Regulation of the expression of carbohydrate digestion/absorption-related genes

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To explore the underlying molecular mechanism whereby nutrients modulate the expression of intestinal digestion/absorption-related genes, we have cloned the 5′ flanking regions of two representing disaccharidase genes, i.e. sucrase–isomaltase (SI) and lactase–phlorizin hydrolase (LPH), and investigated whether the binding activity of putative common nuclear factor(s) binding to the *cis*-elements located in these regions is altered by dietary manipulations. Orogastric feeding of a sucrose-containing diet to rats caused parallel increases in SI mRNA and LPH mRNA levels within 3 h. Among the monosaccharides tested, fructose gave rise to the most prominent increase in the mRNA levels of SI and LPH genes, which were accompanied by a coordinate rise in the mRNA levels of two microvillar hexose transporters, i.e. SGLT1 and GLUT5. Nuclear run-on assays revealed that fructose, but not glucose, increased the transcription of SI, LPH and GLUT5. DNase I footprinting analysis of the rat LPH gene showed that the protected region conserved the same sequence as the *cis*-element (CE-LPH1) reported in the pig LPH gene. Electrophoretic mobility shift assay using CE-LPH1 and the related *cis*-element of SI gene (SIF1) revealed that nuclear extracts from the jejunum of rats fed the high-starch diet gave greater density of retarded bands than those of rats fed the low-starch diet. Force feeding a fructose diet gave rise to an increase in the binding of the dimeric nuclear protein (Cdx-2) to the SIF1 element. These results suggest that the *cis*-elements of CE-LPH1 and SIF1 might be involved in the carbohydrate-induced increases of the transcription of LPH and SI, presumably through a change in the expression and/or binding activity of Cdx-2.

Sucrase–isomaltase: Lactase–phlorizin hydrolase: Transcription: Dietary carbohydrates

In the small intestine, the expression of a group of genes which are associated with the digestion and absorption of nutrients are restricted within the absorptive cells, which represent major populations of small intestinal villus epithelium lining on the surface of the villi. The absorptive cells are derived from the undifferentiated crypt cells, and while migrating toward the tip of the villi, they exhibit abrupt increases in the expression of digestion/absorption-related genes around the villus–crypt junction area. Over a few days, the absorptive cells exhibit variations of the expression of many of these genes in response to dietary manipulations. This phenomenon represents the 'dietary adaptation'.

From the aspect of digestion and absorption of nutrients in the small intestine, nutrients can be divided into two categories according to their physical properties. Distinct from fat-soluble nutrients such as lipids and fat-soluble vitamins, which come relatively easily across the membranes, water-soluble nutrients including carbohydrates require specific machinery in the membranes of the luminal-side surface of the enterocytes to make it possible to transfer the nutrients against the lipid barrier of the plasma membrane into the cells. The machinery is composed of membrane digestive enzymes including sucrase–isomaltase (SI) and lactase–phlorizin hydrolase (LPH), and the transporters.

Abbreviations: SI, sucrase-isomaltase; LPH, lactase-phlorizin hydrolase; SGLT1, sodium-glucose cotransporter; GLUT5, glucose transporter isoform 5; CE-LPH1, *cis*-element of lactase-phlorizin hydrolase; SIF1, sucrase–isomaltase footprint 1.

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Coordinated changes in the expression of intestinal disaccharidase and hexose transporter genes

