Revista Chilena de Historia Natural
73:345-350, 2000

Intestinal Disaccharidases and Aminopeptidase-N in two species of *Cinclodes* (Passerine: Furnaridae)

Disacaridasas y aminopeptidasa-N en dos especies de *Cinclodes* (Paserine: Furnaridae)

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ABSTRACT

It has been postulated that both digestive capacity and intestinal biochemical features are correlated to dietary habits in birds. Therefore, it would be expected to find biochemical constraint to hydrolyze sugars in those species, which predate exclusively on marine invertebrates. In vitro intestinal activities of these enzymes were studied in *Cinclodes nigrofumosus* (d' Orbigny) and *Cinclodes patagonicus* (Gmelling). Due to differences in dietary habits between species I predicted the lack of sucrase activity in *C. nigrofumosus* but not in *C. patagonicus*. Also, low activities of maltase would be expected in both species. On the other hand due to the considerable amount of proteins and trehalose present in preys, high activities of both trehalase and aminopeptidase-N were also expected. Contrary to previous reports in birds, significant activity of trehalase was found. Also lack of sucrase and small amounts of maltase were observed as well as a significant aminopeptidase-N activity in both species. Although the digestive enzyme activities of *C. nigrofumosus* and *C. patagonicus* appear to be correlated with their natural diet, the similarities between species in all enzymes activities suggest an strong effect of phylogenetic inertia.

Key words: digestion, dietary adaptation, disaccharidases, aminopeptidase-N, *Cinclodes*.

RESUMEN

Se ha postulado que en las aves tanto la capacidad digestiva como las características bioquímicas del intestino están correlacionadas con los hábitos dietarios. Por esto, es probable que aquellas especies que se alimentan exclusivamente de invertebrados, posean una restricción bioquímica para hidrolizar azúcares. La actividad intestinal *in vitro* de esas enzimas se estudió en *Cinclodes nigrofumosus* (d' Orbigny) y *Cinclodes patagonicus* (Gmelling).Debido a diferencias en los habitos entre las especies, se predice que sacarasa estará ausente en *C. nigrofumosus*, pero no en *C. patagonicus*. Además, se debiera encontrar una actividad maltásica baja en ambas especies. Por otro lado, debido a las considerables cantidades de proteína y trehalosa presente en las presas, también debiera encontrarse actividades importantes de amnopeptidasa-N y trehalasa. Contrario a reportes previos, se encontró una actividad significativa de trehalasa. Además, en ambas especies se encontró ausencia de actividad de sacarasa y baja actividad de maltasa, asi como una significativa actividad de aminopeptidasa-N. Aun cuando las actividades enzimáticas digestivas de *C. nigrofumosus* y *C. patagonicus* parecen estar correlacionadas con la dieta natural, la similitud de las actividades entre las especies sugiere la existencia de una fuerte inercia filogenética.

Palabras clave: digestión, adaptación dietaria, disacaridasas, aminopeptidasa-N, *Cinclodes*.

INTRODUCCION

Digestive capacity and nutrient extraction efficiency have notorious consequences upon diet selection in vertebrates (Karasov & Diamond 1988). One of the most important components of digestive mechanism are proteins (enzymes and carriers) in the membrane of enterocytes that hydrolyze and transport dietary substrates. Several studies have examined how digestive enzymes characteristics of species matches with contrasting diets in both the laboratory and field (Buddington et al. 1991, Sabat et al. 1995, Sabat et al. 1998). Thus, it is well known that in several vertebrates species, biochemical digestive features are correlated with the natural diet of species (Stevens

(Received May 28, 1999; accepted January, 2000; managed by J. Cancino)

1990, Diamond 1991, Hernandez & Martínez del Río 1992). For example, in birds it has been shown that the lack of a disaccharidase determines the rejection of food items containing the specific substrates hydrolyzed by this enzyme (Martínez del Río & Stevens 1989, Martínez del Río 1990). Through this behavior birds may avoid serious diseases associated with mal absorption of non-hydrolyzed sugars (Rey & Frezal 1967). Furthermore, it has been suggested that the presence of an enzyme would carry enough benefits (e.g., preventing osmotic diarrhea), to avoid the lack of it, even though the corresponding substrate might not be important, but rather an occasional, constituent of the natural diet (Reeder 1970, Sabat et al. 1993).

Sucrase, maltase and trehalase are three intestinal disaccharidases that hydrolize sugars present in both plants and animal tissues. Sucrase (sucrase-isomaltase complex) hydrolyze sucrose, a disaccharide present mainly in fruits and other terrestrial vegetal tissues; maltase (maltase-glucoamilase complex) is an enzyme that hydrolyzes maltose, a main product of the degradation of both vegetal and animal polysaccharides (e.g., starch, glycogen); and trehalase, which hydrolyzes the sugar trehalose present in the insects as well as in marine invertebrates haemolymph (Vonk & Wenstern 1984, Brody 1996). Another important enzyme located at the brush border membrane of enterocytes is the aminopeptidase-N, which participate in the last stages of food protein hydrolysis (Brody 1996).

