Characterization of the 5'-Flanking Region of the Gene for the \( \alpha \) Chain of Human Fibrinogen*

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(Received for publication, August 9, 1995, and in revised form, September 7, 1995)

The 5'-flanking region of the gene coding for the \( \alpha \) chain of human fibrinogen was isolated, sequenced, and characterized. The principal site of transcription initiation was determined by primer extension analysis and the RNase protection assay and shown to be at an adenine residue located 55 nucleotides upstream from the initiator methionine codon, or 13,399 nucleotides downstream from the polyadenylation site of the gene coding for the \( \gamma \) chain. Transient expression of constructs containing sequentially deleted 5'-flanking sequences of the \( \alpha \) chain gene fused to the chloramphenicol acetyltransferase reporter gene showed that the promoter was liver-specific and inducible by interleukin 6 (IL-6). The shortest DNA fragment with significant promoter activity and full response to IL-6 stimulation encompassed the region from –217 to +1 base pairs (bp). Although six potential IL-6 responsive sequences homologous to the type II IL-6 responsive element were present, a single sequence of CTGGGA localized from –122 to –127 bp was shown to be a functional element in IL-6 induction. A hepatocyte nuclear factor 1 (HNF-1) binding site, present from –47 to –59 bp, in combination with other upstream elements, was essential for liver-specific expression of the gene. A functional CCAAT/enhancer binding protein site (C/EBP, –134 to –142 bp) was also identified within 217 bp from the transcription initiation site. An additional positive element (–1393 to –1133 bp) and a negative element (–1133 to –749 bp) were also found in the upstream region of the \( \alpha \)-fibrinogen gene.

Fibrinogen is a plasma glycoprotein that participates in two crucial events in hemostasis. It is an adhesive protein essential for platelet aggregation, and it is converted from soluble fibrinogen to fibrin that polymerizes to form an insoluble fibrin clot at the final stage of the blood coagulation cascade (1). Fibrinogen is synthesized in hepatic parenchymal cells and is secreted into the blood as a heterodimer consisting of two \( \alpha \), two \( \beta \), and two \( \gamma \) polypeptides linked by 29 disulfide bonds. Recent studies have shown that high circulating levels of fibrinogen are associated with an increased risk of myocardial infarction and stroke, suggesting that fibrinogen can be an independent and important cardiovascular risk factor (2, 3). Therefore, studies on the regulation of fibrinogen synthesis may provide an explanation for the high expression levels in these individuals.

The three chains of human fibrinogen are encoded by three distinct single-copy genes, the sequence of which has been determined (4). They are closely linked in a region of approximately 50 kb of DNA located on chromosome 4q23-q32 (5). The three genes are arranged in the order of \( \gamma\alpha\beta \), with the gene for the \( \beta \) chain transcribed in the opposite direction. Studies in rats indicate that the expression of the three chains of fibrinogen is under coordinate regulation at the transcriptional level (6, 7). Recently, the constitutive expression of the human \( \beta \) chain has been shown to be mediated by hepatocyte nuclear factor 1 (HNF-1) (8-10). This transcription factor is also important in the expression of the rat \( \alpha \) and \( \beta \) chains (11).

As an acute-phase protein, the level of fibrinogen in circulation rises in response to trauma and inflammation. Massive defibrination and exposure to fibrinogen degradation products, fragments D and E, lead to a 4-7-fold increase in fibrinogen synthesis in the liver (12) that is apparently mediated by interleukin 6 (IL-6) (13). Response to IL-6 stimulation in the human fibrinogen \( \beta \) chain gene depends primarily on an IL-6 responsive element with the consensus sequence CTGGGA/AA. This element also occurs in the promoter regions of other type II acute-phase proteins, such as \( \alpha_2 \)-macroglobulin and T-kininogen (14, 15).

In order to further understand the regulation and coordinate expression of the three chains of human fibrinogen, it is essential to study the transcriptional regulation of the \( \alpha \) and \( \gamma \) chains in greater detail. In the present report, the sequence of the 5'-flanking region of the gene coding for the \( \alpha \) chain of human fibrinogen has been determined and several regulatory elements, including a HNF-1 site, an IL-6-responsive element, and a C/EBP binding site, have been identified within 200 bp of the transcriptional initiation site. An additional positive and a negative element were also identified in the upstream region of the 5'-flanking sequence.

