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Regulation of ATP-Citrate Lyase Gene Transcription

**Kyung-Sup Kim, Jung-Goo Kang*, Young-Ah Moon
Sahng-Wook Park, and Yoon-Soo Kim**

It has been suggested that glucose metabolites and insulin are the most important factors inducing ATP-citrate lyase (ACL) by a high carbohydrate diet. We have used a primary culture of rat hepatocytes to confirm the role of glucose and insulin in terms of ACL gene expression. The results showed that glucose displayed a direct effect on ACL gene expression and the insulin helps the glucose effect. The nucleotide sequences from −512 to −485 of the ACL promoter are highly homologous (70%) to the sequences surrounding the carbohydrate response element (ChoRE) of the S14 gene. The gel retardation analysis using ChoRE of the S14 gene showed that the ACL promoter which contains the ChoRE-like sequence specifically inhibited the formation of the complex by the nuclear proteins isolated from rat liver. To localize the regions which are involved in the regulation of ACL gene expression, transient expression assay using ACL promoter-CAT (chloramphenicol acetyltransferase) constructs containing various lengths of a 5′ flanking region of the ACL gene were carried out. The proximal promoter region −419 to −1 containing several potential Sp1 binding sites showed the strong enhancing effect, which increases the transcription of CAT genes in the various cell lines, such as the CHO (Chinese hamster ovary) cell, the HepG2 cell, and primary cultured rat hepatocytes. In response to glucose, among the ACL promoter-CAT constructs, only pNP33-CAT (−1342 to −1) showed a 2.64 fold increase in CAT activity by a high concentration of glucose. The activation of ACL gene expression by glucose seems to be regulated in a complicated manner involving interactions between the contexts of the several sequence elements and various transacting factors, which is not a simple mechanism directed only by a short sequence element.

Key Words: ATP-citrate lyase, glucose, expression, primary hepatocyte culture, promoter, regulation

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Department of Biochemistry and Molecular Biology, The Institute of Genetic Science, Yonsei University College of Medicine, Seoul, Korea
*Department of Surgery, Yonsei University College of Medicine, Seoul, Korea
Address reprint requests to Dr. Y.S. Kim, Department of Biochemistry and Molecular Biology, Yonsei University College of Medicine, CPO Box 8044, 134 Shinchon-dong, Seodaemun-ku, Seoul 120-752, Korea. Fax: (82)-(2)-312-5041 Tel: (82)-(2)-361-5187 E-mail: yhahn@bubble.yonsei.ac.kr.

ATP-citrate lyase (EC 4.1.3.8, ACL) is a cytosolic enzyme which catalyzes the formation of acetyl CoA and oxaloacetate from citrate and CoA. The cytosolic acetyl CoA serves several important biosynthetic pathways, including lipogenesis and cholesterogenesis (Kornacker and Lowenstein, 1965). In mammals, the activity of ACL is regulated by diets and hormones in a manner similar to that of other lipogenic enzymes (Katsurada *et al.* 1989; Katsurada *et al.* 1990a; Katsurada *et al.* 1990b; Iritani *et al.* 1992; Kim *et al.* 1992a; Kim *et al.* 1992b). This regulation of enzyme activity according to the state of *de novo* lipo-

genesis is considered to be due to the alteration of the rate of enzyme biosynthesis. Previously we reported that these changes in enzyme synthesis were mediated by the alteration in the corresponding mRNA level. The early phase of mRNA increase was correlated to the increased transcription rate of the gene in nuclei (Kim *et al.* 1992a). These findings strongly suggest that ACL is regulated at the transcription level. To elucidate the mechanism of ACL gene regulation, we cloned the full ACL gene of the rat including the 5′ flanking region (−2370 to −1) (Kim *et al.* 1994; Moon *et al.* 1996) which can direct the expression of chloramphenicol acetyltransferase (CAT) gene fused to it in the CHO (Chinese hamster ovary) cells. The 5′ flanking region contains several consensus sequences defined as promoter elements. These include a CAAT box and Sp1 binding sites, but a TATA box lacks this promoter. This region also contains several sequence elements that may be involved in the transcriptional regulation of the gene.

It has been suggested that insulin and glucose metabolites are the important factors inducing ACL gene expression through a high-carbohydrate diet in the rat (Kim *et al.* 1992a; Park *et al.* 1994). However, *in vivo*, it was impossible to determine the factor that plays the major role in the induction of ACL by refeeding the rat a high carbohydrate diet. These factors lead us to use the primary culture of rat hepatocytes to confirm the roles of glucose and insulin independently. And to further define the molecular mechanism of regulation by carbohydrates, we tested the responsiveness of the 5′ flanking region of the ACL gene to glucose upon introduction into primary rat hepatocytes. Also, we carried out functional assays of the promoter using transient transfections with a reporter gene into various cell lines to analyze the molecular mechanism regulating the expression of ACL.

