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Dietary protein differentially regulates the kinetic behaviour of serine dehydratase and tyrosine aminotransferase of liver and white muscle of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

We have determined the kinetic behaviour of serine dehydratase (SerDH) and tyrosine aminotransferase (TyrAT) in the liver and white muscle of juvenile rainbow trout (*Oncorhynchus mykiss*) fed on a low-protein/high-fat (LP/HF) and control diets. The relationship between the kinetic parameters and tissue-DNA concentration has also been determined. SerDH and TyrAT showed hyperbolic kinetics in all cases. The partial replacement of protein with fat significantly increased hepatic SerDH-specific activity, maximum velocity (V_{max}) and Michaelis constant (K_m) while no significant changes were detected in the values of these parameters in white muscle. Nevertheless, hepatic and white-muscle TyrAT specific activity and V_{max} decreased in trout fed on LP/HF diet with respect to control. The K_m of hepatic TyrAT was also lower in trout fed on LP/HF diet than in control. The TyrAT kinetic parameters expressed per cell unit also changed in the same sense as previously described. These results show that, in trout, SerDH and TyrAT are two enzymes regulated by the partial replacement of dietary protein by fat.

1. Introduction

The amino acids derived from dietary protein and intracellular protein degradation are used by fish both for an energy source as well as for protein synthesis. Thus, an appropriate supply of dietary protein is required for optimum nutrition and growth. This requirement varies among animals. Fish need some 30–50% dietary protein to achieve maximum growth rates (Wilson and Halver, 1986; Cowey, 1992; Peragón et al., 1994), although their net protein utilization for growth is much lower than that of most mammals (Rumsey, 1981; Bowen, 1987) because a greater proportion of the ingested protein is used for energy production. In fish, less than half the total amino-acid pool available for protein synthesis is derived from intracellular protein degradation (Cowey and Luquet, 1983), whereas in mammals this amount is almost 80%. Therefore, the contribution of ingested amino acids to the total intracellular pool is greater in fish than in mammals and thus variations in dietary-protein content should have a direct influence on the intracellular amino-acid pool and hence on the rate of protein synthesis (Lied and Braaten, 1984).

Serine dehydratase (EC 4.2.1.13, SerDH) catalyses the pyridoxal 5′-phosphate (PLP)-dependent deamination of serine and threonine to produce pyruvate and α-ketobutyrate. In rat, this enzyme has a gluconeogenic character and helps to regulate hepatic glucose biosynthesis from serine in different dietary and hormonal states (Snell, 1984). Its activity tends to increase in gluconeogenic situations such as starvation, high-protein dietary uptake, and diabetes mellitus (Pitot and Peraino, 1964; Ishikawa et al., 1965), but decreases during metabolic acidosis (López-Flores et al., 2006) and liver injury caused by thioacetamide ingestion (López-Flores et al., 2005). Such changes in SerDH activity are triggered by a regulation of the transcription of the SerDH gene, resulting in a regulation in SerDH mRNA levels (Ogawa et al., 1991). The stimulation of SerDH gene expression is mediated by cAMP (Kanamoto et al., 1991) and is maximal in the presence of glucocorticoids (Haas and Pitot, 1999). In rainbow trout, SerDH is regulated by long-term starvation/re-feeding cycle (Peragón et al., 2008) and by development (Peragón et al., 2009). SerDH-specific activity increases after long-term starvation and in the first developmental stages (fingerling and first juvenile stages). This enzyme is a metabolic factor that can relate to protein breakdown and glucose metabolism in the rainbow trout.

Tyrosine aminotransferase (EC 2.6.1.5, TyrAT) is the first enzyme in the catabolic pathway of tyrosine. TyrAT catalyses the transamination of

Abbreviations: SerDH, serine dehydratase; TyrAT, tyrosine aminotransferase; V_{max}, maximum velocity; K_m, Michaelis constant; LP/HF, low-protein/high-fat.

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tyrosine to 4-hydroxyphenylpyruvate with the transformation of alpha-ketoglutarate to glutamate, and prevents the accumulation of toxic levels of tyrosine and provides ketogenic and gluconeogenic substrates when the glucose supply to cells is limited and proteins become the major energy source. The enzyme is strongly induced by glucocorticoids (Granner and Beale, 1985; Schmid et al., 1987), hormones that accelerate the gene-transcription rate (Schmid et al., 1989). Glucagon and cAMP also boost TyrAT activity by raising the mRNA-transcription level (Hashimoto et al., 1984; Schmid et al., 1987; Moore and Koontz, 1989). Starvation sharply stimulates TyrAT activity and the level of this enzyme and its mRNA in rats (Rodriguez et al., 1996; Ciechanover et al., 1997). In rainbow trout, TyrAT increases in response to cortisol (Devaux et al., 1992), long-term starvation (Peragón et al., 2008) and development (Peragón et al., 2009).

The aim of this work was to investigate the effects of the level of dietary protein on the kinetic behaviour of trout liver and white-muscle SerDH and TyrAT. For fish in general, and in trout in specific, no studies have examined the capacity of regulation of both enzymes in response to changes in the dietary macronutrient supply. Proteins and amino acids are essential nutrients for fish. Serine and tyrosine are two amino acids with different functions; serine has a gluconeogenic character (Peragón et al., 2008) while tyrosine is an essential amino acid that has been used mainly for protein synthesis (Rollin et al., 2003). Information on the regulation of SerDH and TyrAT by dietary protein will complete our knowledge concerning the role of both enzymes in fish metabolism.