Here I determine the activities of above mentioned enzymes in two passerine birds of the genus *Cinclodes* which inhabit central Chile. I also examine the relationships between enzymatic activity and alimentary habits as well as the inter specific differences in these birds. The genus *Cinclodes* includes several species with different feeding habits. Sea-side cinclodes (*Cinclodes nigrofumosus*, d' Orbigny) forages at intertidal zones (Goodall et al. 1946, Hockey et al. 1987), on almost exclusively marine invertebrates (Paynter 1971, Sabat unpublished results). Dark-bellied cinclodes (*Cinclodes patagonicus*, Gmelling) possesses a wider dietary scope, including insects and other terrestrial arthropods (House 1945, Goodall et al. 1946, Sabat unpublished results). Since preys of *Cinclodes nigrofumosus* do not possess sucrose as sugar storage, I predict a lack of sucrase activity in this species. Nevertheless, as in other vertebrate insectivorous species, the presence of sucrase would be useful to digest some sugars present in the gut content of preys (Reeder 1970, Sabat et al. 1993). If this is true, it is probable that *C. patagonicus*, which include terrestrial preys in their diet, might exhibit a detectable activity of this enzyme. Furthermore it is expected that maltase activity would be present in both species due to ubiquity source of this substrate. Trehalase activity is widespread among insectivorous vertebrates (Zoppi & Shmerling 1969, Hernandez & Martínez del Río 1992, Sabat et al. 1995), but it has not been reported in birds. However, previous studies have been done almost exclusively in granivorous and omnivorous ones and no data in insectivorous and carnivorous birds are available. Based on the above information it is expected to find considerable trehalase activity in both *Cinclodes* species. Moreover, given the high protein loads derived from animal food sources, a high activity of aminopeptidase-N was expected (Bell 1990).

MATERIALS AND METHODS

Animals were captured in El Quisco (33° 24' S, 71° 42' O), a coastal locality in central Chile, using air guns and mist nets during January and February, 1999. Six individuals of *C. nigrofumosus* and four of *C. patagonicus* were obtained. In the field, animals were sacrificed, their digestive tract was excised and washed with a 0.9% NaCl solution, and finally frozen in liquid nitrogen. In the laboratory, the tissues were thawed, and homogenized (30 s in a ULTRA TURRAX T25 homogenizer at maximum setting) in 20 volumes of 0.9 % NaCl solution. Disaccharidase activity was determined according to the method of Dahlqvist (1964), modified by Martínez del Río (1990). Briefly, tissue homogenates (100) mL, were incubated at 40 °C with 100 ml of 56 mmol L^{-1} sugar solutions in 0.1 M Maleate/NaOH buffer, pH 6.5. After 10 min of incubation, reactions were stopped by adding 3 ml of a stop developing Glucose-Trinder (one bottle of Glucose Trinder 500 reagent (Sigma) 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 Sequoia Turner 390 spectrophotometer after 18 min at 20 °C.

Aminopeptidase-N assays were done using L-alanine-p-nitroanilide as a substrate. Briefly, 100 mL of homogenate diluted with 0.9% NaCl solution were mixed with 1 ml of assay mix (2.04 mmol L^{-1} L-alanine-p-nitroanilide in 0.2 mol m L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). The reaction was incubated at 40 °C and arrested after 10 min with 3 ml of ice-cold acetic acid 2 N, and absorbance was measured at 384 nm. On the basis of absorbance, standardized intestinal enzymatic activities were calculated. Thus, the activities of all enzymes are presented as standardized hydrolytic activity (UI/g wet tissue, being UI = m mol hydrolyzed/min). In order to estimate differences of single variables between species, a non parametric Mann-Whitney U test were performed. Data are reported as means ± SD.

RESULTS

Differences in body mass are notorious between species. *Cinclodes nigrofumosus* (78.28 ± 3.42) is bigger than *C. patagonicus* (41.02 ± 2.01). Also differences in standardized length and weight small intestine were found. *Cinclodes patagonicus* possess longest and heaviest mass specific small intestine than *C. patagonicus* (U = -2.55, P = 0.01 and U = –2.34, P = 0.02 for length and mass respectively). For this reason, comparative analysis of the enzyme were performed on the basis of tissue-specific activities (i.e., expressed as mmol min $^{-1}$ g-wet tissue). Contrary to previous reports, and according to expectations, significant activity of trehalase was found in all studied specimens of both species of *Cinclodes*. No differences in trehalase activity were found between species (U = -1.28, P = 0.20, Fig. 1). Also, both studied species showed maltase activity (Fig. 1) and no interspecific differences in the level of activities were found (U = -1.06, P = 0.28). On the other hand no evidence of physiologically significant sucrase activity was found in any specimens of both species. According to the expected aminopeptidase-N activity was also found in both species, and no interspecific differences were disclosed (U = -1.70, P > 0.05, Fig. 1).