MATERIALS AND METHODS

Plasmid construction

The 5′ flanking region of the ACL gene was cloned into pGEM-4Z (Promega, Madison, WI, USA) and named pXH-GEM-4Z (−2370 to +123). Serial 5′ deletion subclones of the ACL promoter were made from pXH-GEM-4Z by being treated with exonuclease III. These clones were selected and confirmed by

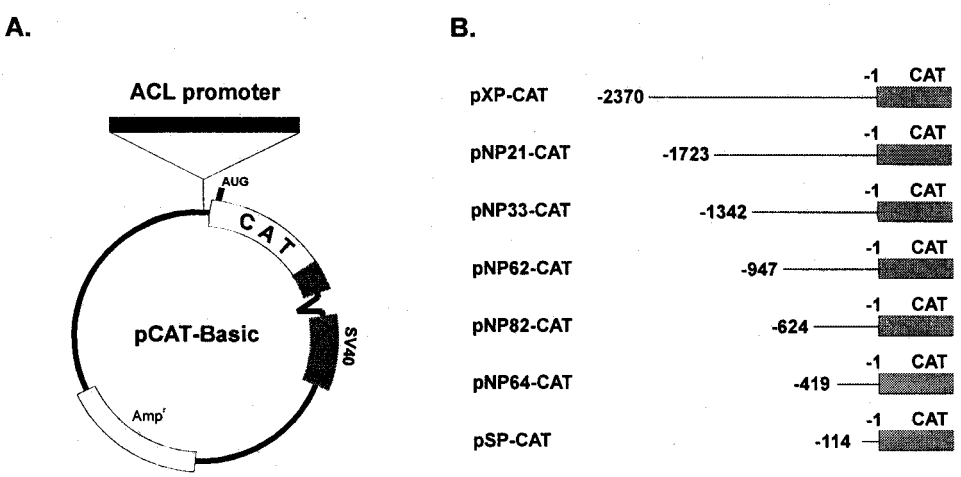

Fig. 1. *Schematic diagram of ACL promoter-CAT constructs. A: The 5′ flanking region of ATP-citrate lyase gene was inserted in front of the CAT (chloramphenicol acetyltransferase) gene of pCAT-Basic vector. B: Serially deleted ACL promoter-CAT constructs.*

restriction mapping and DNA sequencing. PstI fragments of pXH-GEM-4Z were separated and inserted into the PstI site of a pCAT-Basic vector, named pXP-CAT (−2370 to −1). Nested deletion fragments of the ACL promoter were also separated and inserted into a pCAT-Basic vector (Fig. 1). The ACL promoter-CAT plasmid constructs were sequenced from both directions using primers flanking the insertion site.

Nuclear extract preparation and gel mobility shift assay

Nuclear extracts were prepared from the livers of female Sprague Dawley rats weighing approximately 200 g according to Gorski *et al.* (1986). Both strands of carbohydrate response element (ChoRE) sequences of S14 gene (5′-AGCTTACTCTGTTTGCCAGTTCTCACGTGGTGGCCACGCGTA-3′, 5′-AGCTTACGCGTGGCCACCACGTGAGAACTGGCAAACAGAGTA-3′) reported by Shih and Towle (1994) were synthesized. Oligonucleotides were annealed to make double-strand DNA and then labeled by filling the protruding ends with 20 μCi of [α-^{32}P]dCTP (3000Ci/mmole) and Klenow fragments of DNA polymerase I of E. coli. A ^{32}P-labeled probe was purified with Sephadex G50 spun column. 3×10^4 cpm (about 0.5 ng) of probe and 5 μg of nuclear extract were used. DNA-protein binding reaction was performed in 20 μl volume of 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 1 mM DTT, 5% glycerol, and 2 μg poly(dI-dC) for 30 minutes in ice. The reactants were separated on a 4% polyacrylamide gel under 0.5X TBE for 2 hours at 200 V. Unlabeled double-strand ChoRE oligonucleotides of S14 or ACL promoter fragments (−625 to −373 or −420 to −115) were added by a 100 or 200 fold molar excess as competitors.

