and a high labelling of β-alanine, whereas from 1-^{14}C-acetate as a precursor both factors were very low (van Marrewijk & Zandee, 1974). In Crustacea hardly any research has been made into the biosynthesis of β-alanine. Most interesting in this connection are the *in vitro* experiments of Gilles & Schoffeniels (1966), who have shown manometrically β-alanine synthesis by decarboxylation of aspartic acid in muscle extracts of the lobster *Homarus vulgaris* and the crayfish *A. astacus*.

Most data on the occurrence and synthesis of β-alanine in invertebrates refer to insects. In certain species of Diptera pupation is accompanied by marked increases in free β-alanine levels (Levenbook & Dinamarca, 1966). Incorporation of β-alanine into the puparium of the fly *Sarcophaga bullata* is directly related to the process of sclerotization (Bodnaryk, 1971a, b). Studies on the biosynthesis of β-alanine in insects showed that in housefly puparia β-alanine is synthesized from ^{14}C-aspartic acid and ^{3}H-uracil (Hijikuro, 1968; Nakai, 1971; Ross & Monroe, 1972). The experiments were, however, unable to show whether β-alanine synthesis from aspartate occurred through uracil synthesis and catabolism or via a direct aspartate decarboxylase system.

This paper reports incubation experiments *in vivo* of *A. leptodactylus* with 1-^{14}C-aspartate and 4-^{14}C-aspartate, intended to determine whether this crayfish is able to synthesize β-alanine directly by decarboxylation of aspartate. Moreover, the biosynthesis of α-alanine from aspartate in *A. leptodactylus* is discussed.

MATERIALS AND METHODS

Animals

Crayfishes (*Astacus leptodactylus*) imported from Turkey were kept in the laboratory aquarium until used for experiments as described in an earlier paper (van Marrewijk & Ravestein, 1974). Experimental animals were males in the intermolt stage.

Experimental procedure

Incubations of *A. leptodactylus* were performed with 1-^{14}C-aspartate (DL) and 4-^{14}C-aspartate (DL) as precursors. Two animals, total weight 103 g, were injected with 19·40 μCi 1-^{14}C-aspartate (sp. act. 3 mCi/m-mole). To two other animals, total weight 112 g, 12·15 μCi 4-^{14}C-aspartate (sp. act. 2·1 mCi/m-mole) was administered. In both experiments the incubation period was 6 hr. Administration of the radioactive precursors and incubation of the animals, as well as the subsequent isolation of lipids and free amino acids, analysis of amino acids and measurement of radioactivities, were performed as described in an earlier paper (van Marrewijk & Zandee, 1974).

RESULTS AND DISCUSSION

Radioactivities expired and incorporated after a 6 hr incubation period of *A. leptodactylus* with 1- and 4-^{14}C-aspartate are shown in Table 1. From both precursors approximately one-third of the dose

Table 1. Radioactivities expired and incorporated 6 hr after injection of 1-^{14}C-aspartate (DL) and 4-^{14}C-aspartate (DL) into *A. leptodactylus*

	1-^{14}C-Aspartate		4-^{14}C-Aspartate	
	Radioactivity		Radioactivity	
	μCi	%	μCi	%
Dose administered	19·40	100	12·15	100
$^{14}CO_2$ expired*	6·75	34·8	3·43	28·2
Activity dry homogenate	1·40	7·2	0·79	6·5
Activity lipids	0·01	<0·1	<0·01	<0·1
Activity free amino acids	1·09	5·6	0·77	6·3

* Including $^{14}CO_2$ from the water.

administered was recovered in CO_2 expired. Only 6–7 per cent of the radioactivity injected was incorporated into the dry homogenate. Together, radioactivities of CO_2 expired and incorporated into the homogenate accounted for less than one-half of the dose administered. This low recovery can be attributed to the high fixation of CO_2 into the $CaCO_3$ of the exoskeleton (Zandee, 1964, 1966) and several other factors (van Marrewijk & Zandee, 1974). Most of the radioactivity incorporated was found in the free amino acids. Radioactivity of the lipids was very low from both precursors.

Table 2 shows the incorporation of radiocarbon into the free amino acids 6 hr after injection of 1- and 4-^{14}C-aspartate. Only three amino acids were labelled: aspartic acid (precursor), glutamic acid and α-alanine. From 1-^{14}C-aspartate most of the radioactivity was found in α-alanine. For the synthesis of ^{14}C-labelled α-alanine from 1-^{14}C-aspartate two pathways can be mentioned:

(1) Direct synthesis by β-decarboxylation of 1-^{14}C-aspartate.

