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Phenotypic flexibility in the intestinal enzymes of the African clawed frog *Xenopus laevis*

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Abstract

The intestinal plasticity of digestive enzymes of amphibian species is poorly known. The goal of this study was to characterize digestive enzyme profiles along the small intestine of adult frogs, *Xenopus laevis*, in response to an experimental diet. We acclimated adult *X. laevis* for 30 days either to carbohydrate-rich or protein-rich diets, and determined the morphology and digestive enzymes of the small intestine. We found a significant difference of aminopeptidase-N activity between carbohydrate-rich and protein-rich acclimated animals. We also found a little variation in the expression of maltase activity, which contrast with the proposed hypothesis about the existence of digestive tradeoff in vertebrates. This finding supports the adaptive modulation hypothesis and suggests that caution is called for when analyzing physiological data regarding assumed discrete trophic category of species.

Keywords: Aminopeptidase-N; Digestive enzymes; Maltase; Phenotypic plasticity; *Xenopus laevis*

1. Introduction

The study of phenotypic plasticity allows us to understand the responses of organisms to variation in the biotic and abiotic environment (Stearns, 1989; Gotthard and Nylin, 1995). The plasticity of digestive tract and its ecological consequences are important because any constraints on digestive performance may influence life history traits such as growth, reproduction and survival (Karasov and Diamond, 1988; Martinez del Río and Stevens, 1989; McWilliams and Karasov, 2001). It has been proposed that the response of both the morphology and physiology of the digestive tract is limited in adults of dietary specialist vertebrates and more flexible in omnivores (Buddington et al., 1987; Karasov and Diamond, 1983; Sabat et al., 1999). Whereas gut plasticity and digestive performance have received considerable attention among birds and mammals (Martínez del Río et al., 1995; Sabat et al., 1998; Caviedes-Vidal et al., 2000), little is known of the intestinal enzymes of amphibians (Feder, 1992; Sabat and Bozinovic, 1996; Hunt and Farrar, 2002, Naya and Bozinovic, 2004).

Experimental studies report that adult amphibians exposed to different dietary substrates do not show differences in the activity of intestinal enzymes and nutrient transporters, whereas tadpoles do vary intestinal performance with diet (Toloza and Diamond, 1991a,b; Sabat and Bozinovic, 1996). These findings support the adaptive modulation hypothesis proposed by Karasov (1992), which claims that food generalist should exhibit greater flexibility in gut function compared to food specialist because the molecular machinery needed to rapidly regulate gut performance is costly and thus would be eliminated by natural selection in animals that do not vary their diet. Accordingly, Secor (2001) in a comparison across several distantly related lineages of amphibians and reptiles

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reported that frequently feeding species exhibit relatively small postprandial responses in intestinal nutrient transport capacities. This author suggested that animals that naturally feed frequently on small meals benefit energetically by modestly regulating gut performance as opposed to widely regulating gut performance. These few studies do provide the firs step to more thorough investigations of morphology and physiology of the amphibian digestive tracts. Given that studies on adult anurans have been conducted on species with a marked degree of dietary specialization, i.e., insectivorous adults, there is a lack of knowledge of the digestive response of adult anurans with a more generalist diet. In addition, the distribution of digestive enzymes along the intestine in vertebrates has been hypothesized to be modulated by natural selection. Enzyme activities vary along the small intestine according to the concentrations of substrates present in digesta (Diamond, 1986, 1991). In addition, pattern of enzyme activities may be also affected by dietary composition. Hence, studies on profiles of enzyme activities of animals eating controlled diets are needed to know how levels of dietary substrates may affect these profiles. The goal of this study is to characterize digestive enzyme profiles along the small intestine of the adult African clawed frog *Xenopus laevis* in response to different diets. We used disaccharidases (sucrase and maltase) and amino-peptidase-N as indicators of digestive capacity of carbohydrates and proteins, respectively (Vonk and Western, 1984).

