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Reduced carbonic anhydrase and Na-K-ATPase activity in gills of salmonids exposed to aluminium-containing acid water[1]

M. Staurnes, T. Sigholt and O.B. Reite

Department of Zoology, University of Trondheim, N-7055 Dragvoll (Norway), 14 February 1983

Summary. Exposure of young specimens of *S. salar* and *S. gairdneri* to aluminium concentrations of 200 µg/l in water at pH 5 induced reductions of 25–40% in the activity of carbonic anhydrase and Na-K-ATPase in the gills.

Acidification of lakes and streams caused by acid precipitation is a growing environmental problem. Fish death, especially of salmonids, has been reported from many countries[2,3]. Laboratory experiments have shown that addition of acid to the water will produce physiological disturbances and eventually death in freshwater fishes. The most important changes seem to be a reduction of the plasma concentration of sodium and chloride[2]. Corresponding changes have also been found in fish from acidified streams and lakes. However, in the natural waters fish death occurs at a higher pH than would be expected from results obtained in the laboratory experiments. This difference is assumed to be caused by the increased concentration of aluminium in natural waters due to mobilization from the ground, induced by the precipitated acid[4]. Certain aluminium compounds have been shown to be especially toxic to fish at pH levels of about 5, with a marked reduction of sodium and chloride concentrations in blood plasma[5]. In a search for possible mechanisms causing the death of fish under such conditions we have investigated the activities of carbonic anhydrase and Na-K-ATPase in the gills of the salmonids *S. salar* and *S. gairdneri*, comparing the activity in fish exposed to low pH and aluminium with the activity in unexposed fish. Carbonic anhydrase and Na-K-ATPase are important in the osmotic and acid-base regulation across the gills[6].

Specimens of fish were obtained from local hatcheries[1]. They were exposed to tap water to which had been added

Table 1. Effect of exposure to low pH and aluminium on carbonic anhydrase activity in the gills and concentrations of Na^+ and Cl^- in blood plasma of the salmon *(S. salar)*. Values are given as mean ± SD. The 'enzyme unit' (EU) is defined as the activity necessary to halve the time of the uncatalyzed reaction[7]. Experiments were performed in June 1981 (summer) and January/February 1982 (winter). Figures in parentheses indicate the number of fish studied

	Size of fish		Carbonic anhydrase activity	Plasma concentrations	
	Weight (g)	Length (cm)	(EU/g)	Na^+ (mEq/l)	Cl^- (mEq/l)
Summer:					
Control	35.1 ± 6.6 (10)	16.0 ± 1.2 (10)	1438 ± 320 (12)	143 ± 4 (4)	127 ± 2 (4)
Exposed	27.8 ± 7.9 (5)	15.4 ± 1.4 (5)	881 ± 82 (5)	105 ± 7 (3)	88 ± 3 (4)
Winter:					
Control	22.4 ± 4.0 (8)	13.3 ± 0.8 (8)	1351 ± 211 (8)	142 ± 14 (16)	122 ± 11 (14)
Exposed	24.4 ± 3.6 (6)	14.2 ± 1.6 (6)	1037 ± 156 (6)	130 ± 11 (5)	110 ± 5 (5)

Table 2. Effect of exposure to low pH and aluminium on Na-K-ATPase activity in gills and Na^+ and Cl^- concentrations in plasma of rainbow trout *(S. gairdneri)*. Values are given as mean ± SD. Experiments were performed during January/February 1982 with 9 fish in each group

	Size of fish		Enzyme (µmol P_i (mg protein)$^{-1}$ h^{-1})	Plasma concentrations	
	Weight (g)	Length (cm)		Na^+ (mEq/l)	Cl^- (mEq/l)
Control	56.1 ± 15.8	17.4 ± 1.2	2.06 ± 0.89	165 ± 11	132 ± 4
Exposed	57.7 ± 16.1	17.5 ± 1.5	1.47 ± 0.47	101 ± 26	92 ± 21

Al (200 µg/l) in the form of $AlCl_3$. This aluminium concentration is representative of that in the water of lakes and streams in areas receiving acid rain[4]. The acidity of the water was kept at pH 5 by addition of H_2SO_4. Controls were kept in tap water (pH 6.9). Water temperatures were 4–6 °C (winter) or 8–12 °C (summer) and the exposure lasted for 4–7 days. The methods used for determination of carbonic anhydrase and Na-K-ATPase were those described by Maren[7] and Johnson et al.[8], respectively. Chloride was determined by coulometric titration and Na^+ by atomic absorption spectroscopy.