It has been known for many years that sucrase activity in the small intestine varies in response to dietary carbohydrate (Goda & Koldovský 1988), though the molecular mechanism is still unclear. In the early 1980s, an interesting phenomenon was observed that in adult rat jejunum, not only sucrase activity but also lactase activity varied in response to dietary carbohydrate (Yamada *et al.* 1981; Goda *et al.* 1983, 1985). Feeding a low-starch diet to rats caused a parallel decrease in sucrase and lactase activities (Goda *et al.* 1983), and upon the introduction of a high-starch diet, both sucrase and lactase activities were elevated (Yamada *et al.* 1981). It was apparent that this diet-induced change in sucrase and lactase activities was not evoked by the 'substrate' itself, because digestion of starch in the small intestinal lumen produces only substrates for α-glucosidases, e.g. maltase, sucrase and isomaltase, but does not produce the substrate (lactose) for lactase (a β-galactosidase). Furthermore, we also found that sucrose (a substrate for sucrase) was able to induce not only sucrase activity but also lactase activity, and inversely lactose (a substrate for lactase) was able to induce sucrase activity as well (Goda *et al.* 1985). Therefore, we hypothesized that a common regulatory mechanism should be present between these two disaccharidases, and that these coordinated regulations might be mediated by a common constituting monosaccharide or its intermediate metabolites.

Upon cloning the SI and LPH cDNAs, we demonstrated that the carbohydrate-induced changes in sucrase and lactase activities were caused by the accumulation of the respective mRNAs (Goda *et al.* 1995; Yasutake *et al.* 1995). We also found that dietary sucrose was able to evoke a rapid and parallel increase in SI mRNA and LPH mRNA levels, which was detectable within 3 h (Goda *et al.* 1999). To examine at which locus of villus cells along the villus–crypt axis this response to dietary carbohydrate occurs, we force-fed a sucrose-containing diet during the last 6 h to the rats which had been fed a low-carbohydrate diet (Goda *et al.* 1999). Cryostat sectioning of jejunal segments followed by RNA blot hybridizations of the transcripts revealed that, unlike SI mRNA which was expressed maximally in the lower villus, maximal LPH mRNA level was attained at the upper villus. Force-feeding the sucrose diet caused an abrupt increase in SI mRNA level in the lower villus within 3 h, while the rise in the LPH mRNA level occurred in the mid- and upper-villus (Goda *et al.* 1999). These results suggest that LPH gene is maximally expressed in more apical villus cells than SI gene, and that dietary sucrose elicits enhancement of the gene expressions in the villus cells which are accumulating the respective transcripts.

To explore putative signal molecules involved in the carbohydrate-mediated changes in the SI and LPH gene expression, we compared various monosaccharides by their ability to induce SI and LPH gene expression. Among the monosaccharides examined, fructose gave rise to the most prominent increases in the transcripts of SI (Kishi *et al.* 1999a) and LPH (Tanaka *et al.* 1998) genes, which were accompanied by a coordinate rise in the transcripts of Na/glucose cotransporter (SGLT1) (Kishi *et al.* 1999a) and the fructose transporter (GLUT5) genes (Tanaka *et al.* 1998). Force-feeding a glycerol-containing diet also caused an enhancement of the transcript levels of LPH, SI and SGLT1 (Tanaka *et al.* 1998; Kishi *et al.* 1999a). By contrast, feeding the diet containing glucose or α-methylglucoside (a non-metabolizable sugar) generally did not increase the transcript levels of SI, LPH or the intestinal hexose transporters (Tanaka *et al.* 1998; Kishi *et al.* 1999a). To examine whether fructose directly affects the gene expression of SI at the segment where the absorption of this sugar takes place, or the sugar-induced increase in the gene expression of SI is secondary to any possible changes in the levels(s) of certain hormonal factor(s) in the blood stream, a solution containing either fructose or glucose was simultaneously perfused into two consecutive cannulated and irrigated loops of jejunum that were not isolated from blood circulation. Compared with the loop perfused with glucose, the loop perfused with fructose exhibited significantly greater sucrase activity and SI mRNA levels as well as the elevated GLUT5 mRNA level (Kishi *et al.* 1999b). These results suggest that fructose is capable of directly increasing the gene expression of SI and GLUT5 in the confined segment where fructose is absorbed.