MATERIALS AND METHODS

Cloning and Sequencing of the 5'-Flanking Region of the Gene for the \( \alpha \) Chain of Human Fibrinogen—The EcoRI fragments of the recombinant bacteriophage \( \lambda H1-x3 \) (16), which contains a long 3'-flanking sequence of the human \( \gamma \)-fibrinogen gene, were subcloned into the plasmid pUC18; the sequence of each of these subcloned fragments was determined on both strands. Based on these sequences, oligonucleotide primers for the polymerase chain reaction (PCR) were synthesized and used in PCR to determine the orientation and order of their arrangement in the recombinant plasmid.

A fragment which overlaps the human \( \alpha \) and \( \gamma \)-fibrinogen genes was obtained by PCR with primers from the extreme 3'-flanking sequence of the \( \gamma \) chain gene and exon 1 of the human \( \alpha \)-fibrinogen gene using human genomic DNA as templates. The primers were designed with flanking EcoRI recognition sequences, and the PCR product was cloned into the EcoRI site of pUC18. Recombinant plasmids containing the PCR product cloned in both directions were mapped by restriction endonuclease digestions and were unidirectionally deleted by the

* This work was supported by Grant HL16919 of the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4 The abbreviations used are: kb, kilobase(s); bp, base pair(s); HNF-1, hepatocyte nuclear factor 1; PCR, polymerase chain reaction; CAT, chloramphenicol acetyltransferase.
ExovII-mung bean nuclease method after cleavage with SphI and BamHI according to a protocol provided by Stratagene. The sequence was determined on both strands by the dideoxy chain termination method of Sanger et al. (17).

Primer Extension Analysis—The site of transcription initiation was determined by a primer extension method (18) using reverse transcriptase from avian myeloblastosis virus (Pramega). An oligonucleotide primer was designed with a sequence complementary to exon 3 of the α-fibrinogen gene (+86 to +109 bp) and was radiolabeled at the 5’ end with T4 polynucleotide kinase and [α-32P]ATP (3,000 Ci/mmol). The labeled primer was added to 50 μg or 25 μg of total RNA isolated from HepG2 or NIH3T3 cells in the presence of 50 mM Tris-HCl, pH 8.3, 50 mM KC1, 10 mM MgCl₂, 10 mM dithiothreitol, and 0.5 mM spermidine. The reaction mixtures were heated at 60 °C for 30 min and annealed at 30 °C for 2 h. Human recombinant IL-6 (Boehringer Mannheim) was added, and primer extension was performed at 42 °C for 30 min. The reactions were terminated and the extension products were precipitated by the addition of 3 volumes of cold ethanol. The extension products were analyzed on a DNA sequencing gel, and the size was determined by comparison with a DNA sequencing ladder.

CAT Reporter Construct and Mutagenesis—The plasmids pCAT-0 and pSV(β) were obtained from Promega and Clontech laboratories, respectively. The reference plasmid pSV2-CAT was provided by Dr. Carol Miao (19). Seven overlapping fragments spanning from –2975, –2281, –1875, –1621, –1393, –1133, and –749 bp to +109 bp of the 5’-flanking region of the α-fibrinogen gene generated by sequential deletion of the 5’-flanking sequence in pUC18 described above were directionally subcloned into the HindIII and XbaI sites of pCAT-0 with a EcoRI-XbaI adaptor linker. pCAT-217 and pCAT-73 were generated from pCAT-749 by the removal of a HindIII to BglII and a HindIII to Asc restriction fragment, respectively. Mutations in the 5’-flanking sequence were generated by the overlapping PCR method of Ho et al. (20). The mutated fragments were cloned directly into appropriate sites in pCAT-0, and the mutations were verified by DNA sequencing.

