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Enzymatic Activity of α -L-Fucosidase and L-Fucokinase Across Vertebrate Animal Species[‡]

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Abstract

The oligosaccharide portion of glycoproteins is known to modulate protein structure, function, and turnover. Our laboratory is interested in the metabolism of L-fucose, a normal constituent of eukaryotic glycoproteins. L-Fucose is unique in that it is the only levorotatory sugar utilized in mammalian systems. There is considerable interest in understanding the controls which determine the level of L-fucose attached to proteins, in order to generate stable and active glycoforms of protein for the treatment of disease. As part of a program to determine the controls on protein L-fucosylation, we have systematically determined the tissue distribution of the enzymes L-fucokinase and α -L-fucosidase in species across the **vertebrate** animal kingdom. In general, the level of α -L-fucosidase is higher than L-fucokinase level. The tissue with highest enzyme **activity** cannot be generalized, regardless of which enzyme is of interest. Furthermore, there is not a correlation between synthetic and catabolic enzyme activity within a tissue. L-Fucokinase can be detected in all tissues examined. Interestingly, we have also detected β -D-fucosidase activity, present in extraordinary levels in the liver and small intestine of snake. Whether this is due to a specific enzyme or whether it represents a broad specificity of the α -L-fucosidase is currently being investigated.

Keywords

L-fucose salvage; L-fucose synthesis; L-fucose metabolism; species distribution; tissue distribution; L-fucose catabolism

Introduction

The metabolism of glycoproteins represents an area of intense scientific and commercial interest. The oligosaccharide portion of glycoproteins is known to modulate protein function and turnover (Van Den Hamer et al., 1970; Kornfeld and Kornfeld 1980), and is important for recognition events. For example, terminal GalNAc-4-SO₄ on N-linked oligosaccharides is recognized by a Mannose/GalNAc-4-SO₄ receptor that is expressed by hepatic endothelial

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cells of mammals, and that mediates the clearance of glycoproteins bearing these structures from the blood (Fiete et al., 2007).

One naturally occurring moiety of oligosaccharides is L-fucose (Kornfeld and Kornfeld 1976), which is unique in that it is the only levorotatory sugar metabolized in mammalian systems (Flowers, 1981; Werz et al., 2007). L-Fucose can be derived either *via* a *de novo* or a salvage pathway; the *de novo* pathway is considered by most investigators to be more important than salvage. In the *de novo* pathway, the immediate precursor is GDP-mannose, which is converted in the cytoplasm to GDP-L-fucose by GMD (GDP-mannose 4,6-dehydratase, EC 4.2.1.47; Ginsburg, 1960) and a bifunctional enzyme, the FX protein (GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase, EC 1.1.1.271; Sullivan, et al., 1998). The resulting sugar nucleotide is transported into the medial Golgi by a specific transporter (Puglielli and Hirschberg, 1999) for use as a substrate by a group of L-fucosyltransferases with varying specificities.

In the salvage pathway, L-fucose which is derived from dietary sources or from turnover from existing glycoconjugates is taken into cells *via* a specific transport protein (Leck and Wiese, 2004; Wiese et al., 1994). Once within cells, L-fucose is phosphorylated by the enzyme L-fucokinase (ATP: 6-deoxy-L-galactose 1-phospho transferase, EC 2.7.1.52; Ishihara et al., 1968) and converted to sugar-nucleotide by GFPP (GDP- β -L-fucose pyrophosphorylase, EC 2.7.7.30; Quirk and Seley, 2005) before transport into the Golgi and utilization by L-fucosyltransferase enzymes. It has been demonstrated that the vast majority of this L-fucose accumulated is incorporated into proteins (Wiese et al. 1994; Wiese et al. 1996).

Degradation of L-fucose-containing glycoconjugates is carried out by α -L-fucosidase (EC 3.2.1.51, Johnson and Alhadeff, 1991). This ubiquitous lysosomal glycosidase in mammals has an ability to hydrolyze α 1,2, α 1,3, α 1,4 and α 1,6 linkages, and appears to be highly conserved across species as mouse liver α -L-fucosidase significantly cross-reacts with polyclonal antibodies prepared against human enzyme (Johnson and Alhadeff, 1990). It appears that only one mammalian α -L-fucosidase exists, that functions in multimeric form and is known to exist as multiple isoforms. These isoforms in mammals exist as acidic, neutral and basic forms; sialidase treatment converts the acidic to more neutral forms (Johnson and Alhadeff, 1991).

