Expression of Low-Molecular-Weight Kininogen mRNA in Human Fibroblast WI38 Cells

Masaoki Takano, Junya Kondo, Katsutoshi Yayama and Hiroshi Okamoto

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Ikawadani-cho, Nishi-ku, Kobe 651-21, Japan

Received April 5, 1996 Accepted June 5, 1996

ABSTRACT—Expression of the low-molecular-weight kininogen (L-kininogen) mRNA in the human fibroblast cell line WI38 was examined by means of the reverse-transcription polymerase chain reaction and Southern blotting using human L-kininogen cDNA as a probe. The results demonstrated that WI38 fibroblasts expressed L-kininogen mRNA and that the expression was stimulated by 1 mM dibutyryl cAMP or 10 μM prostaglandin E2. Dexamethasone (1 μM) inhibited the stimulatory effect of prostaglandin E2. These results suggest that human fibroblasts supply L-kininogen, a protein precursor of the inflammatory mediator kinins, to connective tissues in response to inflammatory stimuli and that glucocorticoids may exert the antiinflammatory effect in part by inhibiting the local production of L-kininogen.

Keywords: Kininogen, Fibroblast, Prostaglandin E2

Kininogens are endogenous protein substrates for tissue and plasma kallikreins, which by proteolytic cleavage form vasoactive kinin peptides (1). In addition, kininogens contain domains that function as cysteine proteinase inhibitors and act as cofactors for the contact activation of blood coagulation (1). At least three forms have been identified in mammalian plasma: high-molecular-weight (H-kininogen), low-molecular-weight (L-kininogen) and T-kininogen (1). The latter is unique to the rat.

It was thought for many years that the source of kininogens was restricted to the liver. However, various other tissues and cells also express kininogen genes (2–4). We reported that mouse and rat fibroblasts synthesized kininogens in vitro in response to cAMP, prostaglandin E2 (PGE2) and inflammatory cytokines (interleukin-1 and tumor necrosis factor), implying an involvement of fibroblast-derived kininogen in the inflammatory responses (5, 6). However, it remained unknown whether or not human fibroblasts express the kininogen gene. In this study, we examined by means of the reverse-transcription polymerase chain reaction (RT-PCR) whether the human fibroblast cell line WI38 expresses the L-kininogen gene and, if so, whether it is regulated by cAMP or PGE2.

The human fibroblast cell line WI38 and hepatoma HepG2 were obtained from Riken Cell Bank (Tsukuba) and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamycin. At confluence, the total RNA was extracted with acid guanidinium thiocyanate-phenol-chloroform (7).

cDNA was synthesized in a total volume of 20 μl of reaction mixture containing 1 μg total RNA and 0.75 μM reverse primer using a GeneAmp RNA PCR Kit (Perkin Elmer, Norwalk, CT, USA). The mixture was incubated at 42°C for 15 min, and the reaction was terminated by heating at 99°C for 5 min, followed by quick chilling on ice. Target sequences were amplified in a 100-μl reaction mixture containing 0.5 μM forward primer and 2.5 U of Taq polymerase. Denaturing and extension proceeded at 95°C for 1 min and at 72°C for 3 min for both L-kininogen and β-actin cDNAs. The annealing conditions were 57°C for 2 min for L-kininogen cDNA or 55°C for 2 min for β-actin cDNA. There were 35 PCR cycles for L-kininogen cDNA and 18 for β-actin cDNA. The PCR products in 5-μl aliquots were resolved by electrophoresis on a 2% agarose gel and transferred onto nylon membranes. The membranes were prehybridized at 65°C for 30 min and then hybridized in the same buffer at 65°C for 18 hr with human L-kininogen cDNA and human β-actin cDNA as probes, which had been labelled with [32P]dCTP by random priming. The membranes were washed twice in 40 mM sodium phosphate buffer containing 1% sodium dodecyl sulfate and 1 mM ethylenediaminetetraacetic acid for 15 min and then analyzed by Fuji Film Bio Imaging Analyzer BAS 1000 (Fuji Film, Tokyo). Human
L-kininogen cDNA was a gift from Dr. I. Ohkubo, Shiga University of Medical Sciences, Ohtsu (8). The forward primer for human L-kininogen cDNA was 5'-AACGCT GAAGTTTATGTGGTA-3' (exon 9), corresponding to nucleotide positions 1054 to 1074 of L-kininogen cDNA. The reverse primer was 5'-CCGAGGGGAGAAGTAT AAAA-3' (exon 11), corresponding to 1398 to 1418 of L-kininogen cDNA. We predicted that the RT-PCR product for mature L-kininogen mRNA would consist of 365 base pairs. The forward and reverse primers used for human β-actin were 5'-ACCTTCAACACCCCACGGGACGGTACG-3' and 5'-CTGTACCCACATCTGCTGGAA GGTGGA-3', respectively, as reported (9).