Primary hepatocytes culture

Hepatocytes from female rats (weighing approximately 200g) were isolated by the collagenase perfusion method as described in Seglen (1972) and cultured according to the method of Shih and Towle (1992). Freshly isolated hepatocytes were plated confluently and attached for 6 hours on 60 mm plates in 5 ml volume of modified Williams' E media (lacking glucose and methyllinoleate) supplemented with 10% fetal calf serum, 100 unit/ml penicillin, 100 μg/ml streptomycin, 0.1 unit/ml insulin, 10 nM dexamethasone and 5.5 mM glucose. After the attachment period, the media was replaced with the same media but lacks fetal calf serum.

Expression of ACL in primary hepatocytes

After a 5~6 hours attachment period of primary hepatocyte culture, the media was replaced with the media that lacks fetal calf serum and cells were incubated for 8~12 hours before changing to the experimental media. To detect the effect of glucose, modified Williams' E media supplemented with 0.1 unit/ml insulin, 10 nM dexamethasone and either 10 mM lactate, or 5, 25, 50 mM glucose were added to the media respectively. To detect the insulin effect, insulin was added at a concentration of 0.01 or 0.1 unit/ml to the media.

Western blot analysis

After 48 hours under the experimental media, hepatocytes were harvested and cell lysates were prepared in 100 μl of 25 mM Tris-HCl, pH 7.8, 100 mM NaCl, 1 mM DTT by three cycles of freezing and thawing. The protein concentrations were determined by the Bradford method (1976) and 10 μg of protein from each cell lysate was subjected to electrophoresis in SDS-7% polyacrylamide gel, and then blotted on the nitrocellulose membrane followed by immunostaining with anti-ACL serum from rabbit and anti-rabbit IgG horseradish peroxidase conjugated antibodies from a donkey (Amersham, Arlington Heights, IL, USA). ACL bands were detected by the ECL western blot system (Amersham, Arlington Heights, IL, USA). The membrane was exposed to Hyper film-ECL for 1 min.

Northern blot analysis

The changes in the amount of ACL mRNA in primary hepatocytes were analyzed by northern hybridization. Poly(A)$^+$ RNA was isolated from each plate using a Quick Prep

Micro mRNA purification kit (Pharmacia Biotech, Uppsala, Sweden) at a given time, 0, 6, 12, 24 and 48 hr respectively after changing the medium with the given components. Two μg of each RNA was separated in formaldehyde gel. The size fractionated RNAs were transferred onto a nylon membrane and hybridized with ^{32}P-labeled ACL cDNA pGACL1 (Kim *et al.* 1992a) prepared by a random priming method using rediprime DNA labeling system (Amersham, Arlington Heights, IL, USA) according to the manufacturer's instruction. An autoradiogram was obtained by exposing the membrane to an x-ray film for one day at -70℃ with an intensifying screen.

Cell culture and transfection

The CHO cell and the HepG2 cell were cultured in Ham's F-12 media and RPMI 1640 media respectively. The cells were plated at a number of 1×10^5 cells/60 mm plate. Cells were transfected with DNA using lipofectin (Gibco, BRL, Grand Island, NY, USA) according to the manufacturer's instruction in OPTI-MEM media 24 hr after plating. Rat primary hepatocytes were plated at a density of 3.5×10^6 cells/60mm plate in Williams' E media. After a 6 hr attachment period, transfection was performed as the same for CHO or HepG2 cells using Williams' E media instead of OPTI-MEM media. For the CHO cell, 10 μg of experimental ACL promoter-CAT plasmid and 20 μl of lipofectin were used. For HepG2 cells and hepatocytes, 10 μg of ACL promoter-CAT plasmid and 40 μl of lipofectin were used. One μg of pCMV-β-gal plasmid was cotransfected for standardization of transfection efficiency.

CAT assay

Cells were harvested 48 hr after transfection and cell lysates were prepared in 100 μl of 0.25 M Tris-HCl, pH 7.8 by three cycles of freezing and thawing. Using 30 μl of extract, β-galactosidase assay was done according to the method of Edlund *et al.* (1985). For the CHO cell and the HepG2 cell, a CAT assay was done by the modified diffusion method of Neumann *et al.* (1987) as previously described (Kim *et al.* 1994). For hepatocytes, CAT activity was assayed by thin-layer chromatography method of Gorman *et al.* (1982). To 50 μl of cell lysate, 50 μl of 1 M Tris-Cl, pH 7.8, 10 μl of [^{14}C]-chloramphenicol (60 mCi/mmole, 0.1 mCi/ml), 20 μl of 3.5 mg/ml acetyl CoA were added and incubated at 37℃ for 13 hrs. Reaction mixture was extracted with 1 ml of ethylacetate. After centrifugation, the upper organic phase was transferred to a new tube and vacuum dried. Redissolved samples were separated on TLC plates (Sigma, St. Louis, MO, USA) in chloroform : methanol (95:5, v/v) solution. The silica gels were autoradiographed on x-ray film for 24 hrs at room temperature. The acetylated chloramphenicol spots separated on the silica gel plates were extracted and radioactivities were counted by a scintillation counter (Beckman, Palo Alto, CA, USA).

