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Measurement of Antioxidant Activity of Anserine, Taurine, and L-Histidine *in vitro* and Content of Anserine, Taurine, and L-Histidine in Mature and Juvenile Rainbow Trout (*Onchorhynchus mykiss*) Muscle

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Abstract

The content of anserine, taurine, and L-histidine was measured by HPLC in the muscle of mature(670~690g) and juvenile(80~120g) rainbow trout farmed in Chungsun, Korea. The concentration of anserine and taurine was higher in mature rainbow trout than in juvenile, but that of L-histidine was lower in mature than in juvenile. When measured with the chemiluminescence(CL) assay, anserine and taurine showed very powerful antioxidative activity above physiological concentration of rainbow trout. Taurine still showed antioxidative activity below physiological concentration, while anserine showed prooxidative activity below that. L-Histidine was prooxidative dose-dependently. In TBA method, while taurine showed very week antioxidative effect, anserine appeared very powerful antioxidant and L-histidine prooxidant at physiological concentration. There was no synergism between anserine and taurine and anserine inhibited prooxidative effect of L-histidine.

Key words: anserine, taurine, L-histidine, antioxidative activity, TBA method, CL-assay

INTRODUCTION

Rainbow trout(*Oncorhynchus mykiss*) found in fresh water lakes and streams has been cultured in many farms in Korea. Fish has many polyunsaturated fatty acids and high capacity of oxidative stress. But it has also antioxidants which can prevent lipid peroxidation in edible portion, muscle. The general antioxidants in animals are vitamin E, vitamin C, β-carotene, uric acid, ubiquinone and taurine(1). Even though skeletal muscle is the tissue that has the most active oxidative metabolism, the concentrations of the vitamin E, vitamin C and β-carotene in this tissue are not particularly high(2). The dipeptide anserine(N-alanyl-3-methyl-1-histidine) is present in the range of 1~20mM in the skeletal muscles of many vertebrates(3,4) and abundant in water-soluble parts of some fishes including rainbow trout. One function assigned to the elevated levels of this compound is its ability to serve as buffer against lactic acid produced during exercise(5). A second function attributed to the imidazoles is its capacity to function as antioxidants via quenching of singlet oxygen(6), scavenging of hydroxyl radicals(7) and trapping of peroxy radicals(8). Besides anserine, taurine and L-histidine at milimolar concentration level in rainbow trout are thought to be antioxidant compounds as well as other histidine derivatives(9,10).

Antioxidant activity has been usually measured by TBA method. Chemiluminescence(CL) assy was also adopted to measure antioxidant activity for more sensitive measurement(11). Chemiluminescence is a light energy emitted during chemical reaction. The light energy emitted is very weak. So some amplifiers can be used to amplify the light intensity. Cyclic diacylhydrazides like luminol, aminobutylethyl isoluminol (ABEI) have been used as amplifiers. They are substrates of peroxidase and oxidized by peroxidase under H_2O_2. Antioxidants prevent the oxidation reaction, namely inhibit the chemiluminescence light intensity(11).

Anserine, taurine, and L-histidine are present together in physiological state of rainbow trout. So antioxidativity of them together was investigated at physiological concentration of mature rainbow trout.

MATERIALS AND METHODS

Reagents

Anserine, butylated hydroxytoluene(BHT), micro-

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peroxidase, aminobutylethyl ioluminol(ABEI) were purchased from Sigma Chemical Company(St. Louis, MO). L-Histidine was purchased from Janssen Chimical Co. Ltd. Taurine was obtained from Dong-A Pharm. Co. Ltd. Hydroperoxide was product of Kodak. o-Phthaldehyde-3-mercaptopropionic acid(OPA-3-MPA) and borate buffer was supplied by Hewlett-Packard. Rainbow trout(mature: 670~690g, juvenile: 80~120g) were supplied by the Sunpyung Fish Farm in Chungsun, Korea.