We found a marked decrease in the activity of both carbonic anhydrase (table 1; $p < 0.01$) and Na-K-ATPase (table 2; $p < 0.05$) in the gills of exposed fish. The reduction in enzyme activity was associated with a reduction in the plasma concentrations of sodium and chloride (tables 1 and 2; $p < 0.01$, winter experiments in the salmon, $p < 0.05$). Reduction in enzyme activity and loss of Na^+ and Cl^- occurred before the exposed fish showed visible signs of being affected. A similar loss of ions in salmonids in aluminium-free water is observed after acidification to pH 4 by adding H_2SO_4, but at pH 5 the hydrogen ions themselves seemingly represent no physiological stress to the fish[4]. Our results point to the importance of the enzymes studied in fish death induced by aluminium at low pH.

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A new continuous optical assay for isocitrate lyase[1]

E. Giachetti, G. Pinzauti and P. Vanni

Istituto interfacoltà di Chimica biologica, Università di Firenze, viale Morgagni 50, I-50134 Firenze (Italy), 25 February 1983

Summary. A new continuous optical assay method for isocitrate lyase is reported. This is a coupled assay which requires lactate dehydrogenase as an ancillary enzyme. The method yields linear data up to 0.12 units/ml. The assay is also suitable for crude extracts.

Isocitrate lyase (EC 4.1.3.1) catalyzes the reversible cleavage of *threo*-D_s-isocitrate into glyoxylate and succinate. There are 2 widely-used methods for the assay of isocitrate lyase: one discontinuous[2], the other continuous, chemically coupled[3]. A 3rd method, which assays isocitrate lyase continuously in the direction of isocitrate formation (isocitrate dehydrogenase-coupled), has also been reported[4,5], but its application is limited because glyoxylate and succinate inhibit isocitrate lyase from a number of sources[5,6].

Lactate dehydrogenase, isoenzyme I, from pig heart has been reported to catalyze both the oxidation and reduction of glyoxylate[7]. Lactate dehydrogenase can, therefore, be used as an auxiliary enzyme for a continuous assay of isocitrate lyase. This paper is concerned with the reliability of the method. A comparison with other assays currently in use is also made.

Materials and methods. Chemicals and biochemicals. Lactate dehydrogenase (LDH) from pig heart (whole preparation) and NADH were obtained from Boehringer, Mannheim, FRG; lactate dehydrogenase isoenzyme I from pig heart and *threo*-D,L-isocitrate were obtained from Sigma, Chemical Company, USA, and all other chemicals were purchased from Merck, Darmstadt, FRG.

Isocitrate lyase preparation. Isocitrate lyase (ICL) was isolated from the endosperm of *Pinus pinea* germinating seeds by the method previously reported[6]. All the enzyme fractions used in this study were prepared by this purification procedure.

Enzyme assay. All procedures were carried out at 30 °C in 40 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethansulphonic acid) buffer (pH 7). The continuous assay, chemically coupled with phenylhydrazine, is described elsewhere[6]. The discontinuous assay was performed according to Roche et al.[8], with minor modifications of the buffer system (40 mM HEPES, pH 7). This is a colorimetric method which requires a standard curve. 1 unit of enzyme activity is here defined as the amount of enzyme which catalyzes the cleavage of 1 µmol of substrate per min at 30 °C. Statistical analysis of kinetic data was done in the manner described by Wilkinson[9] and Cleland[10].

Results and discussion. The principle of the LDH-coupled continuous method is based upon the following sequence of reactions:

$$\text{isocitrate} \xrightarrow{\text{ICL}} \text{glyoxylate} + \text{succinate} \qquad \text{(a)}$$

$$\text{glyoxylate} \xrightarrow[\text{NADH} + \text{H}^+ \;\; \text{NAD}^+]{\text{LDH}} \text{glycolate} \qquad \text{(b)}$$

Preliminary studies showed that not only isoenzyme I, but the whole preparation of lactate dehydrogenase from pig heart was suitable as an auxiliary enzyme. The amount of auxiliary enzyme required in the assay was determined by the use of the following equation[11]:

$$V_{max}(\text{LDH}) = -\frac{K_{ms} \ln(1 - Fs)}{t} \qquad \text{(c)}$$