RESULTS

Glucose is the key factor inducing ACL expression in hepatocytes

Insulin and glucose metabolites have been suggested as the major factors inducing ACL gene expression by a high-carbohydrate diet in the rat (Kim *et al.* 1992a; Park *et al.* 1994). However, *in vivo*, the various factors changed by refeeding, which may interfere with the elucidation of the exact role of glucose or insulin, should be considered. To eliminate other factors and to confirm the role of glucose and insulin on ACL gene expression, hepatocytes isolated from the rat were cultured under the medium containing various concentrations of glucose and insulin. ACL protein levels in hepatocytes under 25 mM, 50 mM glucose (high concentration) were increased when compared with those under 10 mM lactate and 5 mM glucose (low concentration) (Fig. 2A). High concentrations of glucose without insulin could increase the ACL protein level, although high concentrations of insulin (0.1 unit/ml) alone had no effect. However, 50 mM of glucose together with 0.1 unit/ml of insulin further increased the ACL protein level (Fig. 2B). Whether the increase in ACL protein

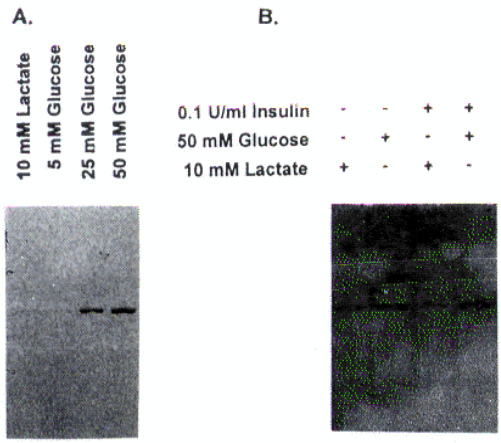

Fig. 2. *Western blot analysis of ACL expression from the primary hepatocyte culture. The hepatocytes isolated from rat were cultured under experimental media described in materials and methods. The cell lysates prepared from the hepatocytes were subjected to electrophoresis in SDS-7% polyacrylamide gel and then blotted onto nitrocellulose membrane, followed by immunostaining using anti ATP-citrate lyase serum. A: The changes of ATP-citrate lyase protein level in hepatocytes cultured in Williams' E media containing 0.1 unit/ml insulin and various concentrations of glucose indicated. B: The effects of glucose and/or insulin on the changes of ATP-citrate lyase protein level.*

level resulted from increase of the mRNA level, mRNA was isolated from the hepatocytes under each glucose concentration and northern blot analysis using ACL cDNA was performed. Under 5 mM glucose, the ACL mRNA level was not changed but under 25 mM, 50 mM glucose, the ACL mRNA level was increased from 6 hr and then its levels were maintained until 48 hr (Fig. 3A). ACL mRNA levels of hepatocytes treated for 12 hr with various concentrations of glucose were compared. In the hepatocytes treated with 25 mM and 50 mM glucose, ACL mRNA levels were highly increased (Fig. 3B). These results suggest that glucose (or its metabolites) may be the key factor which induce the ACL gene expression on transcription level by refeeding the rat with a high carbohydrate diet, and insulin may act as an amplifier.

ACL promoter region homologous to the ChoRE can inhibit the complex formation by ChoRE of S14 and nuclear protein

In some glucose responsive genes, namely L-pyruvate kinase (Liu and Towle, 1995), S14 (Shih and Towle, 1994), and the fatty acid synthase gene (Foufelle *et al.* 1995), the DNA elements (ChoRE; carbohydrate response element) which confer glucose responsiveness have been characterized (Fig. 4). The core sequence of ChoRE has been known as 'CACGTG'. Gel mobility shift assay on Fig. 5 shows that ChoRE of the S14 gene could form a DNA-protein complex with a rat liver nuclear extract and the formation of this complex was markedly inhibited by unlabeled ChoRE oligonucleotides. In the 5′ flanking region of the ACL gene, the sequences from −512 to −485 are highly homologous to the reported ChoRE sequence of the above genes, especially to the S14 gene (70% homology, Fig. 4). In order to examine whether the nuclear factor which binds to the ChoRE of S14 gene also binds to the ACL promoter, a competition assay was performed with the ACL gene fragment (−625 to −373) which contains the ChoRE-like sequence (−512 to −485). The DNA-protein complex formed by ChoRE was significantly diminished by the presence of the ACL promoter fragment from −625 to −373 in a dose dependent manner, but not by the ACL promoter fragment from −420 to −115 which is not related to the ChoRE (Fig. 5).

