The Oocytes of the Goby *Pomatoschistus minutus* III. Determination of Amino Acid Component

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The oocytes of the marine goby *Pomatoschistus minutus* were analyzed for their amino acid content. Most of the amino acids exist as protein, only a little part is free or peptide-bound. Among the protein-bound amino acids, high levels of glutamic acid, proline, alanine, aspartic acid, valine and leucine were detected. These represent more than 60% of the protein amino acids. Among the free acids, glutamic acid, serine and alanine, are dominant. There are no certain proofs of the occurrence of peptide pools in the oocytes of *Pomatoschistus minutus*.

In two previous papers not only egg-membrane and follicle of the marine teleost *Pomatoschistus minutus* were examined by light and electron microscopy [1], but also the morphology of micropyle by light microscopy [2]. As remarkable feature oocytes and eggs of *Pomatoschistus minutus* show up to 220 attaching-filaments, which are connected with the egg-membrane only at the animale pole. After spawning the eggs are fastened to substratum by these attaching-filaments.

In stage III and IV (stages according to Arndt [3]) the oocytes are filled with yolk platelets of different size and composition. Using ultracytochemical methods there was a positive proof of proteins, polysaccharides and lipids in the yolk platelets (Riehl, unpublished data). The quantitative determination of free amino acids and the amino acids of peptides and proteins was demonstrated in oocytes of liminic teleosts [4–6]. Till now such a determination is missing in marine teleosts. Therefore the present report is giving first information on the amino acid component of the yolk proteins in the marine *Pomatoschistus minutus*.

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The material used were females of the marine goby *Pomatoschistus minutus*, which were kept in List/Sylt. After the adult females were anesthetized (MS 222, Sandoz), the ovaries were removed and then cut in little pieces. All oocytes with a diameter more than 0.4 mm were separated (oocytes of stages III and IV).

Oocyte protein was obtained by washing the oocytes briefly in 0.01 M Tris/HCl (pH 8.4) and cutting the oocytes in half. Now egg-membranes were removed by filtration with bolting silk. One part of the oocyte proteins was hydrolyzed and isolated according to Nakano and Yamamoto [5], the other part was prepared according to Simpson *et al.* [7] and Regier *et al.* [8]. Amino acid analysis was performed with a Beckman Model 121 M amino acid analyzer.

Three different fractions were analyzed: the methanole-insoluble fraction contains the free amino acids, the TCA-soluble-methanole-insoluble fraction the peptides and the TCA-insoluble fraction comprises the proteins. Table I demonstrates the amounts of amino acids found in the three fractions. The results are in good agreement with that of Nakano and Yamamoto [5] stated in the limnic teleost *Oryzias latipes*. Most of the amino acids exist as protein, which may be mainly contained in the yolk platelets. Glutamic acid is the dominant amino acid not only in proteins but also in peptides and free amino acids. The protein glutamic acid, proline, alanine, aspartic acid, valine and leucine represent more than 60% of the amino acids. This is

Table I. Amino acids in the oocytes of *Pomatoschistus minutus*. According to Nakano and Yamamoto the values are expressed in µmol/10^3 oocytes (stages III and IV).

Amino acid	Free	Peptide	Protein
Alanine	0.92	1.86	55.2
Arginine	0.05	1.20	17.8
Aspartic acid	0.51	0.94	48.1
Glutamic acid	1.16	2.08	62.7
Glycine	0.15	0.20	20.0
Histidine	0.38	0.72	31.1
Isoleucine	0.12	0.23	21.4
Leucine	0.44	0.51	41.8
Lysine	0.50	0.45	37.1
Methionine	0.01	0.04	4.3
Phenylalanine	0.03	0.14	12.9
Proline	0.21	0.90	60.1
Serine	0.76	2.11	23.9
Threonine	0.08	0.38	26.1
Tryptophan	0.12	0.55	4.2
Tyrosine	0.10	0.77	2.9
Valine	0.24	0.92	47.3

corresponding on the whole with the results of Nakano and Yamamoto [5] and Suyama and Suzuki [6].

The amino acid analysis of *Pomatoschistus* oocytes shows amongst differences also many conformities with the results found in limnic teleosts [5, 6]. On the other hand there are considerable differences of amino acid content in the oocytes of two specimens of rainbow trout [6]. The occurrence of peptide pools in *Pomatoschistus* oocytes is not clear.

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