Although an initial experiment was carried out to detect the transcript of the L-kininogen gene in W138 cells by Northern blotting using human L-kininogen cDNA as a probe, we could not detect it, probably due to the low levels of expression. We then examined RT-PCR amplification of L-kininogen mRNA followed by Southern blotting, a more sensitive method for the detection of mRNA expression. Total RNA extracted from W138 cells was reverse-transcribed and amplified using forward (exon 9) and reverse (exon 11) primers. Since exon 11 specifies the sequence unique to L-kininogen mRNA (1), it specifically anneals to L-kininogen cDNA but not to H-kininogen cDNA. The PCR products were Southern blotted using human L-kininogen cDNA as a probe to increase the sensitivity and specificity. As shown in a representative Southern blot (Fig. 1), a signal corresponding to the transcript from L-kininogen mRNA was detected in non-treated W138 fibroblasts, although it was extremely faint. However, the signal became intense in RT-PCR products of fibroblasts that were cultured in the presence of 1 mM dibutyryl cAMP or 10 μM PGE2 for 24 hr (Fig. 1), but not for 6 hr (data not shown). These results demonstrated that human fibroblast W138 cells express the L-kininogen gene and that the expression is enhanced by cAMP and PGE2. Since PGE2 potently increases the intracellular cAMP level in fibroblasts (10), it seems likely that the PGE2 effect on the expression of the kininogen gene is mediated by increased levels of intracellular cAMP.

The stimulatory effect of 10 μM PGE2 was inhibited by 1 μM dexamethasone (Fig. 1). The specific effects of glucocorticoids on one or more components of the hormone-sensitive adenylate cyclase signal transduction pathway have been identified. These include the modulation of cAMP accumulation, such as by affecting the number of receptors (11), the levels of guanine nucleotide binding regulatory protein (12), and adenylate cyclase activity (13). The PGE2-induced increase in T-kininogen mRNA levels in rat fibroblasts was completely inhibited by cycloheximide (1 μg/ml), suggesting that newly synthesized proteins are involved in the PGE2 effect (M. Takano and H. Okamoto, unpublished data). Mechanisms through which dexamethasone inhibits the PGE2 effect are unknown, but dexamethasone may alter the synthesis of proteins mediating the signal transduction of the PGE2 effect.

