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Nakagawa, Hisaki
Laboratory of Fisheries Chemistry, Faculty of Agriculture, Kyushu University

Tajima, Toshihiro
Laboratory of Fisheries Chemistry, Faculty of Agriculture, Kyushu University

Sato, Tsutomu
Laboratory of Fisheries Chemistry, Faculty of Agriculture, Kyushu University

Watanabe, Midori
The Center of Advanced Instrumental Analysis, Kyushu University

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Evaluation of Erythrocyte 5-Aminolevulinic Acid Dehydratase Activity in the Blood of Crucian Carp, *Carassius auratus langsdorfii*, as an Indicator in Fish of Water Lead Pollution

Hisaki Nakagawa, Toshihiro Tajima, Tsutomu Sato and Midori Watanabe*

Laboratory of Fisheries Chemistry, Faculty of Agriculture,
Kyushu University, Fukuoka 812-81, Japan
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The activity of 5-aminolevulinic acid dehydratase (ALA-D) in the blood of crucian carp, Carassius *auratus langsdorfii*, was measured under a variety of lead exposure conditions. A three-week exposure of crucian carp to water lead concentrations of 3, 10, and 30 ppb inhibited their ALA-D activities with increasing water lead concentrations. When crucian carp were exposed to a water lead concentration of 30 ppb, depressed activity of ALA-D became visible after two days, and this activity became even more depressed with increasing exposure periods. The ALA-D activity of crucian carp after the fish were transferred from a water lead concentration of 100 ppb (two weeks of exposure) to lead-free water recovered to only 40% of the value seen in the fish at the beginning of the exposure test, even after four weeks. Therefore, these characteristics concerning ALA-D in crucian carp blood indicate that this enzyme is useful as an indicator of lead pollution in fresh water.

INTRODUCTION

5-Aminolevulinic acid dehydratase (ALA-D, EC 4.2.1.24) in fish blood is useful as an indicator of water lead pollution since ALA-D activity is inhibited by lead pollution (Hodson, 1976; Hodson et al., 1977; Johansson-Sjobeck and Larsson, 1979; Larsson *et al.*, 1985; Schmitt *et al.*, 1984; Haux *et al.*, 1986; Dwyer et al., 1988; Nakagawa *et al.*, 1995abc).

Crucian carp, Carassius *auratus langsdorfii*, lives extensively in areas of fresh water in northern Kyushu, and the fish are stationary. The fish can be collected easily by a fishing rod. These characteristics indicate that the crucian carp is a suitable fish species for detecting lead pollution in those waters using its blood ALA-D. The present research was undertaken in order to evaluate the suitability of ALA-D in crucian carp blood as an indicator of water lead pollution.

MATERIALS AND METHODS

Test fish

The crucian carp used as test fish were collected by a net cast in the vicinity of Yoshihara bridge in the Umi river. The fish collected were allowed to acclimatize to the

* The Center of Advanced Instrumental Analysis, Kyushu University, Fukuoka 812, Japan.

experimental conditions for at least two months according to the methods described in our previous paper (Nakagawa et al., 1995a). The fish used were of 9.5 — 11.8 cm in body length and 30 — 52 g in weight.

Preparation of dilution water and test waters

Since it is commonly stated that the average level of water hardness of river waters in Japan is 30ppm $CaCO_3$, a dilution water of 30ppm $CaCO_3$ was prepared by adding deionized water to tap water (about 53ppm $CaCO_3$) obtained from the municipal water supply, which was drawn from the Tatara river in Fukuoka city. Lead nitrate was dissolved in 0.1 N nitric acid solution, and a stock solution of 10.0 mg/ml (as Pb) was made. The stock solution was diluted with dilution water, and different concentrations of water lead for the three kinds of exposure tests were made. The dilution water was also used as the control water.

Method for exposure tests

The three kinds of exposure tests described below were conducted according to the methods described in our previous paper (Nakagawa et al., 1995a). Seven fish per tank were used for the exposure tests. The pH of the test waters was within the range of 6.7-7.5 throughout the exposure tests. The exposure tests were conducted at a water temperature of 17 — 21 "C.

