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

diet as single factor, and enzyme activity along the gut considered as repeated measures within each individual. In addition, a posteriori Tukey test was performed to search for specific differences among treatments. To compare morphological measures, we performed both parametric (ANOVA and ANCOVA with body mass as covariate when appropriate) and nonparametric (Mann–Whitney *U*) test for independent samples. Because the results of the analysis using both parametric and nonparametric test were coincident, we only reported the results of the parametric test. All statistical comparisons were performed using Statistica for Windows (1997) and data are reported as mean±S.D.

3. Results

No differences in frog body mass were found between the two diet groups ($F_{1,8}$=0.76, P=0.40). Also, there were no effects of dietary treatment on the main morphological features of the gut (Wilks' Lambda=0.12, P=0.13, Table 2). A repeated-measures ANOVA revealed a nonsignificant effect of dietary acclimation on aminopeptidase-N activity ($F_{1,8}$=3.39, P=0.10), a significant effect of the position along the gut ($F_{1,8}$=15.44, P<0.001), and a very strong interaction between factors ($F_{1,8}$=6.12, P<0.001). For both meal treatments, aminopeptidase-N decreased distally along the length of the small intestine, more pronounced for the protein diet group (Fig. 1).

The a posteriori Tukey test revealed that the first two portions of the intestine of the protein-acclimated toads had aminopeptidase-N activity higher than any portion of the carbohydrate-acclimated toads. In contrast, maltase activity did not differ in respect to diet ($F_{1,8}$=15.44, P=0.52). The activity of maltase showed a decrease from proximal to distal axis ($F_{1,8}$=11.15, P<0.001) but no effect of the interaction was found ($F_{1,8}$=1.31, P=0.28). The a posteriori Tukey test revealed that the proximal two portions of the small intestine exhibited higher maltase activity than the two most distal segments ones (Fig. 1b). No significant physiological activity of sucrase was found in adults of *X. laevis*.

Table 2
Morphological measurements of *X. laevis* acclimated to two contrasted diets

	High carbohydrate diet	High protein-diet	*F*	P
Body mass	133.7±35.2	153.6±36.4	0.76	0.40
Snout–vent length (cm)	11.34±0.66	11.62±0.56	0.51	0.49
Heart mass (g)	0.56±0.21	0.60±0.20	0.15	0.70
Liver mass (g)	4.18±1.08	4.58±1.20	0.24	0.63
Small intestine mass (g)	0.71±0.16	0.53±0.19	3.53	0.08
Small intestine length (cm)	4.70±0.68	4.16±0.84	1.54	0.25
Small intestine area (cm)	1.72±0.35	2.02±0.68	0.27	0.61

Results of ANOVA (for body mass and snout–vent length, degrees of freedom: 1,8) and ANCOVA (for remainder variables, degrees of freedom: 1,7) using body mass as a covariate are showed. Degrees of freedom: (1,8) for the ANOVA and (1,7) for the ANCOVA.

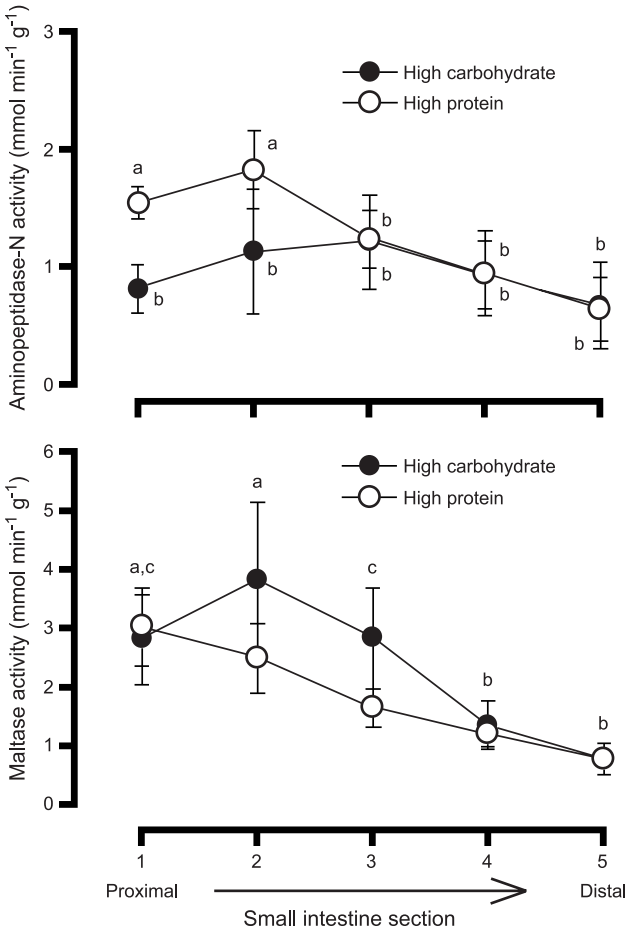

Fig. 1. Mean±S.D. activities of aminopeptidase-N (upper panel) and maltase (bottom panel) in five segments along the small intestine in *X. laevis* acclimated to high- and low protein diets for 30 days. Similar letters indicate nonsignificant differences among segments (a posteriori Tukey test) after repeated measures ANOVA.