These suggest that the hepatic nuclear factor, which binds to the ChoRE of the S14 gene and then mediates the glucose responsiveness, could also bind to the −625 to −373 region of the ACL gene.

ACL promoter activity in primary hepatocytes culture in response to glucose

The ACL gene expression in the hepatocyte was regulated by glucose at the transcription level (Fig. 2, 3), and a hepatic nuclear factor which binds to the ChoRE of the S14 gene

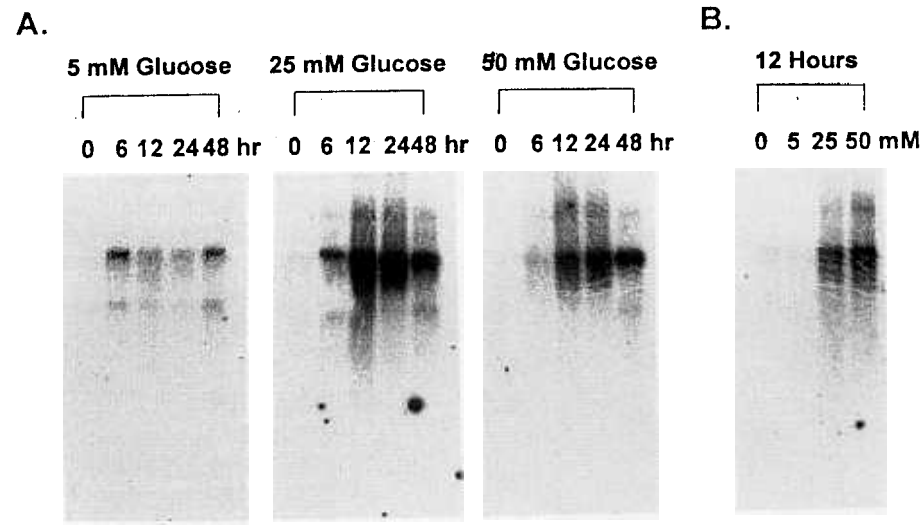

Fig. 3. Northern blot analysis of ACL expression from the primary hepatocyte culture. The hepatocytes isolated from rat were cultured under experimental media described in materials and methods. Poly(A)$^+$ RNAs were isolated from each primary hepatocytes, and subjected to electrophoresis in formaldehyde agarose gel. The size fractionated RNAs were transferred to nylon membrane and hybridized with ^{32}P-labeled ATP-citrate lyase cDNA. A: The changes of mRNA amount of ATP-citrate lyase in hepatocytes cultured under various concentration of glucose with time course. B: Differences of the ATP-citrate lyase mRNA expression under various concentrations of glucose at 12 hr.

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FAS        GGCCGCTGTCACGTGGGCGCC
S14    TCTGTTTGCCAGTTCTCACGTGGTGGCCAC
       .... .. .. ... ..... ... .
ACL    TCTGCTTTCCT.TTCACACGTG.GCGCCTC
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Fig. 4. Comparison of the nucleotide sequences of ChoRE. Nucleotide sequences from +283 to +303 of fatty acid synthase gene (FAS), from −1457 to −1428 of S14 gene and from −512 to −485 of ATP-citrate lyase gene (ACL) are shown. The core sequence of ChoRE are underlined. Dots represent the nucleotide sequence of ACL gene identical to S14 gene.

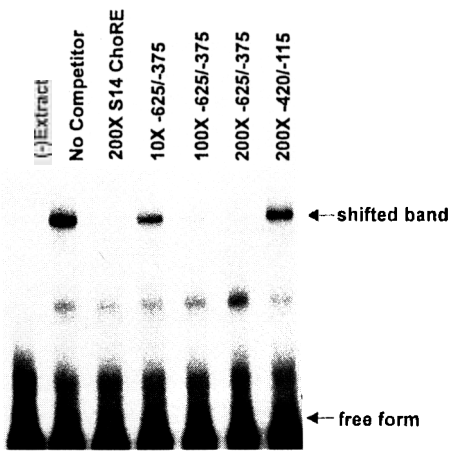

Fig. 5. Competition for the complex formation by hepatic nuclear protein and S14 ChoRE with ATP-citrate lyase promoter. 0.5 ng of ^{32}P-labeled double-strand DNA containing S14 ChoRE sequence was reacted with 5 μg of nuclear protein isolated from rat liver, and then electrophoresed in 4% polyacrylamide gel. The shifted bands were detected by autoradiography. The −625/−375 and −420/−115 used as competitors were corresponded to −625 to −375 and −420 to −115, respectively, of 5′ flanking region of ATP-citrate lyase.