There is considerable interest in understanding the controls that determine the level of L-fucose attached to proteins, in order to modulate glycoforms of protein to control protein half-life of antibodies for the treatment of disease (Natsume et al., 2005). Also increasing our interest in L-fucose are the observations that L-fucose is known to increase in diabetes in both unbound and protein-bound forms (Radhakrishnamurthy et al., 1976) and also in cancer and inflammatory diseases (Thompson et al., 1989, Goodarzi et al., 1995).

As part of a program to determine the controls on protein L-fucosylation, we have previously examined and reported the tissue distribution of L-fucokinase in rodents. These data showed that activity per gram tissue was highest in the brain, with high levels also occurring in the testes, eye and submaxillary gland of both rats and mice. Subsequently, we examined L-fucokinase activity in the brain of other animals and were surprised to find it was not uniformly high. In the present studies, we have systematically determined the tissue distribution of L-fucokinase, and α -L-fucosidase, with the goals of defining the distribution of L-fucokinase in a wide variety of species; establishing the activities of L-fucose catabolic activity in relation to L-fucose salvage; and attempting by species comparison to define states of L-fucose metabolism. In this report, the tissue distribution of these two enzymes, assessed by enzymatic activity, in vertebrate species across the animal kingdom is described.

Materials and Methods

Materials

Standard laboratory materials and chemicals were obtained from Thermo-Fisher (Fair Lawn, NJ, USA) or Sigma-Aldrich (St. Louis, MO, USA), unless otherwise noted. American Radiolabeled Chemicals (St. Louis, MO, USA) provided the L-[5,6-³H]fucose which was greater than 99% pure. Chemicals used were ACS grade unless unavailable. Solutions were prepared with MilliQ-purified water (Millipore, Billerica, MA, USA).

Tissue Preparation

Pigeon (*Columba livia*), snapping turtle (*Chelydra serpentina*), bullfrog (*Rana catesbeiana*), largemouth bass (*Micropterus salmoides*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*) ringneck pheasant (*Phasianus colchicus*), bobwhite quail (*Colinus virginiana*), whitetail deer (*Odocoileus virginianus*), bull snake (*Pituophis cantifer sayi*), cottontail rabbit (*Sylvilagus floridanus*) and jackrabbit (*Lepus californicus*) were obtained locally. Animals collected live from local fishermen or trappers were sacrificed by decapitation. Deer, pheasant, quail, cottontail rabbit and jackrabbit were collected from local hunters as soon as possible after being harvested. Tissues of interest were removed from all species, freed of connective tissues, blotted free of blood, weighed, and transferred to four volumes of fucokinase extraction buffer (1.03 M glycerol, 5 mM β -mercaptoethanol, 1 mM EDTA, 50 mM 3-(N-morpholino)-propanesulfonic acid (MOPS), pH 7.8). Homogenization was performed using a tissumiser homogenizer. Larger tissues were first blended with a Waring blender. All samples were kept on ice until the homogenate was centrifuged for 15 min at 12,000 g at 4 °C. The pellet was subsequently discarded, the supernatant volume recorded, and stored on ice until assayed for enzyme activity and protein content. Thereafter, samples were frozen at -80 °C. Samples can be assayed for at least two years after this storage without significant loss of activity. Three animals were examined in all cases.

L-Fucokinase assay

Tissue homogenate supernates were assayed by the method of Richards and Serif (Richards and Serif, 1977) as modified by Miller and Wiese (manuscript in preparation). An assay cocktail containing MgSO₄, ATP, NaF, DTT, MOPS, pH 7.8, L-fucose and 1.0 μ Ci/mL L-[5,6-³H]fucose was prepared. Two volumes of this cocktail was added to one volume of enzyme solution to be tested. The final concentrations of the components are 6 mM MgSO₄, 5 mM ATP, 8.33 mM NaF, 3.33 mM DTT, 90 mM MOPS, pH 7.8, and 20 μ M L-fucose. Reactions were performed at 37 °C, for a 1 h time period. This has previously been shown to be within the linear range of the assay (Miller et al., 2005), and was verified for these studies. Following incubation, reactions were terminated by spotting 100 μ L on a 2×2 cm DE-81 filter paper and binding for 15 min. Papers were then washed 10× with 0.5 mL portions of water, and placed in a 20-mL scintillation vial. L-[5,6-³H]fucose-1-phosphate was eluted with 0.75 mL of 0.2 M KCl/0.1 M HCl for 15 min with gentle rocking, scintillation cocktail was added, and radioactivity determined in a Packard Tri-Carb Liquid Scintillation Analyzer (Packard, Meriden, CT, USA). Zero-time controls were used to determine non-specific binding and quenching was corrected for by standard techniques.

