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Physiological Constraint on Feeding Behavior: Intestinal Membrane Disaccharidases of the Starling

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Animals clearly choose what they eat and can even choose among chemically different sugars. The physiological and biochemical mechanisms that constrain feeding choices are largely unknown. In this study, European starlings (*Sturnus vulgaris*) preferred mixture solutions of D-glucose plus D-fructose to equimolar (double molar caloric value) solutions of sucrose. Intubation feeding of sucrose did not increase blood glucose levels. Sucrose is a useless energy source for these birds because they lack a single digestive enzyme (sucrase) on the small intestinal brush border membrane. However, the membranes possessed separate maltase and isomaltase disaccharidases. This expression pattern and expression patterns of membrane disaccharidases among mammals suggest a role for intestinal enzymes in the coevolutionary interactions between vertebrates and their plant food sources.

ANIMALS CAN CHOOSE AMONG foodstuffs that differ only subtly in chemical composition (*1–3*). Feeding choices in some birds have been shown to reflect morphological traits (*4*). However, the physiological and biochemical mechanisms that influence animal feeding choices are largely unknown. In the present study, the European starling provided an example of preferences for simple sugars as directly influenced by physiological factors. Our results illustrate how a digestive constraint, the lack of a single enzyme (sucrase), can profoundly influence food preference.

We offered starlings a choice between two paired tubes containing either sucrose or an iso-osmotic mixture (1:1) of glucose plus fructose at three concentrations (*2, 5*). The offerings were alike in all respects except for the type of sugar. At all concentrations, starlings preferred the mixture of glucose plus fructose to sucrose (Fig. 1). When presented with a choice between a sucrose solution and water, starlings preferred water and appeared to develop a conditioned aversion for sucrose (*2*). Starlings fed sucrose solutions developed signs of discomfort and osmotic diarrhea, corroborating earlier reports (*1, 2*).

To test whether starlings are unable to digest sucrose, we measured the concentration of blood glucose after force-feeding starlings with either sucrose (3 g per kilogram of body weight administered in 0.87*M* solutions) or a 1:1 mixture of glucose plus fructose (3 g per kilogram of body weight administered in 1.66*M* solutions). There was no increase in blood glucose concentration after starlings were given a sucrose meal, but substantial increases were found after they were fed the mixture of monosaccharides (Fig. 2).

Next, we investigated the intestinal mechanism responsible for these observations. Using standard techniques (*2, 6, 7*), we measured the activities of maltase (E.C. 3.2.1.20), sucrase (E.C. 3.2.1.48), trehalase (E.C. 3.2.1.28), lactase (E.C. 3.2.1.23), and isomaltase (E.C. 3.2.1.10) in preparations of small intestinal mucosa and brush border membranes (*8*) from 18 starlings. The substrates (28 m*M* each) were maltose, sucrose, trehalose, lactose, and isomaltose (*9*). As predicted, we observed a complete absence of sucrase activity in starlings (Table 1). We were also unable to detect any lactase or trehalase activity. However, the intestinal preparations exhibited both maltase and isomaltase activities (Table 1).

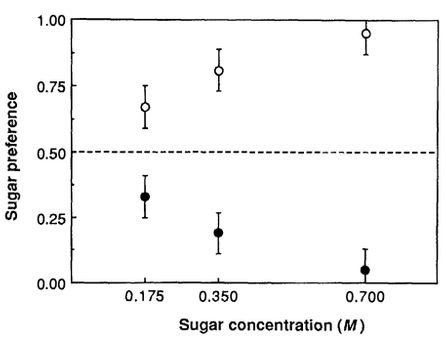

Fig. 1. Starling preferences for sugars. Equimolar solutions of sucrose and a mixture of glucose plus fructose (1:1) were offered paired to starlings (16 birds per sugar concentration test group). Sugar preference is defined as the volume of each sugar solution consumed divided by the total volume of solutions consumed during a 1.5-hour trial. The mixture of glucose plus fructose was preferred to sucrose at all test concentrations. The 95% confidence intervals are shown with mean preferences. A value of 0 would represent complete rejection, a value of 1.0 would represent complete preference, and the dashed line at 0.5 represents no preference. ○, glucose + fructose; ●, sucrose.

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In mammals (*6, 10*), sucrase is transcribed, translated, and then inserted into brush border membranes of small intestine as a single glycoprotein sucrase-isomaltase complex. After membrane insertion, the polypeptide chain is subsequently converted to separate subunits of sucrase and isomaltase. Membrane anchoring occurs via the isomaltase subunit. A review of the avian literature indicates that disaccharidase structural studies have been limited to chickens, ducks, and pigeons (*11*), each showing a sucrase-isomaltase coarrangement similar to that in mammals. The presence of isomaltase in the absence of sucrase is an uncommon phenomenon reported only in isolated human cases of hereditary disaccharidase deficiency (*12*), some marsupials (*13*), alligators (*14*), and in sea lions and fur seals (*Otariidae*) (*10, 13, 15, 16*). In the Tasmanian fur seal (*Arctocephalus tasmanicus*) and sea lion (*Zalophus californianus*), heat inactivation studies indicated that the hydrolysis of isomaltose and maltose likely resulted from a single enzyme with broad substrate specificity (*13, 16*).