Exposure test-l

In order to examine the relationships between ALA-D activity and water lead concentration, between blood lead concentration and water lead concentration, and between ALA-D activity and blood lead concentration, crucian carp were exposed for three weeks to water lead concentrations of 3, 10, and 30 ppb, and to the control water.

Exposure test-2

In order to examine in detail the variations in ALA-D activities and lead concentrations in the blood of lead-contaminated fish according to the exposure period, crucian carp were exposed to a water lead concentration of 30 ppb and to the control water over various exposure periods.

Exposure test-3

In order to examine recoveries of ALA-D activities and lead concentrations in the blood of fish according to each exposure period, crucian carp were transferred to lead-free water and kept there for four weeks, following two weeks of exposure to a water lead concentration of 100 ppb.

Measurement of blood ALA-D activity

Measurement of ALA-D activity, blood collection from fish and measurement of hematocrit followed methods previously reported (Nakagawa *et al.*, 1995abc). The activity was expressed as nmol of porphobilinogen (PBG) which is formed from aminolevulinic acid by 1 ml of erythrocyte (RBC) during 1 h (nmol PBG/ml RBC/h), according to the formula of Hodson *et al.* (1977). Additionally, relative ALA-D activity

was expressed as a ratio of the mean value of ALA-D activity in lead-contaminated fish to that in the control fish.

Analysis of lead in blood and water

Lead analysis of fish blood and test water was conducted using ICP-MS at the Center of Advanced Instrumental Analysis, Kyushu University as described in our previous report (Nakagawa *et al.*, 1995a). We filtrated test water through a 0.45−μm millipore filter and defined the water lead found within the filtrate as the dissolved lead fraction (Nakagawa et al., 1995c).

Statistical analysis

Data were analyzed for statistical significance using the Student's t-test. Significant differences were established at the 5% level.

RESULTS AND DISCUSSION

Dissolved lead concentrations of test waters

Table 1 indicates the mean concentrations of the dissolved fraction of water lead before the beginning of the exposure tests and again after an exposure period of 48 h. Prior to the commencement of the exposure tests, the dissolved lead concentrations of test waters lay within the ranges of 2.42-2.80, 6.88-7.88, 19.12-22.71, and 68.98−77.48 ppb at the nominal lead concentrations of 3, 10, 30, and 100 ppb, respectively, with the dissolved lead concentrations being lower than the nominal concentrations. After an exposure period of 48 h, the dissolved lead concentrations lay within the ranges of 0.37 − 1.00, 1.44-1.88, 4.01-5.89, and 5.64−39.08 ppb, respectively, the dissolved lead concentrations having decreased remarkably. On the other hand, the dissolved lead concentrations of the control waters lay within the range of 0.37-0.51 ppb prior to the

Table 1. Levels of the dissolved fraction of water lead recorded in test waters during the exposure tests.

Nominal Pb concentration (ppb)	Pb concentration determined (ppb)	
	Exposure period (h)	
	0	48
Control	0.45-t 0.05*1	0.11±0.04*2
3	2.64k0.15	0.67f0.25
10	7.50 ± 0.40	1.68±0.19
30	21.27± 1.38	4.63±0.76
100	72.02 ±3.00	19.07± 14.80

*1 Results are expressed as mean ± SD from five water samples.
*2 Mean values at 48 h are significantly different from those at 0 h at 5% level.

commencement of the exposure test and lay within the range of 0.07-0.17 ppb after an exposure period of 48 h, the dissolved lead concentrations having decreased. In the present study, it could be that perhaps the dissolved lead concentrations of the test waters decreased because of the precipitation of lead carbonate caused by the reaction of water lead and carbonate ion (Nakagawa et *al.*, 1995c). Water lead concentrations are expressed as nominal concentrations in this paper.