Quantitation of anserine, taurine, and L-histidine by HPLC

Anserine, taurine, and L-histidine concentrations were determined using the method described by Schuster(12). Analyses were performed with a Hewlett-Packard HP 1090 series liquid chromatograph and Nova-Pak C_{18}, 4μm column(3.9×150mm I.D.). The supernatant that was injected onto the column was prepared from the deproteinized acetonitrile extracts of rainbow trout. Reagents for derivatization were OPA-3-MPA and borate buffer. Mobile phases were composed of buffer A(0.06M sodium acetate, 0.6% THF, pH 8.0) and buffer B(acetonitrile : 0.1M sodium acetate : methanol=14 : 4 : 1).

Data were analyzed by ANOVA using the general linear model procedure of the SAS system. Differences between group means were considered significant at $p<0.05$ using Duncan's test.

Chemiluminescence assay

Chemiluminescence is a light energy emitted during chemical reaction. The light energy emitted is very weak. So amplifiers can be used for amplifying the light intensity. Cyclic diacylhydrazides like luminol, aminobutylethyl isoluminol(ABEI) have been used as amplifiers. They are substrates of peroxidase and oxidized by peroxidase under H_2O_2. Antioxidants prevent the oxidation reaction, thus inhibiting the chemiluminescence light intensity(13). The physiologic concentration of anserine, taurine, and L-histidine was diluted 2 times serially and concentrated 5 and 10 times. Chemiluminescence was assayed according to the method of Birks(13). Two hundred μl of ABEI 0.6μM and 200μl of antioxidant solution were placed in Berthold 9502 polystyrene tube. Hydroperoxide(35% stock solution) and microperoxidase(10mg/ml) were diluted to 1 : 100, and autoinjected to luminometer. Detection of light intensity by chemiluminescence reaction was recorded-performed by Berthold Luminometer (LB 9502, Clilumat) for 2 seconds. Antioxidant activity was expressed as percent inhibition of the sample solution on the ABEI being oxidized by microperoxidase from the reduction of light intensity of chemiluminescence.

Measurement of malondialdehyde(MDA) by thiobarbituric acid(TBA) method

Microsomes were prepared from the muscles of mature and juvenile rainbow trout by standard differential centrifugation techniques(14). The muscle was immediately placed in ice-cold buffer(154mM KCl, 50mM Tris-HCl, EDTA 1mM, pH 7.4) and minced with fine scissors and then homogenized in 4ml buffer per gram of tissue. After standard differential centrifugation, the final supernatant was discarded and the pellet was washed 4 times in 0.25M NaCl. Finally, the pellet was resuspended in 0.25M NaCl and its protein content was determined by the Lowry method(15). Reaction mixtures contained, in a final volume of 1.0ml, 0.5ml phosphate saline buffer(3.4mM Na_2HPO_4-NaH_2PO_4/0.15M NaCl, pH 7.4), 0.2ml microsomal solution(0.8mg/ml), 100μM $FeCl_3$, 100μM ascobate and varying concentrations of each of the compounds dissolved in ethanol. Incubations were at 37°C for 60 min. At the end of this incubation period, 0.1ml of 2%(w/v) BHT was added to each mixture followed by addition of 1ml each of 1%(w/v) thiobarbituric acid(TBA) and 2.8%(w/v) trichloroacetic acid. The solutions were heated in a water bath at 80°C for 20min to develop the $(TBA)_2$-MDA adduct. The chromogen was extracted into 2ml butan-1-ol and the extent of peroxidation measured, in the organic layer, as absorbance at 532nm.

RESULTS AND DISCUSSION

Content of anserine, taurine, and L-histidine in mature and juvenile rainbow trout muscle

The content of anserine, taurine and L-histidine in the acetonitrile extract from mature and juvenile rainbow trout muscle is presented in Table 1. The mature trout muscle contained more anserine and L-histidine than the juvenile fish. The taurine level of juvenile rainbow trout was higher than that of mature one by 42.2%. The study by Coweyl and Parry(16) also showed the taurine content in Atlantic salmon was lower in

Table 1. The content of anserine, taurine and L-histidine in the acetonitrile extracts from mature and juvenile rainbow trout muscle