A signal corresponding to the size of the transcript from L-kininogen mRNA was easily detectable in human hepatoma HepG2 cells. In contrast to fibroblasts, neither dibutyryl cAMP nor PGE2 affected the expression of L-kininogen. Although an initial experiment was carried out to detect the transcript of the L-kininogen gene in W138 cells by Northern blotting using human L-kininogen cDNA as a probe, we could not detect it, probably due to the low levels of expression. We then examined RT-PCR amplification of L-kininogen mRNA followed by Southern blotting, a more sensitive method for the detection of mRNA expression. Total RNA extracted from W138 cells was reverse-transcribed and amplified using forward (exon 9) and reverse (exon 11) primers. Since exon 11 specifies the sequence unique to L-kininogen mRNA (1), it specifically anneals to L-kininogen cDNA but not to H-kininogen cDNA. The PCR products were Southern blotted using human L-kininogen cDNA as a probe to increase the sensitivity and specificity. As shown in a representative Southern blot (Fig. 1), a signal corresponding to the transcript from L-kininogen mRNA was detected in non-treated W138 fibroblasts, although it was extremely faint. However, the signal became intense in RT-PCR products of fibroblasts that were cultured in the presence of 1 mM dibutyryl cAMP or 10 μM PGE2 for 24 hr (Fig. 1), but not for 6 hr (data not shown). These results demonstrated that human fibroblast W138 cells express the L-kininogen gene and that the expression is enhanced by cAMP and PGE2. Since PGE2 potently increases the intracellular cAMP level in fibroblasts (10), it seems likely that the PGE2 effect on the expression of the kininogen gene is mediated by increased levels of intracellular cAMP.

The stimulatory effect of 10 μM PGE2 was inhibited by 1 μM dexamethasone (Fig. 1). The specific effects of glucocorticoids on one or more components of the hormone-sensitive adenylate cyclase signal transduction pathway have been identified. These include the modulation of cAMP accumulation, such as by affecting the number of receptors (11), the levels of guanine nucleotide binding regulatory protein (12), and adenylate cyclase activity (13). The PGE2-induced increase in T-kininogen mRNA levels in rat fibroblasts was completely inhibited by cycloheximide (1 μg/ml), suggesting that newly synthesized proteins are involved in the PGE2 effect (M. Takano and H. Okamoto, unpublished data). Mechanisms through which dexamethasone inhibits the PGE2 effect are unknown, but dexamethasone may alter the synthesis of proteins mediating the signal transduction of the PGE2 effect.

A signal corresponding to the size of the transcript from L-kininogen mRNA was easily detectable in human hepatoma HepG2 cells. In contrast to fibroblasts, neither dibutyryl cAMP nor PGE2 affected the expression of L-kininogen.
Kininogen mRNA in human hepatoma HepG2 cells (Fig. 1). The differential responsiveness of L-kininogen mRNA expression to dibutyryl cAMP or PGE2 in these cells suggests that the mechanisms underlying the regulation of kininogen gene expression differ between fibroblasts and hepatocytes, as seen in rats (6) and mice (5). The regulation of the gene expression between fibroblasts and hepatocytes is similarly distinct in terms of albumin and other liver-specific genes, which are extinguished in fibroblasts (14). A tissue-specific extinguisher encodes a regulatory subunit of protein kinase A, which suppresses the expression of cAMP-responsive genes in fibroblasts, but not in hepatocytes (15). A similar mechanism by which the cAMP-induced expression of the kininogen gene is suppressed in hepatocytes may be involved in the distinct responses to mediators between fibroblasts and hepatocytes. However, further studies will be necessary to resolve this mechanism.

Kininogens are kinin precursors and cysteine proteinase inhibitors (1). Kinins are important mediators of inflammation, producing vasodilation, increased vascular permeability, leukotaxis and pain, and these biological activities are potentiated by PGE2 (16). These lines of evidence and our finding that PGE2 regulates the expression of L-kininogen mRNA in human fibroblasts suggest the involvement of fibroblast-derived kininogen in the inflammatory responses of connective tissues.

Acknowledgments
We thank Dr. I. Ohkubo for the supply of human low-molecular-weight kininogen cDNA. This study was supported by The Science Research Promotion Fund from the Japan Private School Promotion Foundation.

REFERENCES
1 Muller-Esterl W, Iwanaga S and Nakanishi S: Kininogen revisited. Trends Biochem Sci 11, 336–339 (1986)
2 Mann EA and Lingrel JB: Developmental and tissue-specific expression of rat T-kininogen. Biochim Biophys Res Commun 174, 417–423 (1991)
3 Iwal N, Matsunaga M, Kita T, Tei M and Kawai C: Detection of low molecular kininogen messenger RNA in human kidney.