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FREE AMINO ACID CONTENT IN THE SKIN MUCUS OF YELLOW AND SILVER EEL, *ANGUILLA ANGUILLA* L.

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Abstract—1. Free amino acid contents in skin mucus extracts were analysed in sexually undifferentiated yellow eels, female yellow eels and male and female silver eels trapped at the same time from the same freshwater location.

2. Total free amino acid contents in skin mucus were similar in the three groups of sexually differentiated eels (16.2–18.2 μM/g dry weight). In sexually undifferentiated yellow eels, total content was higher (39.6 μM/g dry weight).

3. Free amino acid composition of skin mucus from male and female silver eels were similar. Compared with the two groups of yellow eels, main differences were found in taurine and non-essential amino acid relative contents.

4. Taurine, glycine and alanine concentrations in skin mucus decreased as the level of sexual maturity of the eel increased.

5. Possible influences of nutritional and hormonal factors on the free amino acid content of eel skin mucus are discussed. Potential consequences in osmoregulatory processes and intraspecific chemocommunication of the eel are also considered.

INTRODUCTION

The fish skin mucosa can respond to a variety of environmental fluctuations by altering the quantity as well as the nature of the secretion.

Changes in the amount of mucus produced by a fish have been related to physical (Pickering and Macey, 1977) and chemical aggressions (Zuchelkowski *et al.*, 1981; Burton *et al.*, 1984), to variations in the external tonicity (Olivereau and Lemoine, 1971) and to disease resistance (Pottinger *et al.*, 1984).

In salmonids, changes in the histological characteristics of the skin mucosa have been also related to differences in age and sexual maturity of the fish (Stoklosowa, 1970; Blackstock and Pickering, 1982). In the European eel, *Anguilla anguilla* L., previous histological observations pointed out a sexual dimorphism in the epidermal thickness and in the mucus cell concentration (Saglio *et al.*, in press).

Moreover, skin mucus from immature yellow eel and from male and female silver eels could differ in the chemical composition. In yellow eel, high free amino acid concentrations were found in skin mucus extracts (Fauconneau and Saglio, 1984). Owing to the high chemical sensitivity of the eel to amino acids (Yoshii *et al.*, 1979; Silver, 1980) and to the pheromonal attractivity of the secretion (Saglio, 1982), free amino acids from skin mucus could be involved in intraspecific chemocommunication of eel.

Furthermore, it has been shown that free amino acids can be of primary importance as osmoeffectors in tissue of euryhaline teleosts (Lasserre and Gilles, 1971; Venkatachari, 1974; Vislie and Fugelli, 1975; Fugelli and Zachariassen, 1976; Hegab and Hanke, 1983) but data on the skin are lacking.

Consequently, the aim of this study was to compare the free amino acid content in skin mucus from European eels, at different stages of sexual maturity.

MATERIALS AND METHODS

Collection of skin mucus

Yellow and silver eels were trapped in the lake of Léon (Landes) at the beginning of January and kept for 1 week at low densities in outdoor circular fibreglass tanks, each supplied with a constant flow of water from the Nivelle river (Pyrénées-Atlantiques). No prophylactic treatment was applied and fish were not fed during this period.

Water temperature and pH from the fishing site were close to river values and respectively ranged from 9 to 11°C and 6.8 to 7.1°C. Yellow and silver eels selected on macroscopic characteristics were immersed for 30 sec in liquid nitrogen and left for superficial thawing for 1 hr at 4°C.

Mucus samples were collected by slight scraping from the surface of the dorso-lateral parts of the body skin, from the pectoral fin to the caudal extremity.

For sex determination, a piece of gonad was taken from each fish for histological preparation and microscopic examination, as previously described (Saglio *et al.*, in press). These examinations led us to select mucus samples from four sexual groups of eels:

2 silver eels, females (63 and 69 cm long)
2 silver eels, males (34 and 38 cm long)
2 yellow eels, with small oocytes (35 and 38 cm long)
2 yellow eels, undifferentiated (27.5 and 33 cm long)

Mucus samples from each fish was frozen and freeze-dried.