α -L-Fucosidase assay

Tissue homogenate supernates were assayed by the method of Grove and Serif (Grove and Serif, 1981) using (final concentrations) 1 mM *p*-nitrophenyl- α -L-fucopyranoside, 30 μ g BSA and 20 mM citrate buffer, pH 5.0. Reactions were performed at 37 °C for 1 h overnight, depending on level of enzyme activity, terminated by the addition of 1 M Na₂CO₃, and centrifuged to clarify the denatured protein. Blanks were run using a cocktail

where *p*-nitrophenyl- α -L-fucoside was replaced with *p*-nitrophenyl- β -D-fucoside to control for endogenous absorption and this was subtracted to provide a corrected absorbance. The corrected absorbance at 405 nm was used to determine levels of enzyme activity using the molar extinction coefficient of 18.5×10^3 M.

Protein Assay

Protein was determined by the method of Lowry et al. (1951) as modified by Lees and Paxman (1972) using BSA as the standard. Assays were performed in duplicate, with the appropriate buffer blank subtracted from the absorbance of sample absorbance before calculation of protein amount.

Data Analysis

For the purposes of this work, one unit of L-fucokinase activity is defined as one pmol L-fucose phosphorylated per min, and one unit of α -L-fucosidase activity is defined as one nmol *p*-nitrophenyl- α -L-fucopyranoside hydrolyzed per min. Data are presented as U/g tissue wet mass, $\pm$ SEM. Three animals were examined in all cases, and each assay was conducted in duplicate.

Results

As shown in Fig. 1A, pigeons have a broad distribution of L-fucokinase but, expressed per gram wet mass, most tissues express only a small amount of enzyme activity. The only major organ with a high level of activity is the brain. Liver and kidney express very little activity. Heart, lung and muscle express higher levels of activity. Among the small mass tissues, aorta and nerve express higher levels of activity. In contrast to these findings, when we examined the major tissues of quail and pheasant, brain clearly displayed the highest level of enzyme activity, with the lung being clearly higher than the pigeon. Small tissues were not examined in quail and pheasant and kidney of quail was not examined.

Rabbits show a much different pattern of L-fucokinase activity (Fig. 2A), where a more uniform pattern of expression among tissues was observed. Strikingly, it was also observed that cottontail rabbits express higher levels of activity in all tissues compared to jackrabbit, by as much as 3.5-fold in the case of the kidney.

A similar discordant expression was seen in the case of fish (Fig. 3A), where catfish express much more L-fucokinase than either bass or carp except for the spleen. It was also noted that overall the level of activity is higher in fish than either birds or rodents. In the case of catfish, very high L-fucokinase activity was found in liver, kidney and heart. Activity was more uniform across all tissues of the carp and bass, with similar levels to rodents and birds observed.

When examining reptiles (Fig. 4A), levels of L-fucokinase activity were found to be higher in the mostly water-dwelling frogs and turtles than in mostly land-dwelling snake except for eye and small intestine. Interestingly, the activity in reptiles was also higher than that observed in birds or rodents. The highest level is observed in the spleen of frog; spleen was also the most variable in expression.

α -L-Fucosidase levels are clearly much higher than L-fucokinase levels, at nmol/min·g as opposed to pmol/min·g for L-fucokinase (Fig. 1B). The pigeon was found to have extraordinarily high enzyme activity compared to either quail or pheasant. Much higher activity was present in liver, kidney and small intestine than any other tissue. In quail and pheasant enzyme activity was more uniform at the lower levels. The kidney of quail was not examined.

Intriguingly, whereas L-fucokinase was higher in cottontail than jackrabbit, the α -L-fucosidase was also correspondingly higher for the cottontail than for the jackrabbit (Fig. 2B). The largest amount of enzyme activity was found in the small intestine of the cottontail, and the liver and kidney of the jackrabbit. The jackrabbit had a somewhat more even distribution of enzymatic activity.