4. Discussion

Despite its prominence as the "standard laboratory amphibian" (e.g., Clute and Masui, 1997; Koponen et al., 2004), the digestive physiology of the African clawed frog, *X. laevis*, has been neglected. Here we report measures of intestinal maltase and aminopeptidase-N for adults of *X. laevis* maintained in different diets. Previous studies have differed in their finding on the digestive performance of frogs. For example, Houdry et al. (1979) reported the lack of sucrase activity in *Rana catesbeiana*,

whereas Balogun (1970) found significant levels of sucrase in adults *Bufo regularis*. However, since *B. regularis* were examined using gut homogenates complete with the gut contents, contamination with enzymes in the food or microbial origin cannot be excluded. Further studies are needed to determine whether the lack of ability to digest sucrose is common to another anurans species (but see Sabat and Bozinovic, 1996). Levels of maltase in *X. laevis* are within the range reported for *R. catesbeiana* (2.8 UI/g wet tissue, Houdry et al., 1979). In addition, the profile of maltase expression along the small intestine in *R. catesbeiana* is similar to that of *X. laevis*, i.e., decrease activity distally (Fig. 1). It has been proposed that enzyme activity along the small intestine is correlated with the concentration of its specific substrates (Hume, 1998). We suggest that the decrease in aminopeptidase-N and maltase activity distally is matched with a corresponding decrease of luminal concentration of peptides and disaccharides (McWilliams et al., 1999; Meynard et al., 1999).

A recent study investigating the variation in digestive morphology as a function of experimental diets in larval *X. laevis* found that tadpoles reared on a high protein diets possessed shorter intestines than tadpoles reared on a high fiber diet (Hunt and Farrar, 2002). Our results suggest that gut morphology of *X. laevis* is more inflexible in adults than for tadpoles, given that we found no changes in intestinal morphology in response to different diets (Table 2). Previous studies showed that gut morphology may change seasonally in *Rana temporaria* (Juszczyk et al., 1966) according to the periods of high feeding activity. According, results were obtained by Naya et al. (2003) reporting a seasonal variation in the intestinal length of the South American common frog (*Leptodactylus ocellatus*) related to feeding events. We found that changes in gut size are not related to dietary chemical composition, but we cannot discard that *X. laevis* may exhibit plasticity of gut morphology as an effect of feeding frequency, especially in geographic zones with marked seasonal differences of food availability.

Other studies on the digestive physiology of anurans have shown that only the larvas are able to modify digestive performance in response to diet differences. Toloza and Diamond (1991a,b) reported an up-regulation in amino acid transport in response to high protein diet for larval *R. catesbeiana*. Sabat and Bozinovic (1996) found adult *Bufo spinulosus* not to differ in disaccharidase activities whenever fed a carbohydrate-rich or a protein-rich diet. Contrary to previous reports on digestive enzymes, and intestinal transporters in adult amphibians (see Naya and Bozinovic, 2004), our results in *X. laevis* revealed a significant regulation of aminopeptidase-N when adult animals fed on diets contrasting in protein content. Thus, our aminopeptidase result supports the adaptive modulation hypothesis (Karasov, 1992) that there would be greater aminopeptidase activity in frogs maintained on the high protein diet.

For mammals, disaccharidase activities can be up-regulated by consuming high-carbohydrate diets (Deren et al., 1967; Raul et al., 1978; Sabat et al., 1995). In contrast, birds (including omnivorous species) show remarkable little variation in the expression of disaccharidases when fed diets contrasting in carbohydrate content, (Martínez del Río et al., 1995; Karasov, 1996; Sabat et al., 1998; McWilliams and Karasov, 2001). This coincident trends in enzyme expression found both in frogs and birds, in contrast with the hypothesis proposed by Diamond (1991) that claims the existence of trade off between the ability of the small intestine to hydrolyze proteins and carbohydrates, because of the cost associated to maintain of unused proteins. Alternatively, perhaps the cost of the maintaining the "apparent non used proteins" is outweighed by the benefit of possessing the capacity to digest a broad range of dietary substrates (see Reeder, 1970).

The adaptive modulation of intestinal aminopeptidase-N activity for *X. laevis* is possibly associated with the temporal variation in food composition. The ability to modulate digestive capacity in response to dietary composition may allow frogs to maintain elevated digestive efficiencies if they switch from low to high protein diets. This may explain why *X. laevis* exhibits such a wide trophic niche, and has ability to colonize and exploit different environments (Measey and Tinsley, 1998; Lobos and Measey, 2002). The ability to modulate amino peptidases or even other digestive enzymes is probably not restricted to *Xenopus*, and undoubtedly exists in other frog's species. Unfortunately we have only scarce and partial information of this suggestion, and data on amphibian's digestive enzymes are restricted to a few species representing only a few taxonomic groups. Thus, differences in digestive physiology among the few studied species may be influenced by diet, development stage, ecology, size and phylogeny, which at this point we cannot really support any of those causes not mutually exclusive. For amphibians, future studies that focus on factors influencing modulation of digestive enzymes, along with field studies of foods habits (including detailed chemical analysis of diets) are needed to further understand the adaptive interaction between their food habits and digestive physiology.

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