To test the possibility of a single disaccharidase in starlings, we examined the heat inactivation pattern of disaccharidases in brush border membranes. Inactivation rates for maltase and isomaltase were significantly different (Fig. 3), suggesting the presence of two independent disaccharidases in the intestine of the starling. Together with other evidence (*6, 10–17*), our results suggest that there is no universal membrane arrangement of intestinal disaccharide hydrolyzing systems among vertebrates.

The absence of sucrase activity in the starling has had ecological consequences. Starlings are granivorous and insectivorous, but include fruit as an important seasonal component in their diets (*18*), to the extent that starlings are considered a pest on cultivated fruit with low sucrose:glucose ratios, such as grapes and cherries. The lack of sucrase activity may thus restrict dietary choices of starlings in the field.

Why do starlings lack sucrase? Among birds, it is likely that sucrase activity is correlated with feeding preferences (*2*). Also, in many animals intestinal transport of the disaccharidase hydrolysis product, glucose, is greater in herbivores than in carnivores (*19*). An evolutionary explanation for the starling's lack of sucrase, however, cannot be provided until more comparative data are available for other species of birds. In this regard, data would be especially valuable concerning birds closely related to starlings but with contrasting feeding habits.

Variation in sugar consumption preferences may have played an important role in vertebrate coevolution with plants. Baker and Baker (*20*) have shown that the sugar composition of nectar and fruit pulps follows consistent patterns, such that plants pollinated by hummingbirds offer sucrose-dominated nectars, whereas those pollinated by bats and passerine perching-birds offer nectars containing mainly glucose and fructose. In fruits whose seeds are dispersed by birds, the sugars are primarily glucose and fructose (*20*). By contrast, the fruits consumed by humans often possess sucrose in addition to monosaccharides. The activity of disaccharidases has been measured in relatively few species of birds, such as red-winged blackbirds, grackles, chickens, ducks, pigeons, and sea birds (*11, 21*). As a consequence, we do not know if the absence of sucrase activity in birds that feed on nectar or fruit, or both, is a trait sufficiently widespread to account for the patterns discovered by Baker and Baker (*20*). The morphology of frugivorous and nectarivorous birds has been implicated in the evolution of the characteristics of floral and fruit displays (*22*). Our results suggest that the physiological traits of birds may be associated with the evolution of the chemical composition of fruit pulp and nectar.

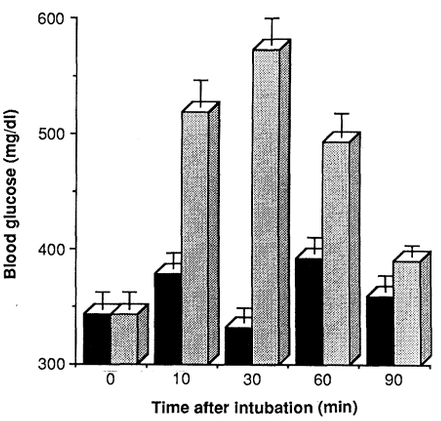

Fig. 2. Sugar tolerance tests in starlings. Blood glucose changes were measured after starlings were fasted overnight and then perorally intubated with either sucrose (3 g per kilogram of body weight), or the same dose of a mixture of glucose plus fructose. The same 15 birds were used in both experiments. Three birds were randomly assigned to each of the five time intervals. Blood was sampled from the brachial vein. The height of each bar represents the mean with 95% confidence interval (n = 3). Solid bars, sucrose; stippled bars, hexoses.

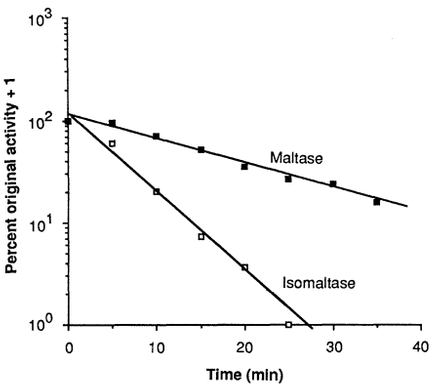

Fig. 3. Heat inactivation of maltase and isomaltase. Intestinal brush border membranes from 18 starlings were exposed to 55°C for various times. After each exposure time, membranes were transferred to ice-chilled containers, then assayed for disaccharidase activities at 39°C. Isomaltase activity was lost at a rate significantly higher than maltase activity (t test for slopes on semi-log transformed data; t = 25.3, df = 9, $P < 0.001$). Means (n = 5) are shown; the 95% confidence intervals were smaller than the size of the symbols. These data suggest that two distinct disaccharidase proteins exist on the membrane of starling small intestine; ■, maltase; □, isomaltase.