Fish expressed the highest amount of α -L-fucosidase in liver, kidney, gill and spleen, with lesser but detectable amounts found all tissues assayed (Fig. 3B). Catfish were not exceptional in their enzyme activity as they were when L-fucokinase was examined. Bass, though, had lower expression levels than carp or catfish. When examining α -L-fucosidase levels in reptiles, the most striking observation was the huge enzyme activity in snake liver, lung and small intestine (Fig. 4B). Interestingly, we also observed that the snake liver and small intestine expressed a high level of β -D-fucosidase. We had been using p-nitrophenyl- β -D-fucopyranoside as a negative control in our assays but observed this was also hydrolyzed by these two snake tissues.

We performed a correlational analysis between the two enzymes under analysis herein by plotting the average level of α -L-fucosidase *versus* the average L-fucokinase activity for each tissue and all species (Fig. 5). It can be stated from this analysis there are some tissues which had a synthetic mode, and others which were in the mode of L-fucose degradation. However, in this analysis if one examines only one tissue across all species, no correlation is observed.

Since two species previously examined were the dog and pig, we also examined the distribution of α -L-fucosidase and L-fucokinase in the deer. As shown in Table 1, activity of both enzymes were found in all tissues examined. α -L-Fucosidase was present in high levels of the kidney and testes as expected, but was also expressed at similarly high levels in the liver and spleen. Brain had quite variable amounts of the enzyme activity. L-Fucokinase was highest in the brain but in quite variable levels. It was also high in testes, lung and spleen, with testes also having a higher variability than other tissues. Kidney and heart had the lowest levels of expression.

Discussion

We recently showed that rats and mice contain L-fucokinase broadly distributed throughout tissues, with the highest level being found in the brain (Miller et al., 2005) and developed a purification scheme for mouse brain L-fucokinase (manuscript in preparation). Subsequently, we attempted purification of L-fucokinase from other species of brain (Honas and Wiese, 2005), to determine whether a general purification could be used on any tissue source. During these studies, we were surprised to find that levels of brain L-fucokinase varied widely among species.

In the present studies, we examined the distribution of both L-fucokinase and α -L-fucosidase in order to have a simultaneous picture of both synthetic (salvage) and catabolic metabolism of L-fucose. To our knowledge the simultaneous analysis has not been previously performed. This is the most complete analysis of L-fucokinase enzyme activity reported in the literature. L-Fucokinase has been studied in both canine and porcine thyroid tissue (Richards and Serif 1977; Grove and Serif, 1981). Dopamine has been shown to increase L-fucosylation in the rat hippocampus (Honza et al., 1983) and hypothesized to act in long-term potentiation (Angenstein et al., 1992). L-Fucose analogs have been used to examine rat liver L-fucokinase (Zeitler et al., 1997). Park, *et al.* have demonstrated L-fucokinase activity in porcine aorta, pancreas, kidney, heart, lung, spleen, brain and liver at varying levels (Park et al., 1998). The only other relatively complete tissue distribution was

performed by Niittymäki, *et al.* who demonstrated mRNA expression in liver, kidney, ovary, testis, spleen, brain, heart and lung (Niittymäki et al., 2004). In this study the authors examined long- and short-splice variants of the gene but as we are examining enzyme activity the results cannot be directly compared. α -L-Fucosidase has been much more extensively studied than L-fucokinase due to its role in fucosidosis (Cabezas et al., 1983) and is considered to have a ubiquitous distribution.

We found that in general, the level of α -L-fucosidase is higher than the level of L-fucokinase. This may be expected if the rate of *de novo* synthesis far exceeds the rate of L-fucose salvage, as discussed below. The tissue with highest levels of enzyme activity cannot be generalized across species, regardless of which enzyme is of interest. The observation that levels of enzyme activity can be so different even in different varieties of the same family (cottontail *versus* jackrabbit, e.g.), makes it tempting to speculate that environmental conditions may have an impact on L-fucose metabolism. It is difficult to see, however, how this could be placed into a manageable scientific study especially in light of current funding levels.

There is not a correlation between levels of salvage and catabolic enzyme activities within a tissue. It is clear from the data of Fig. 5 that tissues can enter a distinctly catabolic state or a distinctly salvage state. However, when a single tissue is examined across all species, it becomes apparent that even this level of correlation is not attained (data not shown). This prompts speculation that developmental, environmental or some other condition influences the overall metabolic state.

