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Higher expression of jejunal LPH gene in rats fed the high-carbohydrate/low-fat diet compared with those fed the low-carbohydrate/high-fat diet is associated with in vitro binding of Cdx-2 in nuclear proteins to its promoter regions

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ABSTRACT

It has been previously demonstrated that the expression of lactase-phlorizin hydrolase (LPH) and sucrase-isomaltase (SI) genes are higher in rats fed a high-carbohydrate/low-fat (HCT) diet than in those fed a low-carbohydrate/high-fat (LCT) diet. In the present study, using a nuclear run-on assay we clearly show that higher expression of LPH and SI genes in jejunum of rats fed the HCT diet compared with those fed a LCT diet was regulated at the transcription levels. DNase I foot printing analysis of the 5′ flanking region of the rat LPH gene demonstrated that by incubating the jejunal nuclear extract the protected region was conserved as the same sequence as the homeodomain protein-binding element designated as CE-LPH1. UV-cross linking and electromobility shift assay in vitro clearly showed that Cdx-2 was including proteins bound to CE-LPH1. Moreover, in vitro binding of Cdx-2 to CE-LPH1 as well as SIF1, a *cis*-element identified as the binding element of Cdx-2 on the SI gene, in jejunal nuclear extracts of rats fed a HCT diet were greater than those fed a LCT diet. These results suggest that in vitro binding of Cdx-2 to CE-LPH1 as well as SIF1 in jejunal nuclear extracts is associated with the higher expression of the LPH and SI genes in rats fed the HCT diet compared with those fed a LCT diet.

Introduction

Lactase-phlorizin hydrolase (LPH) is expressed specifically in the absorptive cells in the mammalian small intestine. LPH is highly expressed at birth and then declines to a low level after weaning, presumably due to transcriptional regulation (Krasinski et al., 1994; Kuranuki et al., 2007). In adult rats, the mRNA levels of LPH (Goda et al., 1995), and sucrase-isomaltase (SI), an enzyme required for final digestion of starch and sucrose (Yasutake et al., 1995), are higher in rats fed a high-carbohydrate/low-fat (containing β-cornstarch as carbohydrate) diet (HCT diet) than in those fed a low-carbohydrate/high-fat diet (LCT diet). These data indicate that carbohydrate and fat are not only important as energy sources, but also for altering mRNA levels of disaccharidases such as LPH and SI genes. It has already been shown that a *cis*-element designated as CE-LPH1 on the promoter region, identified on the pig LPH gene, is important for LPH gene expression (Troelsen et al., 1992). The CE-LPH1 element has also been shown to be located on the LPH gene of the rat (Bosse et al., 2006; Kuranuki et al., 2007). The core sequence of CE-LPH1 was found to be quite similar to that of a *cis*-element of the SI gene designated as SIF1 (Traber et al., 1992). CE-LPH1 and SIF1 are located directly upstream to the TATA box, and have a consensus 5′-TTTAT/C sequence (Traber et al., 1992; Troelsen et al., 1992). Recently, there is much evidence showing that Cdx-2, a caudal homeobox protein which is important for intestinal gene expression and differentiation, directly interacts with CE-LPH1 on the human gene, as well as SIF-1 on human and rodents genes (James and Kazenwadel, 1991; Mallo et al., 1997). Additionally, cell studies in the human intestinal cell line, Caco-2, suggest that Cdx-2 may directly regulate the LPH gene (Mitchelmore et al., 2000; Troelsen et al., 1997) as well as that of SI (Krasinski et al., 2001). Moreover, using a chromatin immunoprecipitation (ChIP) assay we have recently demonstrated that in vivo Cdx-2 binding on the upstream of mouse SI gene was higher in mice fed a HCT diet than in those fed a LCT diet (Honma et al., 2007). However, it is unknown whether Cdx-2 is directly bound to rat CE-LPH1 or whether higher expression of the LPH gene in rats fed the HCT diet compared with those fed a LCT diet is regulated by Cdx-2.

In the present study, we have shown that higher expression of LPH and SI genes in rats fed the HCT diet compared with those fed a

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LCT diet was regulated at a transcriptional level. Furthermore, we found that in jejunal nuclear extracts, the in vitro binding of Cdx-2 to CE-LPH1, as well as to SIF1, was greater in rats fed a HCT diet than those fed a LCT diet.

Materials and methods

Animals

Six-week-old male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were maintained with a 12 h light/dark cycle, and were given free access to one of the isoenergic diets, i.e., either a low-carbohydrate/high-fat diet (LCT diet; 5% energy as β-cornstarch, 73% energy as corn oil) or a high-carbohydrate/low-fat diet (HCT diet; 70% energy as β-cornstarch, 7% energy as corn oil) for 7 days, as described in Table 1 (Goda et al., 1995). The rats were killed by decapitation between 10:00 am and 11:00 am. The experimental procedures used in this study met the guidelines of the animal use committee of the University of Shizuoka.

Preparation of intestinal samples

The entire small intestine was removed and the jejunoileum was divided into three segments of equal length. The proximal two-thirds of the jejunoileum was flushed twice with ice cold 0.9% NaCl solution containing 5 mM dithiothreitol. A 1.0 cm segment of the geometric center of the jejunum and an adjacent segment were excised and immediately used for RNA extraction and disaccharidase assays, respectively. The mucosa was scraped from the remaining with a glass slide and used for preparation of nuclei.

Enzyme assays

Lactase and sucrase activities were assayed as described by Dahlqvist (Dahlqvist, 1968), using 28 mM lactose and sucrose as substrates, respectively. The lactose substrate solution contained p-hydroxymercuribenzoate to inhibit lysosomal β-galactosidase (Koldovsky et al., 1969). Protein was measured according to the method of Lowry et al (Lowry et al., 1951).