Cell Culture and Transfections—HepG2 and HeLa cells were cultured in minimum essential medium supplemented with l-glutamine, pyruvate, fetal bovine sera, antibiotics (penicillin, streptomycin, and neomycin), and fetal calf serum (10%). Rat fibroblasts, NIH3T3 cells, were cultured in Dulbecco’s modified Eagle’s medium supplemented with antibiotics and fetal calf serum (10%). Cells were incubated at 37 °C in a humidified chamber containing 5% CO₂. Cells were grown to about 60% confluence in 10-cm culture dishes and were transfected with a combination of plasmids by the calcium phosphate coprecipitation technique (21). The cells were exposed to a calcium phosphate-precipitated mixture of pCAT construct (15 μg) and a reference plasmid pSVβ (5 μg) that served as an internal control. The transfected cells were further cultured for 48 h, and expression levels of CAT and β-galactosidase were determined. In studies in which IL-6 was used, human recombinant IL-6 (Boehringer Mannheim) was added to the medium to a final concentration of 30 units/ml 24 h after transfection. Stimulation of HepG2 cell lines containing Cαβ and isofib β was performed by cotransfecting CAT reporter constructs with 5 μg of either the expression plasmid pMSV-C/EβP or pMSV-C/EβP, kindly provided by Dr. S. L. McKnight (22). All reporter gene studies were repeated three or more times, and the average result was presented.

CAT and β-Galactosidase Expression Levels—CAT expression levels were determined immunologically by an enzyme-linked immunosorbent assay kit from Boehringer Mannheim, and β-galactosidase activity was determined by the colorimetric method of Horwitz et al. (23). CAT expression levels were normalized to β-galactosidase activities to correct for differences in transfection efficiency and cell number.

Nuclear Extracts and Mobility Shift Assays—Nuclear extracts were prepared by the method of Shapiro et al. (24) as modified by Wengenka et al. (25). Nuclear extracts from HepG2 cells after IL-6 stimulation were prepared from cells that had been exposed to recombinant human IL-6 (30 units/ml) for 24 h. Doublet oligonucleotide des were labeled at the 3’ ends by fill-in synthesis with the Klöwen fragment of DNA polymerase I and [α-32P]CTP. The labeled probe (1 ng, 20,000 cpm) was incubated with nuclear extracts (4 μg of total protein) in a 20-μl reaction containing 10 mM Tris-HCl, pH 7.8, 50 mM NaCl, 5 mM MgCl₂, 1 mM EDTA, 10% glycerol, 0.5 mM dithiothreitol, and 1 μg of poly(d-I-dC) for 20 min at room temperature. The reaction mixtures were analyzed on a 5% acrylamide gel in a low ionic strength buffer (7 mM Tris, 0.1% Nonidet P-40, 50 mM NaCl) at 60 V. In competition experiments, excess unlabeled doublet oligonucleotide competitors were incubated with the nuclear extract simultaneously with the labeled probe.

**RESULTS**

5’-Flanking Sequence of the Gene for the α Chain of Human Fibrinogen—The sequence of a recombinant phage λHiγ13, which contains a portion of the gene for the γ chain of human fibrinogen and approximately 11 kb of the 3’-flanking sequence, was determined. Since there is no overlap between the phage sequence with the known sequence of exon 1 of the α-fibrinogen gene (4), two specific oligonucleotide primers were designed and polymerase chain reaction (PCR) was performed using human genomic DNA as a template to obtain the proximal 5’-flanking region of the gene for the α chain. A single PCR product approximately 3.1 kb in length was obtained, and this fragment was cloned into the EcoRI site of pUC18 and sequenced on both strands (Fig. 1). Overlap of the sequence with λHiγ13 and exon 1 of the α chain gene establishes the intergenic distance between the transcription initiation site of the gene for the fibrinogen α chain and the polyadenylation site of the gene for the γ chain to be 13,399 bp (Fig. 2). This intergenic distance is 3 kb longer than that reported previously (5). No identical sequence was found when a comparison was made with sequences in GenBank™ (version 85). In addition, no significant open reading frames were revealed suggesting that the intergenic sequence is unlikely to contain another gene. Seven Alu sequences which are 59% to 86% identical with the human Alu-Sq subfamily consensus sequence (26, 27) are present with two in the forward direction (−3899 to −3609 and −8290 to −7978 bp) and five in the reverse direction (−1,225 to −901, −7,090 to −6,720, −10,080 to −9,786, −11,508 to −11,162, and −12,717 to −12,416 bp). Sixty-eight percent identity was observed between rat and human 5’-flanking sequences extending for about 330 bp immediately upstream from the translation initiation sites (32). No significant homology was revealed when the 5’-flanking sequence of the gene for the α chain was compared with analogous regions in the genes for the β and γ chains.