2. Materials and methods

2.1. Fish and diets

This study was conducted according to the national and international guidelines on animal experimentation and the experiment was approved by the Ethics Committee on Animal Experimentation of the University of Granada (Spain). Rainbow trout (*Oncorhynchus mykiss*) weighing 114.5 ± 0.65 g were obtained from a local fish farm (Riofrío, Granada, Spain). They were kept in 350-litre fibre-glass tanks, in fresh and continuously aerated water (1.5 L/min and 15.0 ± 0.5 °C) under controlled lighting conditions (08:00–20:00) and with free access to a standard commercial diet (Trouw, Spain S.A. No. R.I.A. 09/40551). After 1 week of adaptation to laboratory conditions the trout were divided into two experimental groups, each composed of 120 randomly selected fish. All the fish were weighed individually and each group was separated into three different tanks, 40 fish per tank. Fish were fed twice daily for 50 days with a control or a low-protein/high-fat (LP/HF) diets. These diets were formulated to meet the requirements of the Nacional Research Council (1993). The general composition of these isocaloric diets is shown in Table 1. The ingredients were blended with sodium alginate and thoroughly stirred with distilled water to a homogeneous moist mixture. Pellets were made by passing the diet mixture through an electric meat grinder fitted with a disc of 2.5 mm hole size. After drying at 30 °C the diet was kept in a deepfreeze at −20 °C. Diets were analysed for crude protein, total lipids and moisture using the Association of Official Analytical Chemists method (AOAC, 1984).

The food consumption of all the groups was recorded daily. The fish were fed manually as near to satiation as possible and the quantity of food supplied at each feeding was recorded. Daily food intake and weekly weight gains were recorded throughout the experiment. The relative daily intake of the fish was calculated by dividing the absolute daily intake by the mean body weight plotted on the growth curve. All the fish were individually weighed at the beginning of the experiment and this process was repeated every two weeks to plot the growth curves. As an initial sample, 12 fish from each experimental group (4 from each tank) were killed by cervical dislocation and the weight of their liver and white muscle (including the bones) recorded. These initial mean values were used for reference in the determination of daily weight gain and growth rate. Every week, 6 fish (2 from each tank) were sampled from each group to weigh their whole body, liver, and white muscle. At the end of the experiment, all the remaining trout were killed to analyse the growth of the entire population and make the experimental analysis.

Table 1
Formulation and composition of control and low-protein/high-fat (LP/HF) diets.

	Control	LP/HF
Formulation, g (kg diet)$^{-1}$		
Fish meal[1]	508.7	254.3
Fish solubles[2]	47.8	23.9
Fish oil	4.2	72.1
Corn oil	40.0	90.0
Pre-cooked starch	230.0	230.0
Vitamin pre-mixture[3]	20.0	20.0
Mineral pre-mixture[4]	50.0	50.0
Cellulose	99.3	259.7
Composition, g (kg dry matter)$^{-1}$		
Protein	400.0	200.0
Lipids	80.0	180.0
Digestible carbohydrates	230.0	230.0
Gross energy[5], kJ kg^{-1}	14,960	14,990
P/E, mg/kJ	26.73	13.34
PE/NPE	1.10	0.35

PE/NPE, protein energy:non-protein energy ratio.
[1] Fish meal was composed of 6.53% fat, 70.77% protein, and 8.72% water.
[2] Fish soluble mixture was composed of 5.36% lipids, 83.55% protein, and 9.65% water.
[3] Vitamin pre-mixture contained – g (kg pre-mixture)$^{-1}$ – thiamine hydrochloride 2.0, riboflavin 3.0, pyridoxine hydrochloride 1.5, calcium pantothenate 7.5, nicotinic acid 12.5, folic acid 0.75, myo-inositol 50, choline chloride 250, biotin 0.15, cyanocobalamin 0.33, ascorbic acid 50, vitamin A 0.0075, vitamin D 0.00375, vitamin E 12.5, vitamin K 1.25, and sucrose up to 1000 g mix.
[4] Mineral pre-mixture contained – g (kg pre-mixture)$^{-1}$ – calcium phosphate monobasic 600, calcium carbonate 130, potassium chloride 50, sodium chloride 80, magnesium sulphate 4.0, ferric sulphate 30, magnesium chloride 0.0435, aluminium sulphate 0.20, and sucrose up to 1000 mix.
[5] The gross energy value of protein, lipids, and carbohydrate was assumed to be 19.6, 39.5, and 17.2 kJ g^{-1}, respectively (Brett and Groves, 1979).

The growth rate of the whole body (G_r) and tissues was calculated as the percentage of weight increase per day using the following equation:

$$G_r(\%\ \text{day}^{-1}) = 100(\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_1 is the mean weight at time t_1 and W_2 is the mean weight at time t_2.

2.2. Determination of liver and white-muscle DNA concentration

After 50 days under these nutritional conditions, nine fish from each treatment (three per tank) were killed by a sharp blow to the head and immediately put on ice. The liver was immediately removed and placed in ice-cold saline solution (9 g L^{-1} NaCl). The head, skin, and fins were removed, the entire muscle was weighed, including the bones, and a section was then removed from beneath the skin, above and on either side of the spine just in front of the dorsal fin, discarding any superficial red muscle. The white-muscle section thus obtained was immediately placed in an ice-cold saline solution before homogenization. Samples of both tissues were used to make the different homogenates needed to measure the concentration of DNA and proteins and determine the enzyme activities.

The DNA concentration of the samples was determined as described by Peragón et al. (2009). Samples of 0.5 g of liver and white muscle were homogenized (1/10 w:v) with cold 0.2 M $HClO_4$ and after centrifuging at 2800 *g* for 15 min at 4 °C, the pellet was washed twice with 0.2 M $KClO_4$. The last pellet was incubated in an alkaline medium of 0.3 M KOH at 37 °C for 1 h followed by acidification in 1.2 M $HClO_4$. The incubation mixtures were centrifuged at 2800 *g* for