RNA extraction and northern blot hybridization

Total RNA was extracted as described by Chomczynski and Sacchi (Chomczynski and Sacchi, 1987). Northern blot hybridization was performed using ^{32}P labeled rat LPH and SI cDNA probes as described previously (Goda et al., 1995; Yasutake et al., 1995). The radioactivity on the membrane was analyzed with an imaging analyzer (BAS 2000, Fuji Film, Tokyo, Japan). Control hybridizations were carried out using a rat 28S rRNA cDNA.

Table 1
Composition of diets

Ingredient	Diet			
	Low-carbohydrate/high-fat		High-carbohydrate/low-fat	
	wt. (g)	Energy (%)	wt. (g)	Energy (%)
Vitamin-free casein	15.9	20.8	15.7	20.9
Cornstarch(β-starch)	3.6	5.5	52.6	70.9
Corn oil	24.7	72.7	2.4	7.2
AIN^{93} mineral mix	2.8		2.8	
AIN^{93} vitamin mix	0.8		0.8	
L-cystine	0.24	1.0	0.24	1.0
Choline bitartrate	0.19		0.19	
2% Agar	51.8		25.3	

All values are given in %. Mineral mix and vitamin mix are based on AIN-93 (Oriental Yeast, Tokyo, Japan). Both diets are isoenergic (12.6 kJ/g).

Preparation of Cdx-2 antiserum

The full-length rat Cdx-2 cDNA (Gene bank ID: NP_076453.1) was subcloned into pET-15b vector (Novagen, CA, USA), and the expression construct was transformed into E. coli BL21 (DE3). The bacterially expressed Cdx-2 was purified by a Ni-NTA agarose column (Qiagen, Tokyo, Japan) according to the protocol of manufacturer. To produce anti-Cdx-2 polyclonal antiserum, New Zealand white rabbit was immunized with the rat Cdx-2 (100–200 μg) 5 times at 2 week intervals. Using an enzyme-linked immuno-sorbent assay (ELISA), we confirmed that the anti-serum for Cdx-2 at 10 weeks after immunity had a pronounced greater titer for purified Cdx-2 protein, while pre-immuno serum was not detected any signal for Cdx-2 protein.

Preparation of nuclei and nuclear extracts

Isolation of pure nuclei was performed according to the method described previously (Tanaka et al., 1998). The nuclei pellets were resuspended in 1 ml of nuclei storage buffer (40% glycerol, 50 mM Tris–HCl pH 8.0, 0.1 mM EDTA, 5 mM $MgCl_2$, and 0.1 mM PMSF), and immediately frozen in liquid nitrogen. The DNA contents of purified nuclei were determined by the method of Labarca and Piagen (Labarca and Paigen, 1980). The nuclei containing 10 μg DNA were suspended in suspension buffer (10 mM HEPES pH 7.9, 420 mM NaCl, 0.1 mM EDTA, 0.1 mM EGTA, 25% glycerol, 1 mM DTT, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin, and 1 μg/ml aprotinin), and the nuclei were incubated on ice with shaking for 30 min to extract the nuclear proteins. The lysed nuclei were ultracentrifuged at 180,000 g for 30 min at 4 °C. The protein concentrations in the nuclear extracts were determined by the method of Bradford (Bradford, 1976).

Nuclear run-on assays

The nuclear run-on assay was performed by the method previously described (Tanaka et al., 1998). The LPH cDNA probe used for the run-on assay was the same as used for Northern blot hybridization. The rat SI cDNA and r28S cDNA were used as previously reported (Tanaka et al., 1998; Yasutake et al., 1995).

DNase I foot print

LPH 5′ upstream was subcloned from rat liver genomic DNA by polymerase chain reaction using Taq/Pfu DNA polymerase (Expand Hi-Fi, Roche Molecular Biochemicals, Tokyo, Japan). The specific primers for the PCR were designed from the sequence of the rat 5′-flanking region (Verhave et al., 1995) as follows: 5′ oligo, 5′-G*CTCTAGA*CACAAGTTGAGGACCTCA (Xba I site italic) and 3′ oligo, 5′-GC*AAGCTT*GTTGAAGGAACTGTTAGA (Hind III site italic). The resulting 900 bp PCR product was subcloned into plasmid Bluescript II SK+ (Agilent, Yokohama, Tokyo) and sequenced by the deoxynucleotide termination method (Sanger et al., 1977). The plasmid was digested with Xba I and Hind III, and then further digested with Alu I. The produced fragment from −230 to +13 was extracted from the agarose gel and end-labeled by filling the 5′ overhang with [α-^{32}P] dCTP using a Klenow fragment. The standard reaction mixture consisted of the following components in a final volume of 100 μl: 20 mM Tris–Cl (pH7.9), 1 mM EDTA, 1 mM $MgCl_2$, 1 mM DTT, 10% glycerol, 10 μg/ml of poly (dI-dC), 15,000 cpm of the labeled probe, and 200–500 μg/ml of nuclear extracts. Following the incubation on ice for 15 min, the DNA probes were digested with DNase I for 2 min in the presence of 2.5 mM $MgCl_2$. The reaction was stopped by adding an equal volume of DNase I stop buffer (0.2 M NaCl, 40 mM EDTA, 1% SDS, 125 μg/ml tRNA, and 100 μg/ml proteinase K) and incubated for at least 15 min at 37 °C. Nucleic acid was extracted with phenol/chloroform and precipitated with ethanol, and then