Identification of Transcription Initiation Sites—The oligonucleotide primer extension technique was used to establish the transcription initiation site on the gene for the fibrinogen α chain. A synthetic oligonucleotide complementary to a region of the signal peptide (Trp² to Leu⁹) the end-labeled and hybridized to total RNA purified from HepG2 and NIH3T3 cells. Primer extension was carried out using avian myeloblastosis virus reverse transcriptase, and the extension products were analyzed on a sequencing gel (Fig. 3). The major extension product obtained with RNA from HepG2 cells was 109 nucleo-

**FIG. 1. Linkage of the human α and γ-fibrinogen genes.** The solid bars represent the structural genes. The arrows represent the direction of transcription. The shaded bars represent sequences contained in the recombinant λ phage and the PCR product.
Fig. 2. Intergenic sequence between the genes for the α and γ chains of human fibrinogen. Transcription initiation sites for the α chain gene determined by primer extension and RNase protection are underlined, and the major initiation site is designated as double-underlined. The upstream sequence is negatively numbered relative to the transcription initiation site. Sequence numbers in parentheses refer to the numbering previously used in the γ chain gene (4). Regulatory sequences established by reporter gene studies and mutation studies are double-underlined.
tides in length and suggested that the adenine residue located 55 bp upstream from the initiation methionine was the major transcription initiation site. Accordingly, this base was designated as 1 bp and preceded the 5′ end of the longest cDNA previously reported by 27 bases (28). Four minor initiation sites corresponding to 2, 22, 23, and 24 were also observed. Transcription initiation at these sites was further confirmed by RNase protection assays (data not shown). An alignment of the 5′-flanking sequences of the human and rat α chain genes shows also that transcription initiates essentially from an identical site with conserved sequences in both genes (31).

Liver-specific Expression of the Gene for Human Fibrinogen α Chain—

In order to assess the tissue specificity of the promoter for the α chain of human fibrinogen, transient transfections...
with a chloramphenicol acetyltransferase reporter gene (CAT reporter) were performed in human HepG2 cells, HeLa cells, and rat fibroblast NIH3T3 cells. In these studies, fusion constructs containing 217, 1393, 1875, and 2975 bp of the 5'-flanking sequence of the fibrinogen α chain gene obtained by sequential deletion were introduced in front of a promoterless CAT reporter gene in the vector pCAT-0. These fusion constructs gave consistent and significant expression levels of the CAT gene in HepG2 cells, but very low to undetectable levels in HeLa and NIH3T3 cells (Fig. 4). The level of expression in HepG2 cells was about 9% compared to that obtained with the SV40 promoter. The level of expression in HeLa cells and NIH3T3 cells were essentially identical with that obtained with the promoterless reference vector pCAT-0 and less than 1% of the SV40 promoter. These results are consistent with the interpretation that the 5'-flanking region of the gene for the α chain of human fibrinogen contains a liver-specific promoter.  

HNF-1 Binding Site—In order to locate regulatory elements in the 5'-flanking region of the gene for the α chain of human fibrinogen, additional deletions of the 5'-flanking sequences were introduced into the pCAT-0 vector, and functional activity in HepG2 cells was examined. The sequence up to 73 bp from the transcription initiation site (pCAT-73) was not sufficient to independently support transcription, while sequence extending to −217 bp (pCAT-217) showed significant CAT expression (9% of pSV2-CAT). However, a complete loss of CAT expression was observed in the construct pCAT-749,144, which contains a specific deletion of 144 bp from the −73 to −217 bp sequence (data not shown). These results indicated that the 144-bp sequence between −73 and −217 bp was necessary for transcription.  