Relationship between ALA-D activity and water lead concentration

The relationship between ALA-D activities in crucian carp blood after three weeks of exposure to various concentrations of water lead is shown in Fig. 1. The activities decreased with increasing water lead concentrations, and the ALA-D activities were negatively correlated to the log of water lead concentrations (r = -0.83). ALA-D activity in the lead-contaminated fish decreased to about 70% of that of the control fish when the crucian carp were exposed for three weeks to a water lead concentration of 10 ppb. At present, the Environmental Water Quality Standard of water lead relating to the Human Health of Japan is 10 ppb. Hodson et *al.* (1977) proposed that fish blood ALA-D could be used as an effective short-term indicator to estimate the long-term effects of lead on fish. If so, a water lead concentration of 10 ppb may cause chronically sublethal effects to crucian carp. A similar result was reported for carp *Cyprinus carpio* following a lead exposure test (Nakagawa *et al.*, 1995c).

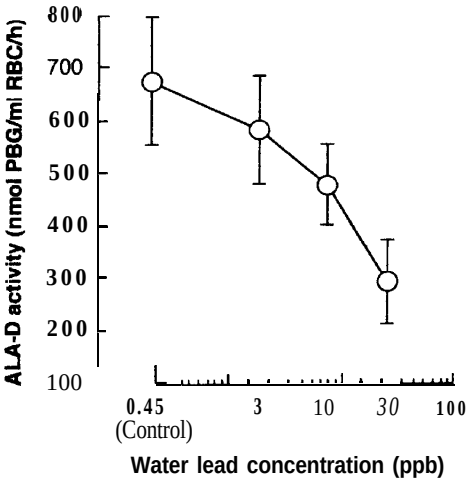

Fig. 1. Variation in erythrocyte ALA-D activities of crucian carp with the log of water lead concentrations. Data were obtained from fish exposed for three weeks to various concentrations of water lead. Values indicate the mean obtained from the measurements of five fish, while the vertical bars show the SE of the mean.

Relationship between blood lead concentration and water lead concentration

The relationship of blood lead concentrations to water lead concentrations after three weeks of exposure is shown in Fig. 2. Blood lead concentrations increased with increasing water lead concentrations, and a positive correlation between blood lead concentrations and water lead concentrations was obtained ($r = 0.94$). It is apparent that blood lead concentrations reflected the degree of lead contamination of the fish. If the fish continue to be exposed to a water lead concentration of 10 ppb (the Environmental Water Quality Standard of water lead), blood lead concentrations will surely increase even more. Moreover, it would seem that such an increasing blood lead concentration would probably cause chronically sublethal effects to crucian carp.

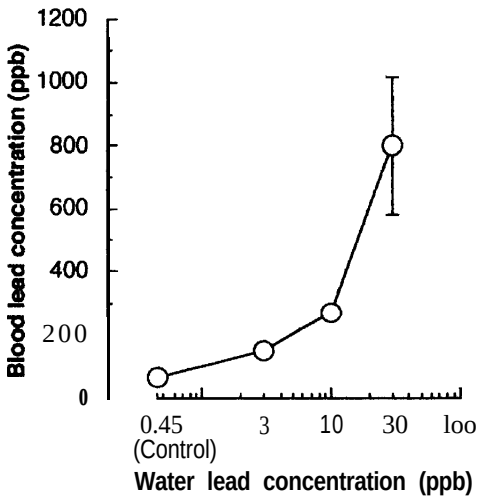

Fig. 2. Variation in blood lead concentrations of crucian carp with the log of water lead concentrations. Data were obtained from fish exposed for three weeks to various concentrations of water lead. Values indicate the mean obtained from the measurements of five fish, while the vertical bars show the SE of the mean.