Nuclear run-on assays revealed that fructose, but not glucose, increased the transcription of SI, LPH and GLUT5 to a similar extent (Fig. 1). This result suggests that fructose (or a metabolite) is capable of increasing the mRNA levels of SI and LPH genes and that the transcriptional regulation might play a pivotal role in the carbohydrate-induced coordinate enhancement of the expression of SI and LPH genes, and presumably the hexose transporter genes as well. Thus, increasing evidence has supported the notion that transcriptional controls are involved in the dietary adaptation of the carbohydrate digestion/absorption-related genes. We hypothesized that some intermediate metabolites or factors derived from fructose metabolism might have evoked an increase in the transcriptional activity of a nuclear factor binding to the *cis*-regulatory elements on the

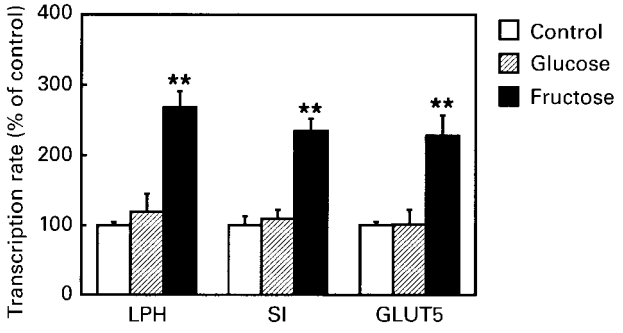

Fig. 1. Effects of force-feeding a low-carbohydrate diet (control), glucose diet and fructose diet for 12 h on the transcription rates of LPH, SI and GLUT5 genes in rat jejunum. Nuclear run-on assay was performed on the nuclei isolated from the jejunum of individual rats. Values are means ± SEM for four animals. The results for each sample normalized for 28S ribosomal RNA were expressed as arbitrary units, representing the mean value of the low-carbohydrate diet group as 100 %. **Denotes a significant difference compared with the control group at $P < 0{\cdot}01$.

SI and LPH genes. Therefore, in the next study, we cloned the 5′ flanking regions of rat LPH and SI genes, and we investigated whether the binding activity of putative common nuclear factors binding to the known *cis*-element was altered by the dietary manipulation.

Putative nuclear factors and *cis*-elements involved

Using DNase I footprinting analysis, we found several nuclear factor-binding regions within the 5′ flanking region of the rat LPH gene. One of the protected region conserving the same sequence as the *cis*-element (CE-LPH1) reported in the pig LPH gene (Troelsen *et al.* 1992) contains a common consensus sequence motif, TTTTAC, which was also seen in the *cis*-regulatory element termed SIF1 (Traber *et al.* 1992) in the mouse SI gene. Several recent studies have suggested that these *cis*-elements are the sites for the binding of certain types of homeodomain proteins. One such candidate is caudal-related nuclear protein called Cdx-2 (James & Kazenwadel, 1991). This homeodomain protein is known to be expressed only in the intestine (James & Kazenwadel, 1991; Suh *et al.* 1994), and it has been suggested that Cdx-2 plays a role in the tissue-specific expression of several intestine-specific genes including SI (Suh *et al.* 1994) and LPH (Troelsen *et al.* 1997). To examine the hypothesis that the carbohydrate-induced changes in the transcription of LPH and SI genes involve the alterations of the expression of Cdx-2, or the change in the binding activity of Cdx-2 to the *cis*-elements, we determined the Cdx-2 binding capability in the nuclear extracts from the jeunal mucosa. Electrophoretic mobility shift assay using CE-LPH1 and the related *cis*-element of SI gene (SIF1) revealed that nuclear extracts from rat jejunum gave rise to retarded bands which disappeared with excess amounts of non-labelled CE-LPH1 and SIF1 probes. The size of the DNA-protein complex was identical to that obtained by the *in vitro*-translated rat Cdx-2. In addition, the retarded bands were 'supershifted' by the inclusion of anti-rat Cdx-2 antibody (Tanaka *et al.* submitted). These results strongly suggest that Cdx-2 in the nuclei of rat jejunum is able to bind both CE-LPH1 and SIF1 elements. In subsequent electrophoretic mobility shift analysis, we found that the animals fed the high-starch diet exhibited greater amounts of Cdx-2 that bound to both the *cis*-element of LPH gene (CE-LPH1) and the *cis*-element of SI gene (SIF1) than the animals fed the low-starch diet (Tanaka *et al.* submitted). This suggests that dietary carbohydrate in general can modulate the binding activity of the homeodomain protein Cdx-2.