	Juvenile		Mature	
	mg/100g wet muscle	mM	mg/100g wet muscle	mM
Anserine	228.6±37.9[1)b]	9.5±1.58[b]	306.4±45.0[a]	12.75±1.87[a]
Taurine	36.4± 8.8[a]	2.9±0.7[a]	25.6± 6.0[a]	2.05±0.48[a]
Histidine	50.5± 8.6[a]	3.3±0.56[a]	64.0±10.5[a]	4.12±0.68[a]

[1)]Values are Means±SE
Means with common superscript are significantly different($p<0.05$)

Table 2. Inhibition of light intensity in the chemiluminescence assay using ABEI-microperoxidase

Anserine(mM)	Inhibition(%)	Taurine(mM)	Inhibition(%)	Histidine(mM)	Inhibition(%)
127.5[1)]	98.2	20.5[1)]	98.7	40.12[1)]	-527.8
63.75[2)]	92.4	10.25[2)]	93.2	20.6[2)]	-305.2
12.75[3)]	22.5	2.05[3)]	82.7	4.12[3)]	-231.4
6.38[4)]	-42.1	1.025[4)]	80.0	2.06[4)]	-36.9
3.19[5)]	-31.2	0.513[5)]	75.2	1.03[5)]	-5.3

[1)]10 times of each compounds in mature rainbow trout, [2)]5 times of each compounds in mature rainbow trout, [3)]Each compounds in mature rainbow trout, [4)]Half of each compounds in mature rainbow trout, [5)]Quater of each compounds in mature rainbow trout

mature marine than juvenile. The content of anserine was higher in mature rainbow trout than in juvenile by 34.0%. The L-histidine content was also increased in mature rainbow trout by 26.7%. These results were consistent with the result of the study performed by Abe(17) who measured anserine and L-histidine in canadian and japanese rainbow trout.

Antioxidant activity based on chemiluminescence(CL) reaction

Table 2 shows the antioxidative activity of anserine, taurine and L-histidine in ABEI-microperosidase-H_2O_2 system measured by LB 9502 luminometer. The antioxidative effect of anserine, taurine, and L-histidine at physiologic, diluted, and concentrated level of mature rainbow trout has determined. Antioxidative activity in chemiluminescence expresses the ability of inhibition in the oxidative reaction between microperoxidase and luminol(ABEI) under H_2O_2 and shows relative capacity of antioxdation(11). There was lower limit of anserine between 12.75mM and 6.38mM. Anserine showed antioxidative activity above physiolosical concentration of mature rainbow trout. But, in lower concentration, half and quarter level of the physiologic concentration(6.38 and 3.19mM), percent inhibition of light intensity was approximately -42.1% and -31.2% indicating the prooxidant function of the anserine at that concentration. The mechanism of this prooxidant activity of anserine at low concentration is not evident. The accumulation of H_2O_2 by anserine serving like SOD was known to occur at very slow rate(18), but might affect the prooxidativity of anserine at the half and qurter level of physiological concentration. The lower limit of anserine appeared higher than other antioxidants, ascorbate, BHT, BHA(11). Taurine seemed to have no lower limit and more than the other two compounds tested here showed a very strong antioxidative activity at the concentration range from 0.513mM to 20.5mM. When concentration of taurine increased 40 times, oxidative reaction was inhibited by 23.5%. Taurine was known well as antioxidant that neutralize hypochlorite anions(ClO^-) (9). There was prooxidativity in L-histidine dose-dependently. L-histidine is a well-known quencher of singlet oxygen(10), but has been reported as a stimulator of peroxidation(7). In liposone added by L-histidine, the initial rate of accumulation of peroxides was higher than in control(19).

Antioxidative activity by CL assay and TBA method

Fig. 1 shows the ability of antioxidation of anserine, taurine and L-histidine at physiologic concentration of mature rainbow trout. CL assay was more sensitive than TBA by 1000 times(11). Malondialdehyde(MDA) reacted with TBA is the products generated by Fe-dependent peroxidation(20).