Relationship between ALA-D activity and blood lead concentration

The relationship between ALA-D activity and blood lead concentration after exposure for three weeks is shown in Fig. 3. The activities decreased with increasing blood lead concentrations and the ALA-D activities were negatively correlated to the log of blood lead concentrations ($r = -0.79$). The reason for the depressed activities with increasing blood lead concentrations can be explained by the following as described in our

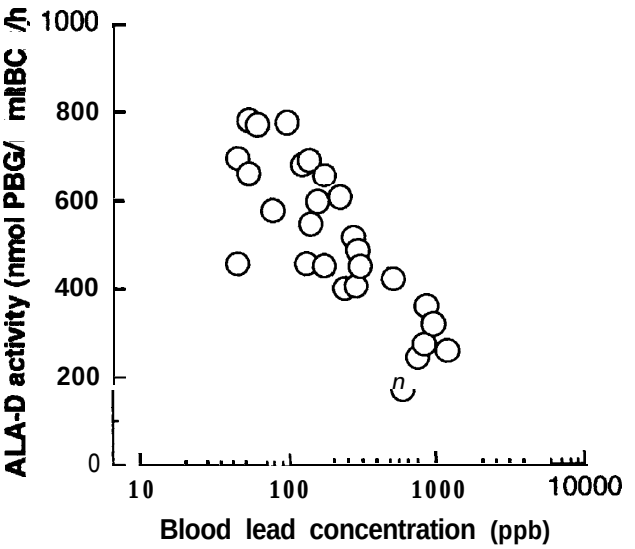

Fig. 3. Variation in erythrocyte ALA-D activities of crucian carp with the log of blood lead concentrations. Data were obtained from fish exposed for three weeks to various concentrations of water lead. Values are those obtained from the measurements of individual fish.

previous report on carp (Nakagawa et al., 1995d). Lead taken up through the gills becomes accumulated at a remarkably high level within the corpuscles. Blood ALA-D is an enzyme which is present in erythrocytes, and erythrocyte ALA-D combines with lead within the blood corpuscles. Therefore, the depressed degrees of blood ALA-D activity caused by lead depend upon the amounts of lead which become accumulated within the blood corpuscles.

Variations in ALA-D activity and blood lead concentration according to the exposure period

Figure 4 shows the variations in ALA-D activity and blood lead concentration according to the exposure period, when crucian carp were exposed to a water lead concentration of 30 ppb and to the control water. Depression of ALA-D activity was observed after only two days of exposure, and this activity became rapidly reduced as the exposure period was extended. Blood ALA-D activity after exposure for eight days decreased to about 65% of that seen in the control fish. A similar result was observed in the exposure test using carp (Nakagawa et al., 1995a). On the other hand, the blood lead concentration was a mean level of 70 ppb at the beginning of exposure, and increasing to a mean level of 950 ppb after eight days of exposure. It is apparent that blood lead concentration increases with an increase in the length of the exposure period. A similar result was observed in an exposure test using carp (Nakagawa et *al.*, 1995a). Therefore, ALA-D in fish blood is closely related to blood lead which reflects the degree of lead

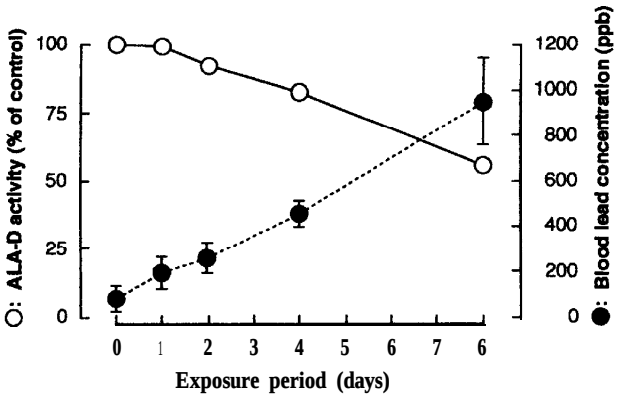

Fig. 4. Variation in erythrocyte ALA-D activities and lead concentrations in the blood of crucian carp exposed to a water lead concentration of 30 ppb according to exposure periods. Values of blood lead indicate the mean obtained from the measurements of five fish, and the vertical bars show the SE of the mean. Relative ALA-D activity was expressed as a ratio of the mean value of lead contaminated-fish to that of the control fish.

contamination of the fish.