Although the sequence up to −73 bp was unable to support transcription by itself, this region contains a sequence that is necessary for efficient transcription in combination with other upstream regulatory elements. A sequence homologous to the hepatocyte nuclear factor 1 (HNF-1) site was identified at −47 to −59 bp (GCCAATGATTAAAC). The lack of apparent independent promoter activity at this site may be attributable to an imperfect match with the palindromic consensus HNF-1 sequence. However, the importance of this sequence in the overall transcription process is shown by the construct pCAT-217 mHNF1 in which mutations in this sequence (−52ATTAAAC to AGGGAC) abolished transcription supported by sequences upstream of this region. These results confirm that the HNF-1 core-like sequence, together with sequences upstream of −73 bp are necessary for transcription of the α-fibrinogen gene.  

Binding of HNF-1 to the HNF-1 Core-like Sequence—Binding of nuclear proteins to the HNF-1 core-like sequence was assessed by the mobility shift assay. The duplex oligonucleotide probe contains the HNF-1 core-like sequence extending from −45 to −67 bp in the promoter of the fibrinogen α chain. The presence of protein-DNA complexes were detected by the appearance of bands with reduced electrophoretic mobility in a polyacrylamide gel. As shown in Fig. 5, two major complexes were formed with both nuclear extracts prepared from IL-6-stimulated and unstimulated HepG2 cells. The upper band was completely abolished by competition with a 10-fold excess of an unlabeled oligonucleotide with an HNF-1 consensus sequence of GTTAAATATTAAAC. Furthermore, this upper band became more intense upon mutagenesis of the sequence to a perfect palindromic HNF-1 consensus sequence (data not shown). These results suggest that HNF-1 binds specifically to the HNF-1 core-like element in the promoter of the human fibrinogen α chain. A second lower band probably resulted from nonspecific DNA-protein interactions since a 2-fold excess of a nonspecific competitor poly(dI-dC) essentially eliminated complex formation. These results are in agreement with the current understanding that the regulatory function of the HNF-1 site is mediated by interactions with liver-specific nuclear factors.  

IL-6-responsive Element—Since fibrinogen is an acute-phase protein regulated at the transcriptional level by IL-6, it was of interest to examine the effect of IL-6 on the reporter gene expression in HepG2 cells. This was of particular interest since six potential sequences identical to the IL-6 consensus sequence of CTGGGA/AA are located in the 5'-flanking region of the gene: they are located at −127 to −122, −228 to −223, −1100 to −1095, −1769 to −1764, −2231 to −2226, and −2439 to −2434 bp, respectively. IL-6 stimulation of HepG2 cells transfected with the various constructs (pCAT-217 to pCAT-2975) resulted in a 2-fold increase in the expression of CAT (Fig. 6A). Since the ratio of CAT expression with and without IL-6 stimulation remained relatively constant upstream from the −217 bp sequence, and no further increases were observed, it seemed likely that there is only a single active IL-6 responsive element, and this element is located from −127 to −122 bp in the 5'-flanking sequence. To further confirm this possibility, the CTGGGA sequence at −127 bp as well as a second CTGGGA sequence located at −228 bp were examined in greater
detail by specific mutations (Fig. 6B). Constructs in which the CTGGGA at −127 bp was converted to CTCTAG (pCAT-749m1) strongly reduced the CAT expression of pCAT-749 when stimulated with IL-6. This mutation also decreased the basal promoter activity of pCAT-749. The CTGGAA sequence at −228 bp, when mutated to CTCTAG (pCAT-749m2), did not significantly change the promoter activity relative to the control. These results suggest that the sequence at −228 bp, although identical with the consensus sequence, was not functional in the IL-6 response in the promoter of the α chain gene. CAT expression levels obtained with the double mutant pCAT-749m1m2 were similar to those of the single mutant pCAT-749m1. Confirming that the second sequence at −228 bp is apparently inactive in the α chain gene. In addition, as shown in Fig. 6A, the region from −749 to −1133 bp showed a strong negative element, while the sequence from −1133 to −1393 bp contained another positive element.