could also bind to the ACL promoter (Fig. 5). Thus, we have attempted to localize the region which is involved in the regulation of ACL gene expression in response to carbohydrate by use of CAT assay. CAT constructs which contained various lengths of the 5′ flanking region of the ACL gene (Fig. 1) were introduced into hepatocytes. Following trans-

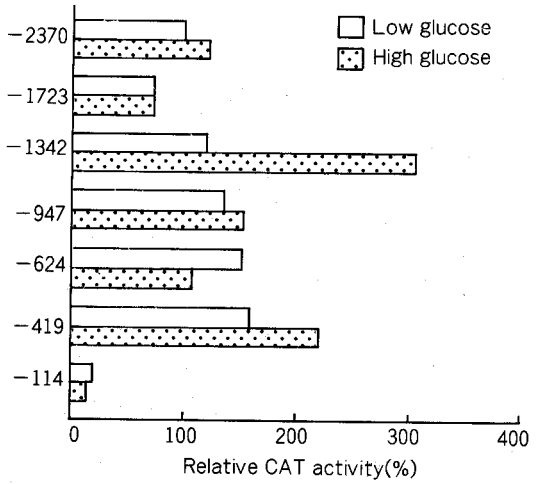

Fig. 6. *Regulation of ATP-citrate lyase promoter activity in primary hepatocytes by glucose. The ACL promoter-CAT constructs were transfected into primary hepatocytes. Cells were cultured in media containing 5.5 or 27.5 mM glucose for 48 hrs. CAT assay was performed by thin layer chromatography. The fast migrating acetylated chloramphenicol spots were extracted and their radioactivities were counted. Values are expressed relative CAT activity compared with pXH-CAT in low glucose media. Each value represents the average from triplicate plates.*

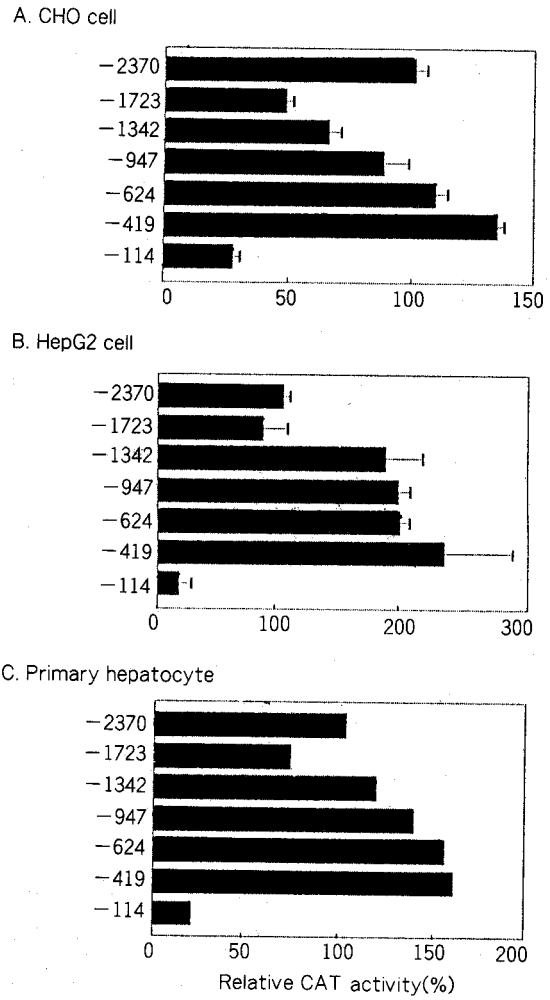

Fig. 7. *Transient expression of CAT directed by ATP-citrate lyase promoter in CHO cell (A), in HepG2 cell (B), and in primary hepatocyte under 5.5 mM glucose (C). All values are expressed relative CAT activity compared with value of pXH-CAT. Each value in panel A. and B. represents the average of three independent experiments from triplicate plates of cells, and each value in panel C represents the average from triplicate plates of cells.*

fection, cells were cultured in a media containing either 5.5 mM (low glucose concentration) or 27.5 mM (high glucose concentration) glucose in the presence of insulin (0.1 unit/ml). Most CAT constructs containing a ChoRE-like sequence of the ACL promoter did not activate the promoter by glucose except pNP33-CAT(−1342 to −1), which showed a 2.64 fold increase in CAT activity by high concentrations of glucose compared with the activity under low glucose concentration (Fig. 6).