X. laevis is a very opportunistic forager, feeding on aquatic vertebrates, vertebrates and algae (Measey, 1998; Lobos et al., 1999). We hypothesized that adult *X. laevis* exhibit adaptive plasticity of intestinal enzyme activity in response to different diets.

2. Materials and methods

2.1. Animals and treatment

Adult female *X. laevis* (Daudin) (n=10) were obtained from a feral population in San Antonio, a mesic coastal locality of central Chile (33°34′ S, 71°36′ W), characterized by a warmer summer and rainy and cold winters (mean annual precipitation 441.3 mm, di Castri and Hajek, 1976). Animals were trapped in winter 2004, transported to the laboratory and randomly assigned to two dietary treatments different in protein and carbohydrate content but nearly isocaloric. In order to obtain an extreme response of animals to diets, and also for comparative purposes (see Sabat and Bozinovic, 1996, Naya et al., 2003), diets were prepared contrasting in protein (10% and 70%) and carbohydrate content (75% and 15%) (Table 1). Animals were maintained in separate plastic aquarium of 10×20×30 cm with aerated tap water for 30 days in a climatic chamber with a photoperiod 12:12 D/L and at 20±2 °C. Water was replaced each day when food was provided (1.5% of the body mass/day). After dietary acclimation, animals were weighed (±0.05 g), measured (snout–vent length) and sacrificed. Animals were abdominal dissected and digestive organs were removed. Once supporting mesenteries had been cut, small intestine was weighed and then aligned along a ruler and measured to the nearest 0.1 cm. Liver and heart were also dissected, and then weighed (±0.05 g). Each intestine was separated into five sections of similar length, washed with a 0.9% NaCl solution, and immediately frozen in liquid nitrogen. Tissues were thawed and homogenized (30 s in an Ultra Turrax T25 homogenizer at maximum setting) in 20 vol of 0.9% NaCl solution.

Table 1
Composition of experimental diets expressed as weight percentage

	High carbohydrate diet (%)	High protein diet (%)
Soy protein	10	70
Cornstarch	75	15
Cellulose	7	7
Corn oil	8.0	8.0
Energy (kJ/g)	19.3	19.6

2.2. Enzyme assays

Disaccharidase activity was determined according to the method described by Martínez del Río et al. (1995). We measured enzyme activity in whole-tissue homogenate to avoid the underestimation of activity. Hence, the activities of all enzymes are presented as standardized hydrolytic activity, (UI/g wet tissue, where UI=μmol hydrolyzed/min; see Martínez del Río et al. (1995) for an explanation of the use of this standardization). Briefly, tissue homogenates (100 μL) were incubated at 25 C with 100 μL of 56 mmol L^{-1} sugar solutions (maltose and sucrose) in 0.1 M maleate/NaOH buffer, pH 6.5. After 10 min of incubation, reactions were stopped adding 3 mL of a stop/develop glucose-Trinder (one bottle of Glucose Trinder 500 reagent (Sigma, St Louis, MO, USA) in 250 mL 0.1 mol l^{-1} TRIS/HCl, pH 7 plus 250 mL of 0.5 NaH_2PO_4, pH 7). Absorbance was measured at 505 nm with a spectrophotometer after 18 min at 20 °C.

Aminopeptidase-N assays were done with L-alanine-*p*-nitroanilide as a substrate. Briefly, 100 μL of homogenate diluted with 0.9% NaCl solution was mixed with 1 mL of assay mix (2.04 mmol L^{-1} L-alanine-*p*-nitroanilide in 0.2 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 25 °C and arrested after 10 min with 3 mL ice-cold acetic acid 2 N, and absorbance was measured at 384 nm. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. The selected pHs for measuring the activities were the optimum for each enzyme, which were determined previously by measuring enzyme activities in a range of pH from 4.0 to 9.0.

2.3. Statistics

We statistically evaluated enzyme activity as a function of intestinal position by repeated measures ANOVA using