PE-33

Black Porgy Prolactin cDNA Sequence, Its mRNA Expression and Blood Physiological Responses During Freshwater Acclimation

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Introduction

Prolactin (PRL) is a peptide hormone secreted from a pituitary gland, and is known as ion regulator by facilitating retention/uptake of Na^+ in freshwater (McCormick, 2001). Thus PRL plays an important role in osmoregulation in freshwater. The salt change in aquaculture causes a variety of physiological stress responses including plasma hormones, energy metabolism and electrolyte equilibrium due to an environmental stressor. The aim of the study was to explain the role of PRL in black porgy during freshwater acclimation. To this end, we first analysed the base sequence by cloning the full length of PRL cDNA of black porgy, and based on this result we measure the level of pituitary mRNA. We also studied on the stress response and osmoregulatory ability of black porgy with regard to freshwater acclimation by measuring the level of plasma cortisol, glucose, AST, ALT, ion and osmolality.

Materials and methods

Freshwater acclimation and sampling procedure: Freshwater acclimation of black porgy was performed as follows. Briefly, put the underground water in the tank and keep them at 10% seawater for 24 hours and add underground water again to convert it freshwater. Blood and tissues were sampled from 6 black porgy each time at the time of seawater, 10‰ seawater acclimation for 24 hours, freshwater acclimation for 24, 48 and 72 hours. Plasma sample separated by centrifugation and collected pituitary were stored at -80℃ until analysis.

Rapid amplification of cDNA 3′ and 5′ ends (RACE): The primers were designed for RACE as follows: 5′RACE gene specific primer (5′-GSP: 5′-TTG TCA TTG GGT GTC TGT AGA GAG GAG GTA T-3′), 3′RACE gene specific primer

(3'-GSP: 5'-TCA CTG CCC TAC AGA GGC TCC AAT GAC A-3'). PCR was carried out for 35 cycles as follows: denaturation at 94°C for 45 s, annealing at 58°C for 45 s and an extension at 72°C for 90 s, followed by 1 cycle of 5 min at 72°C for extension. The PCR product was amplified, cloned into a pGEM-T Easy Vector, and sequenced.

Plasma parameters analysis: Plasma cortisol was analyzed by RIA using RIA kit. Plasma glucose, AST, ALT, Na^+, K^+ and Cl^- were examined with Biochemistry Autoanalyzer. Plasma osmolality was examined with Vapor Pressure Osmometer.

Results and Summary

The PRL cDNA of black porgy consists of 1492 bases, in which 212 amino acids are encoded including 14 signal peptides. In our experiment, expression of a pituitary gland PRL mRNA of black porgy showed significant increase during freshwater acclimation. Also, we studied stress responses and osmoregulatory ability of black porgy in a time of salinity change from seawater to freshwater by endoctrinological and blood physiological methods. Plasma cortisol increased six times from 5 ng/ml in seawater to 32 ng/ml at 24 hours in freshwater, however after this, it decreased. Glucose, AST and ALT showed similar changes as cortisol such that they increased from 10‰ seawater, and showed maximum values at 24 hours in freshwater, and returned back to the seawater at 48 hours in freshwater. In contrast with them, plasma ions (Na^+, Cl^-, Ca^{2+}) and osmolality decreased up to 24 hours in freshwater, then increased gradually. These results suggests that PRL (or cortisol) play hormonal regulation in osmoregulatory organs, thereby improving the hyperosmoregulatory ability of black porgy in freshwater.

References

McCormick, S. D. 2001. Endocrine control of osmoregulation in fish. Am. Zool. 282, 290-300.