ACL promoter activity in cell lines

To define the DNA elements which are required for general regulation of ACL gene transcription, ACL-promoter CAT constructs were introduced into the CHO cell, the HepG2 cell and hepatocytes in which we carried out transient expression assay. In all cells, pXH-CAT(−2370 to −1) displayed a high level of CAT activity. Deletion extending down to −1723 decreased the CAT activity but deletion extending down to −419 increased CAT

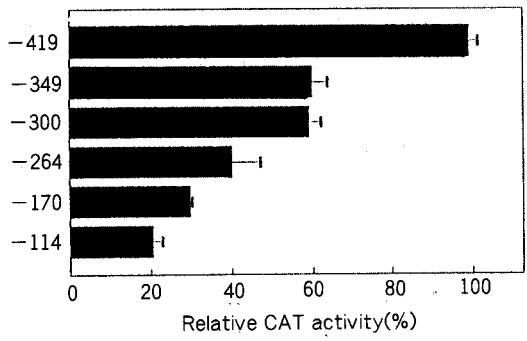

Fig. 8. *CAT activities in CHO cell transfected with ACL promoter-CAT constructs containing various lengths of ATP-citrate lyase promoter region from −419 to −1. All values are expressed relative CAT activity compared with the activity of pNP64-CAT (−419 to −1). Each value represents the average of three independent experiments from triplicate plates.*

activity gradually. pNP64-CAT(−419 to −1) induced the highest CAT activity and deletion to −114 (pSP-CAT) decreased CAT activity abruptly. pNP64-CAT(−419 to −1) may contain several elements which are important for ACL gene transcription (Fig. 7). We attempted to analyze the DNA elements within the region −419 to −1 in detail. As shown in Fig. 8, increasing the length from −114 to −300, CAT activities were increased. −300 to −349 had little effect but −349 to −419 can increase CAT activity.

DISCUSSION

In the liver or adipose tissue of rats, the activities of lipogenic enzymes are known to be regulated by diet or hormones (Katsurada *et al.* 1989; Fukuda *et al.* 1990; Katsurada *et al.* 1990a; Katsurada *et al.* 1990b; Iritani *et al.* 1992). The activities of enzymes are not only regulated by covalent bonding such as phosphorylation but also by the change of enzyme concentration. ACL is an enzyme of which activity is regulated by diet and various hormones (Kim *et al.* 1992a; Park *et al.* 1994). The concentration of enzymes was increased by 20 to 30 fold when the rats were subjected to fasting and then to refeeding. This increase of enzyme concentration was consistent with its RNA concentration and transcription rate.

In rat induced diabetes by streptozotocin treatment, insulin can increase the ACL mRNA level and ACL gene transcription rate in the nucleus (Park *et al.* 1994). Therefore, it has been suggested that insulin may be the important factor that induces the expression of ACL. However, in the experiments using cultured cells, insulin alone could not induce the expression of L-pyruvate kinase (one of the glycolytic enzyme) and fatty acid synthase (one of the lipogenic enzyme), but glucose alone could induce the expression of the above genes (Foufelle *et al.* 1992; Brun *et al.* 1993; Marie *et al.* 1993; Foufelle *et al.* 1994). Thus, it is also possible that the induction of ACL expression in the diabetic rat after insulin treatment was caused by the increase of glucose concentration in the cell by the insulin action. To confirm the key factor for the induction of lipogenic enzyme gene expression, only the cell culture experiment which can exclude other effects would be the choice. In the present experiment using primary hepatocyte culture, we observed that glucose alone can increase the ACL expression in both the protein and mRNA level, but insulin alone can not induce ACL expression but helps the glucose effect. Thus, it seems that the key factor for ACL induction after high carbohydrate diet is glucose, and it acts on the transcription level.

Recently, the sequence of carbohydrate response element(ChoRE) which can mediate the response to the glucose was reported in the S14 gene (−1448 to −1428) (Shih and Towle, 1994) and FAS gene (+283 to +303) (Foufelle *et al.* 1995). The ChoRE of the S14 gene can refer the glucose response to its promoter in the isolated hepatocyte. In the 5′ flanking region of the ACL gene, the consensus sequence of ChoRE was found in the region of −512 to -485 (Fig. 4). In the S14 or FAS gene, the ChoRE sequence was CACGTGNNNGCC but the sequence of the region −512 to −485 of ACL was CACGTGNNGCC. In the gel mobility shift

assay, the shifted band formed between certain nuclear factor-ChoRE of the S14 gene was specifically inhibited by the ACL gene fragment from −625 to −373 which contained a ChoRE-like sequence. This result suggests that the nuclear protein which binds to the ChoRE sequence of S14 can also bind to this region of ACL gene promoter and may mediate the transcriptional regulation by glucose. But the further studies may be needed to prove whether the ChoRE-like sequence in the ACL promoter is the real binding region.