Table 1. Selected enzymes of small intestinal mucosa and enterocyte brush border membranes in *Sturnus vulgaris*. Values are means ± SE for three experiments. In each experiment the mucosa of six individuals was used. Enzyme initial rates of hydrolysis were assayed at 39°C. All sugar substrate concentrations were 28 m*M* and the alkaline phosphatase substrate, *p*-nitrophenyl phosphate, was 5 m*M*.

Fraction	Enzyme activity (μmol min^{-1} gram $protein^{-1}$)			
	Maltase	Isomaltase	Sucrase	Alkaline phosphatase*
Brush border membranes	2103 ± 50	14.0 ± 0.3	0	335 ± 17
Mucosal homogenate	276 ± 23	2.9 ± 0.1	0	56 ± 5
Membrane enrichment	7.6-fold	4.6-fold		6-fold

*Alkaline phosphatase was used as a brush border marker (*8, 10*). Sucrase was never detected in any starling's intestine.

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Disruption of the Yeast *N*-Myristoyl Transferase Gene Causes Recessive Lethality

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The structural gene for *N*-myristoyl transferase (*NMT1*) has been cloned from the budding yeast *Saccharomyces cerevisiae*. The gene encodes a polypeptide of 455 amino acids (M_r = 52,837) that has no identifiable significant primary sequence homology with any protein in currently available databases. Overexpression of NMT activity was achieved by means of the yeast episomal plasmid YEp24 without obvious effects on growth kinetics, cell morphology, or acylprotein metabolic labeling patterns. Insertional mutagenesis of the *NMT1* locus on yeast chromosome XII caused recessive lethality, indicating that this protein acyltransferase activity is necessary for vegetative cell growth.

N-MYRISTOYL TRANSFERASE (NMT) catalyzes the co-translational (*1*) attachment of myristic acid (C14:0) via an amide linkage to the NH_2-terminal glycine residues of certain cellular and viral proteins. Myristoyl proteins are targeted to several cellular locations, including the plasma membrane, cytosol, and endoplasmic reticulum, and are involved in such diverse processes as protein phosphorylation, signal transduction, and oncogenesis (*2*). Viral myristoyl proteins include the VP4 and VP2 capsid components of some picornaviruses and papovaviruses, respectively, the *gag* polyprotein precursors of many mammalian retroviruses, and the human immunodeficiency virus *nef* (*3′-orf*) gene product (*3*). Abolishing myristoylation of certain oncoproteins and retroviral *gag* proteins can have profound biological effects. For example, site-directed mutagenesis of the Gly^1 residue of $pp60^{vsrc}$ to Ala^1 or Glu^1 prevents myristate attachment, interferes with the protein's stable association with plasma membranes, and blocks its ability to transform cells (*4*). Analogous mutagenesis of the *gag* polyprotein precursors of some mammalian retroviruses suggests that the myristoyl moiety is critical for their association with plasma membranes and proper assembly of mature virions (*5*). However, since the various myristoyl proteins are distributed widely among both membrane and cytosolic compartments, myristoylation cannot be solely responsible for targeting a specific protein to a particular intracellular location.

NMT has been purified from *Saccharomyces cerevisiae* and its fatty acyl coenzyme A (CoA) thioester and peptide substrate specificities examined in vitro (*6*). The enzyme shows remarkable specificity for myristoyl CoA, excluding other fatty acids based on chain length rather than hydrophobicity, and has an absolute requirement for NH_2-terminal glycine. Binding of the fatty acyl CoA appears to influence subsequent binding of peptide substrates. Comparative kinetic analyses indicate that the substrate specificities of NMT are highly conserved among yeast, plant, and mammalian cells. The gene for NMT has now been cloned from *S. cerevisiae* to determine the structure of its primary translation product, and to take advantage of the genetic manipulability of yeast to probe the role of NMT in cell growth.

NMT was purified to apparent homogeneity from *S. cerevisiae* strain BJ405 as previously described (*6, 7*), except that the final fast protein liquid chromatography (FPLC) step was deleted and a microbore C4 high-performance liquid chromatography (HPLC) step substituted. The principal peak contained a single 55-kD protein, as defined by silver staining of an SDS-polyacrylamide gel. NMT activity coeluted with this polypeptide as measured by an in vitro assay (*8*). Sequence analysis of the purified 55-kD protein revealed that its NH_2-terminus was blocked. The protein was therefore digested with trypsin and the resulting peptides separated by C18 reversed-phase HPLC and subjected to Edman degradation (*9*). After consulting yeast codon usage tables (*10*), we synthesized nondegenerate oligonucleotides (45 and 48 bases long) on the basis of the sequences of two of these peptides.

Blot hybridization of total yeast DNA indicated that both oligonucleotides hybridized to 5.4-kb Hind III and 5.7-kb Pst I fragments. Hind III– or Pst I–digested genomic DNA fragments were fractionated on agarose gels to enrich for the appropriate sized pieces and introduced into pUC13 DNA. The resulting two libraries were screened for bacterial colonies that hybridized specifically with both oligonucleotides.

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