In an elegant study, it was shown that in HeLa cells, exogenously added [3 H]fucose underwent a 9- to 10-fold dilution of specific activity (Yurchenco and Atkinson, 1975). From this it was concluded that most L-fucose is synthesized *versus* salvaged. Based on some experimental data though, we wonder whether this is always true. In particular, we have shown a marked, hyperbolic and saturable increase in L-fucosylated glycoconjugates in HepG2 cells when exogenous L-fucose is added to culture medium (unpublished data). It may be that some cell types rely more on salvage than on synthesis. Recently, a model system was developed by generating FX-null mice (Smith et al., 2002). The fact that this knockout prevented L-fucosylation and caused embryonic lethality is evidence that synthesis is critically important. In these studies though, rescue of FX(-/-) embryos with maternal L-fucose supplementation was marginally or not effective even though L-fucosylated glycans could be detected in surviving embryos. These authors suggest the possibility that the salvage pathway is absent from some cells, or that there are some cell types for which a complete defect in L-fucosylation will confer lethality upon an embryo. We have considered that perhaps the *de novo* pathway controls the gross level of L-fucosylation and the salvage component of the pathway fine-tunes the expression of L-fucosylated glycans. Furthermore, the L-fucose synthetic pathway is considered to be constitutive (Becker and Lowe, 2003). However, as it is widely accepted that L-fucose levels increase in disease states, it must be wondered what the mechanism of this increase is. Further studies are needed to address these questions.

L-Fucokinase activity can be detected in all tissues examined. It seems likely that the enzyme is present in all tissues that synthesize glycoproteins. Regardless of whether L-fucose is synthesized *de novo* or being salvaged the sugar precursor must be phosphorylated prior to conversion to the active sugar nucleotide form. The pattern is not perfect, but it seems that L-fucokinase expression is higher in tissues important to immune function (spleen, e.g.). There is also a tendency to believe tissues higher in blood content display higher L-fucokinase activity. This leads to speculation that perhaps cells of the immune system are particularly dependent on L-fucosylation *via* the salvage pathway. The

observation that water-dwelling animals have higher levels of activity would also seem to favor this hypothesis. Water-dwelling animals seem more likely to suffer immune insult than would land-dwelling animals. The fact that L-fucokinase levels vary so widely across tissues within an animal could be viewed as additional evidence that the relative importance of synthesis *versus* salvage is not always the same. Furthermore, we know that L-fucokinase activity of developing mouse brain is developmentally regulated (unpublished data). Additional mechanisms of regulation, including post-translational modifications of L-fucokinase, represents an area of current interest which should be addressed. One project currently underway is synthesis of an inhibitor of L-fucokinase. A further line of investigation is to determine whether isoforms of L-fucokinase exist. There is currently no published data indicating different L-fucokinase protein isoforms, though it is known there are long- and short-splice variants of the mRNA (Niittymäki, et al., 2004)

Interestingly, we have also detected β -D-fucosidase activity, that is present in extraordinarily high amounts in the liver and small intestine of the snake. Whether this is due to an enzyme specific for β -D-fucose, or whether it represents a broad specificity of the α -L-fucosidase is currently being investigated.

In conclusion, we have demonstrated enzyme activity of both L-fucose catabolism and salvage enzymes in all tissues and all species examined, at levels that range across two orders of magnitude. Because α -L-fucosidase activity is high in tissues where L-fucokinase is not, it is clear that not all L-fucose is salvaged within a tissue. Regulation of the L-fucose salvage pathway must be addressed to more clearly define the relative importance of L-fucose synthesis *versus* salvage.

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Abbreviations

BSA	bovine serum albumin
DTT	dithiothreitol
EDTA	ethylene diamine tetraacetic acid
β-ME	beta-mercaptoethanol
MOPS	3-(N-morpholino)-propanesulfonic acid

References

- Angenstein F, Matthies H Jr, Staack S, Reymann KG, Staak S. The maintenance of hippocampal long-term potentiation is paralleled by a dopamine-dependent increase in glycoprotein fucosylation. *Neurochem Int.* 1992; 21:403–408. [PubMed: 1338901]
- Becker DJ, Lowe JB. Fucose: biosynthesis and biological function in mammals. *Glycobiology.* 2003; 13:41R–53R.
- Cabezas JA, Reglero A, Calvo P. Glycosidases (Fucosidases, galactosidases, glucosidases, hexosaminidases and glucuronidase from some molluscs and vertebrates, and neuraminidase from virus) [Review]. *Int J Biochem.* 1983; 15:243–259. [PubMed: 6299816]

