Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

Effect of cadmium on the extracellular Na^+, K^+, and Ca^{2+} in the gill and small intestine of Goldfish *Carassius auratus*

Dongwu Liu[a,b], *Hong Guo*[b], *Zhiwei Chen*[a,*], *Ying Wang*[b]

[a] *School of Life Sciences, Shandong University of Technology, Zibo, Shandong Province 255049, China*
[b] *Analysis and Testing Center, Shandong University of Technology, Zibo, Shandong Province 255049, China*

ARTICLE INFO

Article history:
Received 5 November 2013
Received in revised form
26 January 2014
Accepted 1 February 2014
Available online 8 February 2014

Keywords:
Cadmium
Cation
Ion chromatograph
Gill
Small intestine

ABSTRACT

In this study, the toxic effect of cadmium on extracellular Na^+, K^+, and Ca^{2+} in the gill and small intestine of goldfish *Carassius auratus* was determined with the technique of ion chromatograph. Two-way ANOVA indicated that the two factors (Cd^{2+} treatment and time) and the interaction factor had significant effect on the level of Na^+, K^+, and Ca^{2+} in the small intestine and gill. 1.0 mg/L Cd^{2+} significantly increased Ca^{2+} level in the small intestine, but Ca^{2+} level in the gill was significantly decreased by 1.0 and 5.0 mg/L Cd^{2+} at 24, 48, and 72 h. Na^+ and K^+ level in the small intestine and gill was increased by 1.0 mg/L Cd^{2+} at three time points, but increased by 5.0 mg/L Cd^{2+} at a certain different time. In addition, Na^+ level was significantly decreased by 5.0 mg/L Cd^{2+} at 24 or 48 h in the small intestine and gill. The results indicated that Cd^{2+} played an important role in regulating the level of Na^+, K^+, and Ca^{2+} in the small intestine and gill of goldfish *C. auratus*. A method was constructed to investigate the extracellular Na^+, K^+ and Ca^{2+} in the tissues of gold fish with ion chromatography.

1. Introduction

It is known that Na^+ and K^+ are essential to organisms and play a significant role in regulating the physiological functions. The balance of Na^+ and K^+ is important for maintaining the pressure of cell infiltration and nerve excitability. Ca^{2+} mainly derives from the extracellular Ca^{2+} or intracellular Ca^{2+} in the stores of endoplasmic reticulum and mitochondria, and further participates in the neurological disorders and signaling transduction pathways (Ohashi et al., 2009; Yamakage and Namiki, 2002). As a second messenger, Ca^{2+} regulates the metabolism, motility, necrosis, apoptosis, and transport in the cells (Cummings et al., 2004; Pozzan et al., 1994).

The ions in the tissues were usually detected with the method of analytical chemistry, which needs the complex procedures. Ion chromatography has high precision and the parameters can be determined in one run (Tartari et al., 1995; Marchetto et al., 1995). The conductivity detectors combined with chemical suppression are suitable for the determination of inorganic ions (Buldini et al., 1997). Furthermore, ion chromatography can simultaneously determinate the ions of interest in a short time, and yield the precise and reproducible data. Thus ion chromatography has been widely used to analyze the inorganic ions (Ohta and Tanaka, 1999).

* *Corresponding author at*: School of Life Sciences, Shandong University of Technology, 255049 Zibo, China. Tel.: +86 05332781329; fax: +86 05332781329.
E-mail address: 12chen@163.com (Z. Chen).
1382-6689/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.etap.2014.02.002

The aquatic environment is being polluted by the increasing number of industrial and agricultural chemicals. Cadmium is not an essential element for biological functions, but it is a serious environmental threat for its mutagenic, clastogenic, teratogenic, and carcinogenic toxicity (Mandel and Ryser, 1984; Degrave, 1981). Moreover, cadmium is recognized as one of the most deleterious heavy metal pollutants, which may interact metabolically with nutritionally essential metals (Nath et al., 1984). Fish are recognized as a sensitive monitor of water pollution and respond with great sensitivity to changes in the aquatic environment (Aas et al., 2001; Mondon et al., 2001). The mechanisms of cadmium on Na^+, K^+, and Ca^{2+} in fish tissues remain unclear until now. The aim of this study was to determine the effect of cadmium on the extracellular Na^+, K^+ and Ca^{2+} in the gill and small intestine of goldfish *Carassius auratus* with ion chromatography.