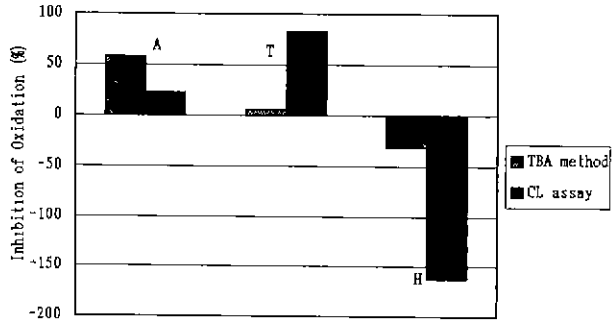

Fig. 1. Inhibition on oxidation by anserine, taurine and L-histidine measured by TBA method and CL assay
Concentration of anserine, taurine and L-histidine is 12.75, 2.05 and 4.12mM, respectively.

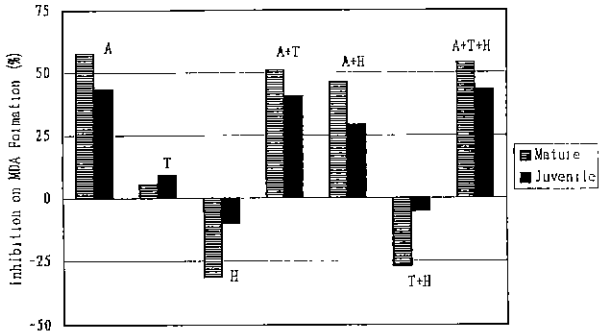

Fig. 2. Inhibition on malondialdehyde(MDA) formation by anserine, taurine and L-histidine in Fe-dependent peroxidation system
Concentration of anserine, taurine and L-histidine in mature is 12.75, 2.05 and 4.12mM and in juvenile is 9.5, 2.9 and 3.3mM, respectively.

$$2LOOH \xrightarrow[\text{Complexes}]{\text{Fe(II) or Cu(I)}} LO\cdot + LO_2\cdot + H_2O \downarrow MDA$$

Lipid hydroperoxides(LOOH) are relatively stable(3). The formation of MDA is necessary for hydroperoxides to be decomposed to secondary products during the reaction by metal ions *in vivo*(21). While anserine was the most powerful antioxidant by TBA method in physiological concentration of mature rainbow trout, taurine was the most powerful by CL assay. That means taurine has very high potency of antioxidation. In TBA method, taurine showed very weak antioxidativity. This is consistent with the result of Alvarez who showed that the taurine has not reduced MDA much(22). The reason anserine was effective antioxidant when measured by TBA method may be explained that the anserine not only inhibited the formation of MDA, but also reduced the formed MDA(20). The ability of histidine to accelerate Fe-dependent peroxidation has been reported by forming a stable chelate with ferrous ion(7,23). The inhibition percentage of L-histidine was higher in CL assay than TBA method, indicating the difference in sensitivity between the two assay systems. The prooxidative activity of L-histidine was due to not only stabilizing ferrous ion but also other factor.

Antioxidative activity of anserine, taurine and L-histidine at physiological concentration of mature and juvenile rainbow trout

There was no synergism observed between anserine and taurine, but anserine masked the prooxidative effect of L-histidine(Fig. 2). The similar results about synergism of histidyl dipeptides-anserine, carnosine, and 1-methyl histidine have been reported(24). The masking ability of anserine on L-histidine was also observed with CL assay. Synergistic effect of anserine and taurine and masking effect of anserine about prooxidative activity of L-histidine were also investigated. Percent inhibition on oxidation measured by CL assay by anserine, taurine, L-histidine, anserine+taurine, anserine+L-histidine and taurine+L-histidine at concentration of mature rainbow trout was 22.5, 82.7, -163.0, 95.6, 20.2 and -78.7%, respectively. The mechanism of the effect of masking is not known. When anserine, taurine, and L-histidine were present together at physiological concentration *in vitro*, antioxiant effect of them was a little higher in physiological concentration of mature rainbow trout than in juvenile.

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