- Fiete D, Mi Y, Oats EL, Beranek MC, Baenziger JU. N-Linked oligosaccharides on the low density lipoprotein receptor homolog SorLA/LR11 are modified with terminal GalNAc-4-SO₄ in kidney and brain. *J Biol Chem.* 2007; 282:1873–1881. [PubMed: 17121844]
- Flowers H. Chemistry and biochemistry of D- and L-fucose. *Adv Carbohydrate Chem Biochem.* 1981; 39:279–345.
- Ginsburg V. Formation of guanosine diphosphate L-fucose from guanosine diphosphate D-mannose. *J Biol Chem.* 1960; 235:2196–2201. [PubMed: 13850250]
- Goodarzi MT, Turner GA. Decreased branching, increased fucosylation and changed sialylation of α 1-proteinase inhibitor in breast and ovarian cancer. *Clin Chim Acta.* 1995; 236:161–171. [PubMed: 7554283]
- Grove DS, Serif GS. Porcine thyroid fucosidase. *Biochim Biophys Acta.* 1981; 662:246–255. [PubMed: 7317440]
- Honas BJ, Wiese TJ. Fucokinase distribution varies widely within the animal kingdom. *MWRM.* Oct 27.2005 40:19. (Abstract).
- Honza R, Jork R, Lossner B, Matthies H. The effect of dopamine on L-fucose incorporation into hippocampal and striatal slices of dopamine-supersensitive rats. *Biomed Biochim Acta.* 1983; 42:419–422. [PubMed: 6684921]
- Ishihara H, Massaro DJ, Heath EC. The metabolism of L-fucose. III The enzymatic synthesis of α -L-fucose 1-Phosphate. *J Biol Chem.* 1968; 243:1103–1109. [PubMed: 5646161]
- Johnson SW, Alhadeff JA. Mammalian α -L-fucosidases. *Comp Biochem Physiol [B] Biochem Mol Biol.* 1991; 99:479–488.
- Johnson SW, Alhadeff JA. Isoform, kinetic and immunochemical characterization of rodent liver α -L-fucosidases. *Comp Biochem Physiol [B] Biochem Mol Biol.* 1990; 97:713–717.
- Kornfeld R, Kornfeld S. Comparative aspects of glycoprotein structure. *Ann Rev Biochem.* 1976; 45:217–237. [PubMed: 786150]
- Kornfeld, R.; Kornfeld, S. Structure of glycoproteins and their oligosaccharide units. In: Lennarz, WJ., editor. *The Biochemistry of Glycoproteins and Proteoglycans.* Plenum Press; New York: 1980. p. 1-34.
- Leck JR, Wiese TJ. Purification and characterization of the L-fucose transporter. *Protein Expr Purif.* 2004; 37:288–293. [PubMed: 15358349]
- Lees MB, Paxman S. Modification of the lowry procedure for the analysis of proteolipid protein. *Anal Biochem.* 1972; 47:184–192. [PubMed: 5031110]
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951; 193:265–275. [PubMed: 14907713]
- Miller EN, Rupp AL, Lindberg MK, Wiese TJ. Tissue distribution of L-fucokinase in rodents. *Comp Biochem Physiol [B] Biochem Mol Biol.* 2005; 140:513–520.
- Natsume A, Wakitani M, Yamane-Ohnuki N, Shoji-Hosaka E, Niwa R, Uchida K, Satoh M, Shitara K. Fucose removal from complex-type oligosaccharide enhances the antibody-dependent cellular cytotoxicity of single-gene-encoded antibody comprising a single-chain antibody linked the antibody constant region. *J Immunol Methods.* 2005; 306:93–103. [PubMed: 16236307]
- Niittymäki J, Mattila P, Roos C, Huopaniemi L, Sjöblom S, Renkonen R. Cloning and expression of murine enzymes involved in the salvage pathway of GDP-L-fucose. L-Fucokinase and GDP-L-fucose pyrophosphorylase. *Eur J Biochem.* 2004; 271:78–86. [PubMed: 14686921]
- Park SH, Pastuszak I, Drake R, Elbein AD. Purification to apparent homogeneity and properties of pig kidney L-fucose kinase. *J Biol Chem.* 1998; 273:5685–5691. [PubMed: 9488699]
- Puglielli L, Hirschberg CB. Reconstitution, identification, and purification of the rat liver Golgi Membrane GDP-fucose transporter. *J Biol Chem.* 1999; 274:35596–35600. [PubMed: 10585436]
- Quirk S, Seley KL. Substrate discrimination by the human GTP fucose pyrophosphorylase. *Biochemistry.* 2005; 44:10854–10863. [PubMed: 16086588]
- Radhakrishnamurthy B, Berenson GS, Parfaonkar PS, Voors AW, Srinivasan SR, Plavidal F, Dolan P, Dalferes ER. Serum-free and protein-bound sugars and cardiovascular complications in diabetes mellitus. *Lab Invest.* 1976; 34:159–165. [PubMed: 1249917]