Dietary fructose induces modifications of the structure of a nuclear protein

Recently, we also found that a certain type of dietary carbohydrate (fructose) is able to modify the Cdx-2 binding characteristics to the *cis*-element of SI gene. Force feeding a fructose diet gave rise to an increase in the binding of the Cdx-2 dimer to the SIF1 element, which was previously reported to express greater transcriptional activity than the Cdx-2 monomer (Suh *et al.* 1994). The density of another band that represents the binding of Cdx-2 monomer was rather reduced by the fructose diet (Kishi *et al.* submitted). Therefore, it is likely that the abrupt increase in the transcription of SI gene that is induced by dietary fructose may involve a modulation of the structure of Cdx-2 leading to dimer formation. We recently observed that phosphorylation of the jejunal nuclear proteins by the action of protein kinase A caused a decrease in the fraction of Cdx-2 dimer, and inversely, dephosphorylation of the nuclear proteins by the action of protein phosphatase I produced a greater fraction of Cdx-2 dimer (Kishi *et al.* submitted). These results support the notion that the dimer formation of Cdx-2 is regulated by phosphorylation/dephosphorylation of the Cdx-2 protein.

Conclusion

Our results suggest that the *cis*-elements of CE-LPH1 and SIF1 might be involved in the carbohydrate-induced increases of the transcription of LPH and SI, presumably through a change in the expression and/or binding activity of Cdx-2 (Fig. 2). However, the route of signal transduction starting from fructose and leading to modifications of the nuclear factor remains to be elucidated. The evidence shown in this study tempts us to speculate that nutrient-induced increases in the gene expression in the small

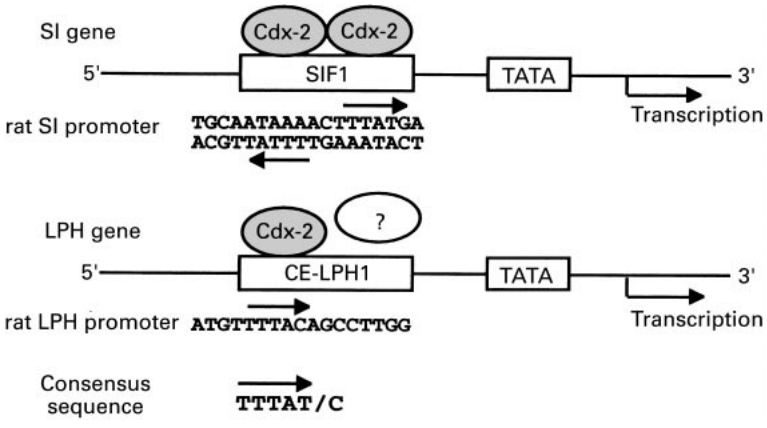

Fig. 2. A model of a coordinated regulation of sucrase–isomaltase (SI) and lactase–phlorizin hydrolase (LPH) genes through a homeodomain protein Cdx-2.

intestine occurs at a transcription level through the activation of nuclear receptors or nuclear transcription factors. The concept of 'nuclear receptor-mediated transcriptional control' was first established for steroid hormones, and subsequently adopted for the function of some nutrients including vitamin A and vitamin D. Further vigorous attempts are required to examine whether this concept is generally applicable to other nutrients including carbohydrates.

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