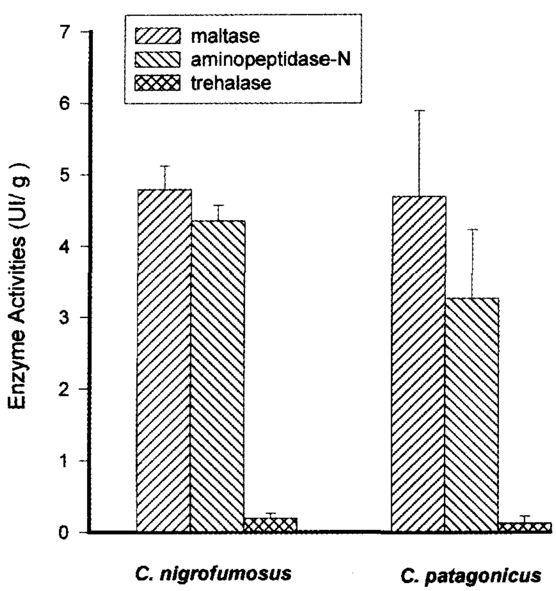

Fig. 1. Digestive enzyme activities in two species of genus *Cinclodes*. Activities are expressed as UI/g wet tissue, being UI = m mol hydrolyzed/min (mean ± 1SD). No statistical differences in the activities of all enzymes were found between species.

Actividades enzimáticas digestivas en dos especies del género *Cinclodes*. Las actividades se expresan como UI/g de tejido húmedo, siendo UI = m moles hidrolizados/min (promedio ± 1DE). No se encontraron diferencias estadísticas entre las especies en todas las enzimas.

DISCUSSION

Presence of trehalase in *Cinclodes* is the first record in birds. However, in contrast with other disaccharidases, as maltase, the correlation with animal diets is not unequivocal. Other birds, as the European crane (*Grus grus*) and the quail (*Coturnix chinensis*), do not posses trehalase, even tough trehalose is present in some mushrooms consumed by this species (Zoppi & Shmerling 1969, Vonk & Wenstern 1984). Even though in *Cinclodes* the levels of trehalase activities were comparatively lower than maltase and amino-peptidase-N, it probably matches with natural presence of dietary substrate, as happens in other biochemical systems (Hochachka & Somero 1984). The differences in enzyme activities exhibit by *Cinclodes* suggest that trehalose are not as concentrated in the diet as others substrates (e.g., proteins and maltose). However, is possible that even a low concentration of trehalose in the natural diet may be physiologically relevant. As already mentioned, if trehalase carry enough benefits as for example to prevent osmotic diarrhea, their evolutionary lack may be avoided. On the other hand, this enzyme is widespread among vertebrates (Vonk & Wenstern 1984) and would be not surprisingly to find it in other birds. Further studies are need to determine how widespread this enzyme is in birds, particularly in insectivorous/carnivorous species.

Contrary to the expectation no differences in sucrase activity were found between species. Even though *C. patagonicus*, as *C. nigrofumosus*, consumes only invertebrates, their diet includes also terrestrial preys. Some preys which may forages on terrestrial plants, probably have sucrose in their gut. Sabat et al. (1993) suggest that the presence of sucrase in a insectivorous marsupial may be related to the ingestion of sucrose-loaded preys. Hence, I expected to find some sucrase activity in *C. patagonicus*. It is probably that in spite of dietary differences, the levels of sucrose to digestion in both species would be negligible. Like in other passerine groups, as Sturnidae-Muscicapidae lineage, the absence of sucrase activity appears to be related with the low concentration or to the complete absence of sucrose found in the species' natural diets (Martínez del Río & Stevens 1989, Martínez del Río 1990, Martínez del Río et al. 1992). This absence of sucrase activity is due to the lack of the sucrase-isomaltase complex (see Vonk & Wenstern 1984). The absence of sucrase in *C. patagonicus* and *C. nigrofumosus* probably reflects the relaxed selection to maintain sucrase. As pointed out by Diamond (1986), disused proteins become genetically lost by natural selection if the cost of synthesis and maintenance exceeds the benefits of its possession. This phenomenon based on a cost and benefit hypothesis appears to be universal for proteins related with metabolic pathways (see Dikhuizen 1978). Alternatively, as happens in other groups of birds, the lack of sucrase activity would be due to a phylogenetic constraint (Martínez del Río 1990, Martínez del Río et al. 1995). Whether or not the lack in sucrase activity is due to a phylogenetic constraint in the genus *Cinclodes*, or even in the Furnaridae family, will be only elucidated on the basis of studies in other members of the group.