- Richards WL, Serif GS. Canine thyroid fucokinase. *Biochim Biophys Acta*. 1977; 484:353–367. [PubMed: 20962]
- Smith PL, Myers JT, Rogers CE, Zhou L, Petryniak B, Becker DJ, Homeister JW, Lowe JB. Conditional control of selectin ligand expression and global fucosylation events in mice with a targeted mutation at the FX locus. *J Cell Biol*. 2002; 158:801–815. [PubMed: 12186857]
- Sullivan FX, Kumar R, Kriz R, Stahl M, Xu GY, Rouse J, Chang XJ, Boodhoo A, Potvin B, Cumming DA. Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis in vitro. *J Biol Chem*. 1998; 273:8193–8202. [PubMed: 9525924]
- Thompson S, Kelly CA, Griffiths ID, Turner GA. Abnormally-fucosylated serum haptoglobins in patients with inflammatory joint disease. *Clin Chim Acta*. 1989; 184:251–258. [PubMed: 2611998]
- Van Den Hamer CJ, Morell AG, Scheinberg IH, Hickman J, Ashwell G. Physical and chemical studies on ceruloplasmin. IX The role of galactosyl residues in the clearance of ceruloplasmin from the circulation. *J Biol Chem*. 1970; 245:4397–4402. [PubMed: 4322435]
- Werz DB, Ranzinger R, Herget S, Adibekian A, von der Lieth CW, Seeberger PH. Exploring the structural diversity of mammalian carbohydrates “Glycospace” by statistical databank analysis. *ACS Chem Biol*. 2007; 2:685–691. [PubMed: 18041818]
- Wiese TJ, Dunlap JA, Yorek MA. L-Fucose is accumulated by a specific transport system in eukaryotic cells. *J Biol Chem*. 1994; 269:22705–22711. [PubMed: 8077222]
- Wiese TJ, Dunlap JA, Yorek MA. Effect of L-fucose and D-glucose concentration on L-fucoprotein metabolism in human Hep G2 cells and changes in fucosyltransferase and α -L-fucosidase activity in liver of diabetic rats. *Biochim Biophys Acta*. 1996; 1335:61–72. [PubMed: 9133643]
- Yurchenco PD, Atkinson PH. Fucosyl-glycoprotein and precursor pools in HeLa cells. *Biochemistry*. 1975; 14:3107–3114. [PubMed: 167817]
- Zeitler R, Danneschewski S, Lindhorst T, Thiem J, Reutter W. Inhibition of L-fucokinase from rat liver by L-fucose analogues in vitro. *J Enzyme Inhib*. 1997; 11:265–273. [PubMed: 9208369]

Fig. 1. L-Fucokinase and α -L-Fucosidase Activity in Birds. A. Tissues of pigeon (*Columba livia*), bobwhite quail (*Colinus virginiana*) and ringneck pheasant (*Phasianus colchicus*) were isolated, extracted and centrifuged. The resulting supernates were reacted with L-fucokinase cocktail containing L-[5,6- 3 H]fucose for 1 h. Samples were then spotted on DE-81 filters and L-[5,6- 3 H]fucose-1-phosphate determined after binding, washing and scintillation counting. Each data point is the mean activity per gram tissue wet mass, $\pm$ sem of three animals assayed in duplicate. B. Identical aliquots of tissue supernates were reacted with 1 mM p-nitrophenyl- α -L-fucopyranoside in BSA/citrate buffer, pH 5.0 for 1 h – overnight. Samples were then terminated and measured by spectrophotometry using an $\epsilon = 18.5 \times 10^3$

M. Each data point is the mean activity per gram tissue wet mass $\pm$ sem of three animals assayed in duplicate.

Fig. 2. L-Fucokinase and α-L-Fucosidase Activity in Rodents. A. Tissues of jackrabbit (*Lepus californicus*) and cottontail rabbit (*Sylvilagus floridanus*) were isolated, extracted and centrifuged. The resulting supernates were reacted with L-fucokinase cocktail containing L-[5,6-³H]fucose for 1 h. Samples were then spotted on DE-81 filters and L-[5,6-³H]fucose-1-phosphate determined after binding, washing and scintillation counting. Each data point is the mean activity per gram tissue wet mass, ± sem of three animals assayed in duplicate. B. Identical aliquots of tissue supernates were reacted with 1 mM p-nitrophenyl-α-L-fucopyranoside in BSA/citrate buffer, pH 5.0 for 1 h – overnight. Samples were then terminated and measured by spectrophotometry using an $\epsilon = 18.5 \times 10^3$ M. Each data point is the mean activity per gram tissue wet mass ± sem of three animals assayed in duplicate.