Sequence-specific interaction of nucleotides containing IL-6 responsive elements with nuclear proteins was studied by mobility shift assays. In these studies, labeled duplex oligonucleotides containing sequences around the functional IL-6 site (−127 bp), a nonfunctional site (−228 bp), and a mutant IL-6 site (−127m1) were incubated with nuclear proteins prepared from HepG2 cells with and without prior stimulation with IL-6. As shown in Fig. 7, all three oligonucleotides formed multiple complexes with HepG2 nuclear proteins. The difference in the pattern produced by a functional and a nonfunctional IL-6 responsive element is shown in the relative intensity of the bands rather than a distinct appearance or disappearance of bands. Thus, all three oligonucleotides formed complexes A and C. Competition with a core IL-6 consensus sequence (acute phase response element) reduced the abundance of complex A. In the case of the functional IL-6 sequence (−127 bp), this reduction was accompanied by an increase in complex C, whereas with the mutant IL-6 sequence (−127m1) and the nonfunctional sequence (−228 bp), no accompanying increase in complex C was observed. Similar studies were also performed with two additional nonfunctional IL-6 sequences from the fibrinogen α chain gene, the results are comparable to the nonfunctional site at −228 bp (data not shown), and only quantitative differences in the ratio of the complexes were observed. Nuclear proteins prepared from IL-6 stimulated HepG2 cells gave rise to an additional complex (Band B) with all three oligonucleotides. Formation of complex B was also abolished by competition with the core IL-6 consensus sequence. The formation of this complex is attributed to the presence of novel protein or a post-translationally modified protein in IL-6 stimulated HepG2 cells. These binding studies do not show readily the difference between a functional and a nonfunctional IL-6
site and suggest that sequences surrounding these sites may have a contribution.

C/EBP Site—In other reporter gene and mutation studies, a site for the CCAAT/enhancer binding protein (C/EBP) has also been identified in the 5'-flanking region of the α-fibrinogen gene. The C/EBP site (−134 to −142 bp) is located immediately upstream of the functional IL-6 responsive element. A mutation in the C/EBP sequence (pCAT-217mC/EBP) reduced not only basal expression, but abolished completely the response to IL-6 (Fig. 8A). Furthermore, cotransfection of CAT reporter constructs with cDNAs expressing either the b or d isoforms of rat C/EBP increased the expression of CAT reporter gene approximately 3.5-fold and 2.5-fold respectively (Fig. 8B). These responses were abolished by mutations in the C/EBP site as shown by the construct pCAT-217mc/EBP. These studies show that the C/EBP site in the α-fibrinogen gene is functional, and increased abundance of C/EBP isoforms in HepG2 cells led to a corresponding increase in CAT expression. The close proximity of the C/EBP site to the IL-6 responsive element suggests that C/EBP isoforms may directly modulate the IL-6 response by binding to a nearby site and interacting with factors bound to the IL-6 site.

DISCUSSION

Studies on the assembly of fibrinogen showed that only fully assembled six-chain fibrinogen molecules were secreted (29–31) and in HepG2 cells the β chain is present in limiting amounts. However, disproportionate β chain synthesis may be attributable to, in part, variations in the origin of cell lines and culture conditions in vitro. Preliminary studies with another human hepatoma cell line, HuH7, showed that the α chain and not the β chain is limiting.2 Therefore, studies on the regulation of the α- and γ-fibrinogen genes are equally important, particularly in an acute-phase response, in which the increase in total fibrinogen synthesis far exceeds the amount that a compensatory increase in β chain synthesis alone can account for. A concerted increase in the synthesis of all three chains is necessary to account for the observed increase. To better understand the regulation of fibrinogen synthesis, we have focused on the α chain gene in the present studies.

The intergenic sequence between the genes for the α and γ chain of human fibrinogen present in the phage λH1γ13 is

\[\text{CAT expression by reporter constructs.} \, \text{pCAT-217mc/EBP contains a mutation of } {\text{ACCTAGCAA}}^{134} \text{ to } {\text{ACCTAGCAA}}^{142} \text{ in the C/EBP site of construct pCAT-217. A, effect of mutation on IL-6 stimulation. B, effect of co-expression of C/EBPβ and C/EBPδ isoforms. Control, cotransfection with plasmid Bluescript. Uninduced pCAT-217 expression levels were used as reference (100%).} \]
incomplete since there is no sequence overlap between its 3' end and exon 1 of the \( \alpha \) chain gene. The polymerase chain reaction was used to obtain the overlap between these two genes and establish the intergenic distance to be 13,399 bp, about 3 kb longer than previously reported (5). Furthermore, as shown in reporter gene studies, this newly isolated 3-kb fragment contains elements that are functionally important in the transcriptional regulation of the human \( \alpha \)-fibrinogen gene.