The level of maltase enzymatic activity found was near five-fold lower than the ones reported in comparable studies on other omnivorous and granivorous passerines (Martínez del Río et al. 1995, Sabat et al. 1998, Caviedes-Vidal et al. 2000) and comparable to those reported for two mainy insectivorous species of the Muscicapidae family (Martínez del Río 1990). Like in other vertebrates, in birds the maltase activity of the sucrase-isomaltase is accomplished by the maltase-glucoamilase complex but also by the sucrase-isomaltase complex. This sucrase-isomaltase enzymatic complex contributes considerably on the total maltose hydrolysis in birds (Martínez del Río 1990). Then, the lack of sucrase activity in this *Cinclodes* species would explain the low maltose hydrolysis rates, as probably occur also in those above mentioned Muscicapidae species. Even though this enzyme has an apparent low activity, which probably reflects the natural maltose load to digestion (scarce maltose source in animal tissues), their presence suggests that maltose would be well assimilated by the small intestine in these birds.

Surprisingly, aminopeptidase-N activity was not higher than that documented for other herbivorous and omnivorous birds (Sabat et al. 1988, Meynard et al. 1999). However, the enzymatic activity is not the only variable that influence the efficiency by which birds hydrolyze and assimilate nutrients. Differences in retention time of food also play a significant role in the assimilation of dietary substrates (Karasov 1996). It is possible that even when the enzymatic activities for a particular substrate are low, birds might still efficiently break down substrates if the retention time is sufficiently low (Afik et al. 1995). Probably the ratio between the activities of aminopeptidase-N and maltase might be a better predictor of the relative amount of proteins and carbohydrates in bird's natural diet. In an analogous way, the ratio observed in several vertebrates between glucose and amino acids uptake by the intestine are well correlated to diet with different proportions of protein and carbohydrates (Buddington et al. 1987, Karasov & Levey 1990). As pointed out by these authors, these interspecific differences are thought to be physiological adaptations selected in

TABLE 1

Summary of aminopeptidase-N/ maltase ratio and natural diets in passerine birds

Resumen de la razón aminopeptidasa-N/maltasa y la dieta natural en aves paserinas

Species	A-N/Maltase ratio	Diet	Source
Cinclodes nigrofumosus	0.91	C	This study
Cinclodes patagonicus	0.69	C/I	This study
Zonotrichia capensis	0.22- 0.34	S/I	Sabat et al. 1998
Diuca diuca	0.16 - 0.19	S	Sabat et al. 1998
Passer domesticus	0.03 - 0.09	S/F/I	Caviedes- Vidal et al.2000
Sturnus vulgaris	0.35 - 0.46	I/F	Martinez del Río et al. 1995
Dendroica coronata	1.36 - 2.24	I/F/S	Afik et al. 1995
Phytotoma rara	0.025	L/F	Meynard et al. 1999

Nomenclature of diet: C = crustaceans and marine invertebrates, I = insects, S = seeds, L = leaves and F = fruits.
Nomenclatura de la dieta: C = crustáceos e invertebrados marinos, I = insectos, S = semillas, L = hojas y F = frutos.

evolutionary time. Therefore, the ratio between the activities of aminopeptidase-N and maltase in *Cinclodes* species is higher than those found in herbivorous and other omnivorous passerine species, and comparable to those found in one omnivorous birds that seasonally switch diet between fruits and insects (Table 1).

Although the digestive enzyme activities of *C. nigrofumosus* and *C. patagonicus* appear to be correlated with their natural diet, to some extent the similarities between all enzymes activities suggest an strong effect of phylogenetic inertia (see Harvey & Pagel 1991). As pointed out by Feder et al. (1987) a conventional evolutionary wisdom asserts that an animal's first response to a selective pressures are behavioral. Apparently this is the case of *Cinclodes* species, which have remarkably similarity in their biochemical digestive physiology but show some differences in their feeding behavior and other ecological features. It is also possible that differences in dietary habits between these *Cinclodes* species may be related to other physiological features, for example their specific nutritional requirements, and not only to digestive hydrolysis capacity.

ACKNOWLEDGMENTS

The work was supported by FONDECYT 3980027. M. Rosenmann, F Bozinovic, and two anonymous referees improved previous versions of the manuscript.

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