Fig. 3.

L-Fucokinase and α -L-Fucosidase Activity in Fish. A. Tissues of largemouth bass (*Micropterus salmoides*), carp (*Cyprinus carpio*), and channel catfish (*Ictalurus punctatus*) were isolated, extracted and centrifuged. The resulting supernates were reacted with L-fucokinase cocktail containing L-[5,6- 3 H]fucose for 1 h. Samples were then spotted on DE-81 filters and L-[5,6- 3 H]fucose-1-phosphate determined after binding, washing and scintillation counting. Each data point is the mean activity per gram tissue wet mass, $\pm$ sem of three animals assayed in duplicate. B. Identical aliquots of tissue supernates were reacted with 1 mM p-nitrophenyl- α -L-fucopyranoside in BSA/citrate buffer, pH 5.0 for 1 h – overnight. Samples were then terminated and measured by spectrophotometry using an $\epsilon = 18.5 \times 10^3$ M. Each data point is the mean activity per gram tissue wet mass $\pm$ sem of three animals assayed in duplicate.

Fig. 4.

L-Fucokinase and α-L-Fucosidase Activity in Reptiles. A. Tissues of snapping turtle (*Chelhydra serpentina*), bull snake (*Pituophis cantifer sayi*), and bullfrog (*Rana catesbeiana*) were isolated, extracted and centrifuged. The resulting supernates were reacted with L-fucokinase cocktail containing L-[5,6-³H]fucose for 1 h. Samples were then spotted on DE-81 filters and L-[5,6-³H]fucose-1-phosphate determined after binding, washing and scintillation counting. Each data point is the mean activity per gram tissue wet mass, ± sem of three animals assayed in duplicate. B. Identical aliquots of tissue supernates were reacted with 1 mM p-nitrophenyl-α-L-fucopyranoside in BSA/citrate buffer, pH 5.0 for 1 h – overnight. Samples were then terminated and measured by spectrophotometry using an $\epsilon = 18.5 \times 10^3$ M. Each data point is the mean activity per gram tissue wet mass, ± sem of three animals assayed in duplicate.

Fig. 5. Correlation between α -L-fucosidase and L-fucokinase activities. The average of each species' α -L-fucosidase activity are plotted *versus* the average of each species' L-fucokinase activity. The error bars are omitted for clarity.

Table 1 **α -L-Fucosidase and L-Fucokinase Activity of Deer Tissues**

Tissue	α -L-Fucosidase nmol/min·g	L-Fucokinase pmol/min·g
Liver	406.7 $\pm$ 68.2	186.5 $\pm$ 20.4
Kidney	539.4 $\pm$ 50.4	71.6 $\pm$ 12.6
Heart	98.7 $\pm$ 29.0	76.5 $\pm$ 17.0
Lung	104.0 $\pm$ 29.7	340.1 $\pm$ 50.8
Spleen	324.9 $\pm$ 221.8	239.2 $\pm$ 87.1
Brain	220.1 $\pm$ 137.2	530.3 $\pm$ 388.2
Testes	365.5 $\pm$ 55.0	287.6 $\pm$ 160.3

Supernatant of tissues from whitetail deer (*Odocoileus virginianus*) were reacted with L-fucokinase cocktail containing L-[5,6- 3 H]fucose for 1 h in duplicate. Sample was then spotted on DE-81 filters and L-[5,6- 3 H]fucose-1-phosphate determined after binding, washing and scintillation counting. A second set of duplicate aliquots of tissue supernate were reacted with 1 mM p-nitrophenyl- α -L-fucopyranoside in BSA/citrate buffer, pH 5.0 for 1 h – overnight. Sample was then terminated and measured by spectrophotometry using an $\epsilon = 18.5 \times 10^3$ M. Data are expressed per gram tissue wet mass $\pm$ sem; n=3