2. Materials and methods

2.1. Experimental animals

The goldfish *C. auratus* weighing 9.0–11.0 g (6 months) were purchased from a market in Zibo, Shandong, China, and acclimated in laboratory for two weeks prior to each experiment. All fish were maintained at $20 \pm 1.0\,^{\circ}C$ in dechlorinated and oxygen saturated water (pH 7.0 ± 0.1). The fish were fed once daily in the morning with commercial dry pellets (Sanyuan fish food, Beijing Sanyou Beautification Feed Tech. Co., Ltd.). The fish were randomly assigned into three groups and each group had 10 fish. The different concentration of Cd^{2+} standard solution (1.0 g/L) (purchased from the standard solutions center in Shanghai, China) was added into the aquarium water, and the fish were treated with the dose of 0, 1.0 mg/L and 5.0 mg/L Cd^{2+} for 24, 48, and 72 h, respectively.

2.2. Preparation of tissue supernatant

For ion chromatographic analysis, the gill and small intestine were cut from goldfish. The tissues were weighed and washed with the sodium phosphate buffer (PBS, pH 7.4). Then a certain amount of purified water was added and oscillated for 30 min with an ultrasonic oscillator. To acquire the supernatant, the tissues were centrifuged at 10,000 r/min for 15 min. The supernatant was diluted twenty-five times with purified water and filtered through 0.22 μm Nylon filters and Dionex OnGuard C18 before ion analysis.

2.3. Cation analysis

All reagents, eluents, and standard solutions were prepared using water purified with a Milli-Q system (Millipore). Na^+, K^+, and Ca^{2+} standard solutions (1.0 g/L, purchased from the standard solutions center in Shanghai, China) were used for cation determinations, and methanesulphonic acid (MSA, Fluka) was used for preparation of cation determinations.

A Dionex ICS-2000 ion chromatograph with a Dionex gradient pump, eluent degassing module, and conductivity detector was used. Cations were separated with a CS12 A ion-exchange column (4.0 mm I.D.) and CG-12 A guard column, and detected after suppression with CSRS 300 (4.0 mm I.D.) cation electrical self-regenerating suppressor. Isocratic elution was used for Na^+, K^+, and Ca^{2+} determination with 20 mM MSA as eluent (1.0 mL/min). The injection volume was 25 μL, the run time was set to 15 min.

Table 1 – Optimum condition for ion chromatography.

Optimum condition for ion chromatography	
Concentration of MSA	20 mmol/L
Flow rate of eluent	1.0 mL/min
The electric current of suppressor	59 mA
Chromatographic column	CS12 A ion-exchange column
Guard column	CG-12 A guard column

2.4. Statistics

Two-way ANOVA followed by the appropriate post hoc test was carried out to calculate the interaction of the two factors, "Cd^{2+} treatment" and "time", on the levels of Na^+, K^+, and Ca^{2+} in the tissues of goldfish. One-way ANOVA followed with comparison test revealed the significant differences of the factor "Cd^{2+} treatment" on the levels of Na^+, K^+, and Ca^{2+}. All statistical tests were performed at the significance level of $p < 0.05$.

3. Results

3.1. Separation condition

Na^+, K^+, and Ca^{2+} were successfully separated with the optimum chromatographic conditions summarized in Table 1. The standard solutions with increasing concentrations were used for calibration for Na^+, K^+, and Ca^{2+} determinations. The calibration was linear for Na^+ ($y = 0.281x + 0.520$; $r^2 = 0.999993$), K^+ ($y = 0.196x + 0.041$; $r^2 = 0.99999$), and Ca^{2+} ($y = 0.371x + 0.307$; $r^2 = 0.999790$) (x is the amount of cations, and y is the area of the chromatogram). The typical chromatogram obtained for Na^+, K^+, and Ca^{2+} standard solutions was shown in Fig. 1.

3.2. Effect of Cd^{2+} on Ca^{2+} content in the small intestine

Fig. 2 shows the chromatogram of Na^+, K^+, and Ca^{2+} in the small intestine of the goldfish. Two-way ANOVA indicated that the two factors (Cd^{2+} treatment and time) and interaction factor had significant effect on Ca^{2+} level in the small intestine (Table 2). Ca^{2+} level was significantly increased by 1.0 mg/L Cd^{2+} at 24, 48, and 72 h. However, Ca^{2+} level was significantly increased by 5.0 mg/L Cd^{2+} only at 24 h (Table 2).

3.3. Effect of Cd^{2+} on K^+ content in the small intestine

Two-way ANOVA revealed that the two factors (Cd^{2+} treatment and time) and interaction factor had significant effect on K^+