(2) Conversion of aspartate to oxaloacetate and malate, followed by decarboxylation to phosphoenolpyruvate or pyruvate and transamination to α-alanine.

From our results it could not be concluded whether in *A. leptodactylus* β-decarboxylation of aspartate takes place. As far as we know aspartic acid decarboxylase activity in animal tissues has been reported only once (Gilles & Schoffeniels, 1966). These authors have found both α- and β-decarboxylation of aspartic acid in muscles of lobster and crayfish.

The ability of synthesizing α-alanine from aspartic acid via the second pathway is present in *A. leptodactylus*. The labelling of glutamic acid from 1-^{14}C-aspartate (Table 2) implies a randomization of the label in fumarate or succinate between the C-atoms 1 and 4, via the reverse reactions of the tricarboxylic acid cycle. Thus oxaloacetate and malate labelled in the C-atoms 1 and 4 will be available. Moreover, in *A. leptodactylus* we have shown activity of the enzymes which catalyse the decarboxylation of oxaloacetate and malate, as will be discussed in a later publication. This pathway can also account for the formation of ^{14}C-labelled α-alanine from 4-^{14}C-aspartate.

From 1- or 4-^{14}C-aspartate no radiocarbon was incorporated into β-alanine (Table 2). α-Decarboxylation of 4-^{14}C-aspartate would have resulted directly in the formation of 1-^{14}C-β-alanine. From the fact that no labelling was found in β-alanine it can be concluded that during the incubation period no β-alanine has been synthesized by α-decarboxylation of aspartate. This suggests that the enzyme aspartate-α-decarboxylase is not present in *A. leptodactylus*. Thus our results disagree with those of Gilles & Schoffeniels (1966), who reported the formation of both α- and β-alanine by decarboxylation of aspartate in muscles of decapods. From their experiments it could not be concluded, however, whether one enzyme or two enzymes are involved.

In mammals β-alanine is formed in the degradation of pyrimidines (Fink *et al.*, 1956; Fritzson & Pihl, 1957). Also in the housefly *Musca domestica* the pyrimidine base uracil was by far the best precursor to β-alanine from various probable precursors tested (Ross & Monroe, 1972). The high labelling of β-alanine after a prolonged incubation of *A. leptodactylus* with U-^{14}C-glucose and the very low

Table 2. Concentration and radioactivity of free amino acids labelled 6 hr after injection of 1-^{14}C aspartate and 4-^{14}C-aspartate into *A. leptodactylus*

	1-^{14}C-Aspartate				4-^{14}C-Aspartate			
	Concentration	Radioactivity			Concentration	Radioactivity		
	μmoles	dis/min × 10^3	% of the total	dis/min per μmole × 10^3	μmoles	dis/min × 10^3	% of the total	dis/min per μmole × 10^3
Aspartic acid	0·34	4·1	17·0	12·1	0·56	5·7	31·0	10·2
Glutamic acid	1·04	5·9	24·5	5·7	2·78	6·4	35·2	2·3
α-Alanine	3·85	14·2	58·5	3·7	6·83	6·2	33·8	0·9
β-Alanine	0·37	—	—	—	0·35	—	—	—

The results refer to free amino acids isolated from 100 mg dry homogenate.

incorporation of radiocarbon from 1-^{14}C-acetate we found in earlier experiments (van Marrewijk & Zandee, 1974) suggests that also in the crayfish β-alanine is synthesized from the pyrimidines. For synthesis of these bases ribose 5-phosphate is needed, which can easily be formed from glucose via the pentose phosphate pathway. During this synthesis aspartic acid is incorporated into the pyrimidine ring, while by degradation of the ring β-alanine is formed of which the C-atoms correspond with C-atoms 2, 3 and 4 of the aspartic acid incorporated. The reason we did not find labelling in β-alanine from ^{14}C-aspartate then could be a too short duration of the incubation period (6 hr) for synthesis of β-alanine through the metabolism of pyrimidines.

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Key Word Index—Amino acid metabolism; β-alanine; biosynthesis of α- and β-alanine; decarboxylation reactions; crayfish; *Astacus leptodactylus*; crustaceans.