Recoveries of depressed activity of ALA-D and residual blood lead concentration in lead-contaminated fish

Figure 5 shows the variations in ALA-D activity and blood lead concentration when crucian carp were exposed to a water lead concentration of 100 ppb for two weeks, and then transferred to lead-free water. After two weeks of lead exposure, ALA-D activity in the lead-contaminated fish decreased to about 30% of that seen value in the fish at the beginning of the exposure test. After the fish were transferred to lead-free water, the depressed activity of ALA-D recovered slowly. However, four weeks after the transfer to lead-free water, the activity had increased to only 40% of the value in the fish at the beginning of the exposure test. Thus, it is apparent that the once depressed activity of ALA-D does not recover easily to a normal level of activity, even if the lead-contaminated fish have been transferred to lead-free water. Similar results were observed in exposure tests using rainbow trout Oncorhynchus *mykiss* (Hodson et *al., 1977;* Johansson-Sjobeck and Larsson, 1979), brook trout Salvelinus fontinalis (Hodson *et al.,* 1977), and carp (Nakagawa et *al.,* 1995a). On the other hand, after two weeks of lead exposure, the residual blood lead concentration reached a high level of 2,000 ppb. After their transfer to lead-free water, blood lead levels in the lead-contaminated fish did not drop rapidly and only a very slight decreasing trend was observed. As mentioned above, the activities of erythrocyte ALA-D are closely related to the lead levels of the erythrocyte. Therefore, it

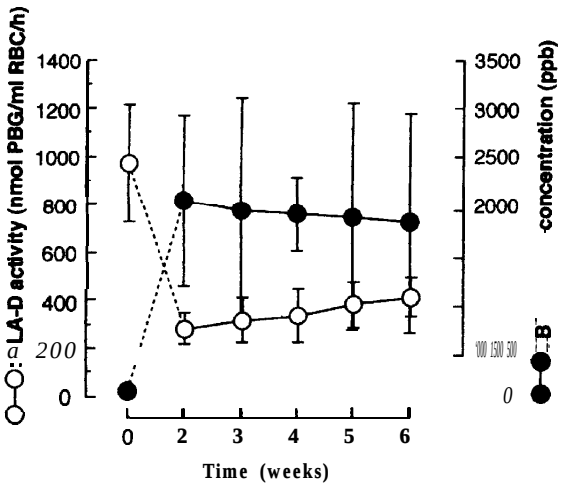

Fig. 5. Recoveries of erythrocyte ALA-D activities and blood lead concentrations of crucian carp from the effects of lead exposure, following transfer to lead-free water. The fish were exposed to a water lead concentration of 100 ppb for two weeks, and were then transferred to lead-free water. Values indicate the mean obtained from the measurements of five fish, while the vertical bars show the SE of the mean.

is only natural that the depressed ALA-D activities recover slowly, since the blood lead levels in the lead-contaminated fish fail to recover to the levels of blood lead seen in the fish at the beginning of the exposure test, even four weeks after their transfer to lead-free water. Throughout this exposure test, the blood lead concentrations varied largely with each individual fish. It is thought that this simply reflects differences among individuals of wild crucian carp, because the test fish used for these experiments were allowed to acclimatize gradually to the experimental conditions after their collection from the Umi river.

Throughout the three kinds of exposure tests, no mortality occurred, and no symptom of lead poisoning such as loss of appetite or abnormal swimming was observed.

The results described above indicate that ALA-D in crucian carp blood is useful as an indicator of lead pollution in fresh water because of the following characteristics: 1) The ALA-D activity reflects the degree of lead contamination that crucian carp have suffered in the past. 2) The inhibition of ALA-D activity is induced by short-term lead exposure. 3) The depressed ALA-D activity does not recover even if fish are transferred to lead-free water for at least four weeks.

At present, we are planning to assess the actual conditions of water lead pollution in areas of fresh waters in northern Kyushu using crucian carp blood ALA-D.

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