However, in the analysis of CAT activity of the ACL promoter-CAT constructs changing glucose concentration, only the activity of pNP-33 CAT(−1342 to −1) was increased to 2.64 fold in 27.5 mM glucose concentration when compared with the activity in 5 mM glucose concentration. The CAT activities of other constructs pNP82-CAT(−624 to −1), pNP62-CAT(−947 to −1), pNP21-CAT(−1723 to −1), pXP-CAT(−2370 to −1), even though they contain a ChoRE-like sequence, were not increased when the glucose concentration was raised. Shih and Towle (1994) reported that a 21 base pair fragment between -1448 and -1428 was sufficient to mediate glucose induction. In the promoter of the S14 gene −4316 to +18, when -2111 to −290 region was deleted, except −1448 to −1428, or was replaced by +283 to +303 region of the FAS gene, the S14 gene promoter region could increase the CAT activity to 10 fold by a high concentration of glucose in the primary hepatocyte culture. However, the nuclear protein which could bind to ChoRE was not yet defined and their results imply that it may be possible that the sequence −4316 to −2112 or −291 to +18 was needed for responding to the high glucose level. Sudo *et al.* (1993) reported that ChoRE existed in the S14 gene promoter region from −1583 to −1069 but these sequences could not induce the response to glucose when inserted into the heterologous promoter, the thymidine kinase promoter, or ligated with the S14 promoter region −285 to +19, and that a thyroid hormone response element (TRE) expanding from −3261 to −2110 was needed for ChoRE function. Therefore, even though the consensus sequence of ChoRE was reported, ChoRE alone may not be sufficient for the induction of a response to a high glucose level, but interaction with another sequence together with ChoRE is needed. In the present study, only the pNP33−CAT(−1342 to −1) construct could respond to the high level of glucose among the several constructs containing the ChoRE − like sequence. The sequence from −1342 to −947 may be needed for ChoRE at −485 or another ChoRE may exist in the region from −1342 to −947. In the ACL promoter, at −1288, there is a TRE sequence even though its functional study has not been initiated. So, as it did in S14 gene, it is possible that the TRE sequence at −1288 in the ACL promoter has an important role for ChoRE to respond to glucose, but it cannot be explained why other constructs containing the region from −2370 to −1342 could not respond to glucose. It seems that the mechanism of induction of gene transcription by glucose is very complex and several *trans*−acting factors other than single consensus DNA element may be involved, and these *trans*−acting factors and their interactions should be researched.

The ACL gene is expressed in most tissues (Elshourbagy *et al.* 1990). In fact, this gene has a character of a housekeeping gene; that is, the TATA box was absent in the ACL promoter and it would be a reason that transcription of ACL is initiated over several bases. And four conserved Sp1 consensus sequences existed in the region from −310 to −30 (Kim *et al.* 1994). To compare the expression of CAT gene among ACL promoter-CAT constructs in the CHO cell, the HepG2 cell and primary culture of hepatocytes, a series of the constructs have been introduced into the cells and CAT activities have been assayed. The proximal region (−419 to −1) containing several potential Sp1 binding sites showed the strong enhancing effect, which increase the transcription of CAT gene in the various cell lines. The regions of −300 to −114 and −419 to −349 were considered to be involved in enhancing the expression. The region from −1723 to −419 decrease efficiency of the ACL promoter which suggests that there may be various silencer sequences. The

region from −2370 to −1723 contains the enhancer recovering the expression decreased by the extinguisher region (−1723 to −419). In all cell lines, −2370 to −1723 region could increase the CAT activities of −1723 to −1 construct and this effect was prominent in CHO cell. It would be needed to test whether the region from −2370 to −1723 has an simple enhancing effect to the ACL promoter activity or has an inhibitory effect to silencers located in −1723 to −419. The construct containing −114 to −1 region is thought to be a basic region where the CAAT box and a Sp1 binding site are, and −114 to −419 region is thought to be a region which induces the gene expression to a high level. ACL gene expression would be regulated by several *trans*-acting factors and *cis*-acting DNA elements. Further studies are obviously required to clarify these *trans*-acting factors and *cis*-acting DNA elements which regulate the ACL promoter activity under various conditions and their interactions on regulatory mechanism.

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