Sample preparation

Free amino acids in the skin mucus were extracted by homogenization in trichloracetic acid (TCA) solution (10% w/v) and centrifugation (5000 *g*, 15 min). This operation was repeated four times and the supernatants were combined.

Table 1. Free amino acid content in skin mucus of yellow and silver eels (μmol/g of freeze-dried mucus)

Sexual stage	Silver eel				Yellow eel			
	Female		Male		Female		Undifferentiated	
Amount of mucus collected per fish (g of freeze-dried mucus)	2 g		0.6 g		0.43 g		0.35 g	
	μmol/g	% total content	μmol/g	% total content	μmol/g	% total content	μmol/g	% total content
Aspartic acid	4.19	25.85	4.60	25.25	1.72	10.21	2.93	7.39
Glutamic acid	4.77	29.43	5.45	29.92	3.51	20.84	4.82	12.17
Serine	0.76	4.68	1.15	6.31	1.18	7.00	2.15	5.42
Histidine	0.14	0.86	0.20	1.09	0.37	2.19	1.74	4.39
Glycine	0.37	2.28	0.24	1.31	1.00	5.94	6.35	16.03
Threonine	0.13	0.80	0.52	2.85	0.32	1.90	0.65	1.64
Arginine	0.24	1.48	0.14	0.76	0.41	2.43	0.25	0.63
Alanine	0.66	4.07	0.65	3.56	0.99	5.88	2.11	5.32
Tyrosine	0.10	0.61	0.07	0.38	0.05	0.29	ND	
Methionine	0.59	3.64	0.80	4.39	0.28	1.66	0.62	1.56
Valine	0.36	2.22	0.53	2.90	0.23	1.36	0.34	0.85
Phenylalanine	0.12	0.74	0.07	0.38	0.17	1.00	0.24	0.60
Isoleucine	0.37	2.28	0.30	1.64	0.18	1.06	0.31	0.78
Leucine	0.77	4.75	0.57	3.12	0.74	4.39	1.57	3.96
Lysine	0.45	2.77	0.63	3.45	0.57	3.38	0.84	2.12
Taurine	2.20	13.57	2.30	12.62	5.12	30.41	14.70	37.11
Total	16.22		18.22		16.84		39.62	
Essential amino acids	3.17	19.50	3.76	20.60	3.27	19.40	6.56	16.50
Non essential amino acids (taurine included)	13.05	80.50	14.46	79.40	13.57	80.60	33.06	83.50

The TCA was removed from the supernatant by three successive extractions (mixing and decantation) with diethyl ether (93°C).

Remaining diethyl ether was removed from the extract by evaporation under vacuum and recovery in double-distilled water.

Amino acid analysis

The amino acid content of the samples were measured after ortho-phtaldialdehyde (OPA) derivatization with mercaptoethanol as a reducing agent to produce iso-indol derivates.

The amino acid derivates were separated by HPLC (Vista 5000, Varian France) on C 18 micropack column (Varian S.A.). Using a water/methanol eluting gradient (Hogan *et al.*, 1982). The derivates were detected by fluorimetry. The gamma-amino butyric acid was used as an internal standard. Secondary amines proline and hydroxy proline, were not analysed.

RESULTS

In each fish skin mucus extract, 16 OPA positive compounds were detected at significant levels (>0.05 μmol/g dry weight extract). Eels at the same sexual stage showed negligible variations in the free amino acid concentrations of mucus and mean values per sexual group are indicated in Table 1.

Total free amino acid content in skin mucus appeared similar in the three groups of sexually differentiated eels (16.2–18.2 μmol/g dry weight).

In sexually undifferentiated yellow eels, total content was higher (39.6 μmol/g dry weight) than in other groups.

Taurine, glycine and, to a lesser extent, alanine, histidine and serine mainly contributed to the higher total free amino acid content found in skin mucus of undifferentiated yellow eels.

In this group, taurine and glycine were present in high amounts and accounted for 53.1% of total content.

In the female yellow eels, taurine and glycine showed lower levels with 36.3% of total content, while in silver eels, those two compounds only accounted for 15.8% (female) and 13.9% (male).