Significant sequence identity (68%) was observed between the 5'-flanking region of the human and rat \( \alpha \) chain gene extending to about 221 bp upstream from the transcription initiation site. Sequence conservation suggests that this region may contain functionally conserved regulatory elements. The transcription initiation sites determined by primer extension analyses and RNase protection assays showed one major and several minor sites. Transcription initiation appears to occur at conserved sites in the rat and human \( \alpha \)-fibrinogen genes. Both transcription initiation sites are associated with a potential upstream TATA-like sequence (\(-31\text{TATAA}-27\)). There are potential CCAAT-like sequences in the promoters of the human \( \alpha \), \( \beta \), and \( \gamma \) chain genes, but the CCAAT sequence from \(-58\) to \(-54\) bp in the \( \alpha \) chain gene is not likely to be functional since it overlaps with the HNF-1 binding site and is not conserved in the rat \( \alpha \) chain gene.

No significant homology for at least 3 kb was observed in the 5'-flanking sequences of the three human fibrinogen genes and the three genes appear to be regulated independently. However, several potential common cis-acting elements were noted in the promoters for the human \( \alpha \) and \( \beta \) chain genes. Both genes contain HNF-1, IL-6, and C/EBP sites that apparently contribute to the overall transcription of the two genes. The HNF-1 binding sites in the promoters of the human \( \alpha \) and \( \beta \) chain genes appear to mediate constitutive liver-specific expression to differing extents (8, 33). The functional importance of the HNF-1 sequence in the human \( \alpha \) chain promoter is further confirmed by mutation studies.

Fibrinogen is up-regulated by IL-6 during the acute-phase response. The expression level of the human fibrinogen \( \alpha \) chain was stimulated 2- to 3-fold in HepG2 cells by IL-6, which is comparable in magnitude to the stimulation observed in vivo (13). This is in contrast to the 5- to 10-fold induction reported for the IL-6-responsive element in the human \( \beta \) chain gene (10). Our present studies on six potential IL-6 responsive sequences in the \( \alpha \)-fibrinogen gene show that sequences adjacent to the consensus sequence, e.g., the C/EBP site, may determine which sequence would in fact be functional and the magnitude of the response. This interpretation is in agreement with observations on the IL-6 responsive element in the human \( \beta \) chain gene that an adjacent C/EBP binding site may be modulating and further increasing the magnitude of the IL-6 response (8, 36). Mobility shift assays show that multiple protein-DNA complexes are formed by the IL-6 responsive sequence with nuclear proteins suggesting the involvement of several proteins. One protein that binds to the IL-6 consensus sequence, designated as the acute phase response factor, also known as Stat-3, has been cloned and was found to be ubiquitously expressed (34, 37). However, Stat-3 does not bind to the IL-6 consensus sequence of the rat \( \alpha \)-fibrinogen gene, suggesting that the IL-6 response may involve a novel transcription factor (38).

In preliminary studies, dexamethasone elicited a small but reproducible increase in CAT reporter gene expression in HepG2 cells from the first 217 bp of the 5'-flanking sequence of the \( \alpha \)-fibrinogen gene (data not shown). However, this region does not contain sequences homologous to a typical glucocorticoid responsive element, and HepG2 cells have been shown to contain limiting amounts of glucocorticoid receptor (35). Further studies are necessary to define the effect and mechanism of dexamethasone regulation of the \( \alpha \)-fibrinogen gene expression.

Since the level of circulating fibrinogen that correlates with a high risk of myocardial infarction and stroke does not exceed the normal range by a 2-fold increase, subtle functional differences in promoters and enhancers in each of the three fibrinogen genes may be crucial in determining the level of fibrinogen in circulation. These considerations further emphasize the necessity for detailed characterization of the regulatory elements in each of the three fibrinogen genes.

Acknowledgments—We thank Jun Mizuguchi and Carol H. Miao for numerous helpful discussions.

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