Free amino acid composition in skin mucus from female and male silver eels were similar.

In silver eels, relative contribution of aspartic acid and glutamic acid was higher (approx. 55% of total content) than in female yellow eels (31%) or in sexually undifferentiated yellow eels (19.5%).

The ratio of essential to non-essential amino acids was lower in skin mucus from undifferentiated yellow eels (0.19) than in other groups (0.24–0.26).

DISCUSSION

Variations in free amino acid content of skin mucus

Differences found between groups of eels suggest that free amino acid contents of skin mucus are related to the level of sexual maturity rather than to the sex of the fish. Variations were shown between the three stages of sexual maturity but free amino acid contents of skin mucus in male and female silver eels were roughly similar. On the other hand, female yellow eels and female silver eels showed noticeable differences in the free amino acid content.

In the rainbow trout, *Salmo gairdneri*, and in the lake whitefish, *Coregonus clupeaformis*, Hara *et al.* (1984) also found similarities in the free amino acid composition of skin mucus of male and female, but in both species concentrations were significantly greater in the male than in the female.

Such intersexual differences did not appear in the present work, but we found that taurine, glycine and alanine concentrations decreased as the level of sexual maturity of the eel increased. Free amino acid concentrations in skin mucus could be influenced by sexual endocrine control. In the rainbow trout,

steroid sex hormones affected transport of amino acids (Habibi *et al.*, 1983).

Besides, it had been shown that taurine, glycine and alanine are particularly involved in intracellular adjustment of euryhaline teleosts to changes in the external salinity (Huggins and Colley, 1971; Lasserre and Gilles, 1971; Colley *et al.*, 1974). However, all groups of eels were trapped at the same time from the same freshwater location and kept in fresh water before analysis. Therefore, variations observed here in taurine, glycine and alanine concentrations cannot be merely explained in terms of osmotic adjustment.

In Atlantic salmon, *Salmo salar* L., variations in the free amino acid content of tissue were found without changes in the external tonicity, especially during parr/smolt transformation. Fontaine and Marchelidon (1971) pointed out a decrease in the free amino acid content of the muscle during smoltification, particularly in taurine, glycine and histidine concentrations.

Same decreases were observed here in the skin mucus from the yellow to the silver stage of the eel. Those variations could originate from a similar physiological mechanism in both species and constitute a preliminary step in adjustment to marine life.

The decrease found in free amino acid concentrations of skin mucus from the yellow to the silver stage of the eel could result from different nutritional conditions. In skin mucus of goldfish, *Carassius auratus* L., the main change produced by long-term fasting was a fall in taurine and some non indispensable amino acids while the content of essential amino acids was maintained (Saglio and Fauconneau, 1985).

Differences in free amino acid concentration of skin mucus between the yellow and the silver eel could also be due to endogenous hormonal control.

In mammals, it had been shown that a number of growth hormones and growth factors had a general stimulatory effect on amino acid transport (Nutting and Coates, 1977; Heindel and Riggs, 1978; Albertsson-Wikland *et al.*, 1980; Freeman and Handwerger, 1983).

In silver eel, histochemical studies from Olivereau and Lemoine (1971) suggested that skin mucus production was under prolactin control.

Further investigations are needed to evaluate the effect of endocrine regulation on the free amino acid content of eel skin mucus.

Possible influence in pheromonal communication

Free amino acid concentrations in skin mucus were high in all sexual groups of eel ($>10^{-5}$ mol/fish). Owing to the intraspecific attractivity of eel skin mucus extracts (Saglio, 1982) and to the extreme gustatory and olfactory sensitivity of this fish to amino acids (Yoshii *et al.*, 1979; Silver, 1980), free amino acids from skin mucus could interfere in pheromonal communication.

In rainbow trout and in lake whitefish, electrophysiological data (Hara *et al.*, 1984) indicated that free amino acids present in the mucus were fully responsible for olfactory stimulation.

Variations found in the free amino acid composition of skin mucus of eels at different stages of growth and sexual maturity could play a role in precise identification of conspecifics.

This hypothesis requires further olfactometric experiments.

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