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DIRECT EVIDENCE OF TRANSCRIPTIONAL CONTROL OF FIBRINOGEN AND ALBUMIN SYNTHESIS IN RAT LIVER DURING THE ACUTE PHASE RESPONSE.

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Summary
Using immunoprecipitation technique we have purified fibrinogen polypeptide mRNAs to homogeneity as demonstrated by translation in a wheat germ cell free system and by hybridization kinetics. By measuring the sequence contents of fibrinogen polypeptide mRNAs and albumin mRNA, using tritiated DNAs complementary to fibrinogen polypeptide mRNAs and to albumin mRNA respectively, a dramatical increase in mRNA content of fibrinogen polypeptides (7 fold) and a decrease in albumin mRNA content (2 fold) have been found in the liver of rats 24 hours after i.m. injection of 1.0 ml turpentine. These results were consistent with the findings in cell free translation under the direction of poly A^+RNA prepared from livers of experimental animals and suggest that the fibrinogen and albumin synthesis during the acute phase response is reciprocally regulated at the transcriptional level.

Introduction

Fibrinogen is a plasma glycoprotein which consists of a dimeric molecule of three non-identical polypeptides, namely 2 Aα, 2 Bβ, and 2 γ chains. In rat fibrinogen the three chains have molecular weights of 61,000, 58,000 and 51,000 respectively (1,2). Based on studies using indirect methods, it has been shown that fibrinogen polypeptide chains are synthesized separately under the direction of three different messenger RNAs and is not first synthesized as a single large protein which is then processed into its three component chains (3,4). Albumin is a single polypeptide chain and has a molecular weight of 68,000. During the acute phase (inflammatory) response, it has been documented that the plasma concentration of fibrinogen increases as a result of enhanced liver synthesis (4-8), and the albumin concentration decreases. The decreased plasma concentration of albumin has been ascribed to the increased degradation rate while little is known about albumin production during the acute inflammatory reaction (9-11). Although a substantial amount of informations has been compiled concerning biochemical features, plasma concentration and the synthetic rate under physiological and pathological circumstances (8,12), little is known of the molecular regulatory

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mechanism of fibrinogen and albumin biosynthesis in the liver.
Recently, using immunoprecipitation technique we have isolated rat liver polyribosomes synthesizing fibrinogen polypeptides. Messenger RNAs of these polypeptides have been purified from these polyribosomes to homogeneity as demonstrated by a wheat germ translation system and by hybridization kinetic analysis. By measuring the sequence contents of fibrinogen polypeptide mRNAs and albumin mRNA, using radiolabelled complementary DNAs specific for fibrinogen polypeptide mRNAs and for albumin mRNA respectively, we have found a dramatic increase of mRNA content of fibrinogen polypeptides in the liver of rats 24 hours after treatment with turpentine. In contrast, the albumin mRNA content decreases. These findings suggest that fibrinogen and albumin synthesis during the acute phase response is reciprocally regulated at the transcriptional level.

Materials and methods

Male Spraque Dawley rats weighing 300-350 grams were used throughout and were maintained on standard Purina Chow and water ad libitum. Acute inflammatory response was induced by a 1.0 ml intramuscular injection of commercial turpentine.
Purified rat fibrinogen was prepared as previously described (1). The goat antibodies to rat fibrinogen polypeptides were made RNAase free by a CM-52 and DEAE-52 column chromatography as previously described (13).
Purification of mRNAs of fibrinogen polypeptides was performed using indirect immunoprecipitation of liver polyribosomes as reported previously for isolation of albumin mRNA (14,15). Hybridization kinetics of poly A^+ RNA prepared from immunoprecipitated polyribosomes and translation of this RNA fraction in a wheat germ cell free system show that the purified RNAs represent mRNAs of fibrinogen polypeptides (see results).
Analytical RNA-cDNA hybridization was performed according to the method of Housman et al as previously described (14). Tritium labelled cell free translation products were examined on sodium dodecyl sulfate (SDS)- polyacrylamide gel electrophoresis as described by Laemmli (16). Autoradiographs were obtained by exposure to X-ray film after treatment of the gels as described by Bonner et al (17).

Results and discussion

By immunoprecipitation technique using antibodies against rat plasma fibrinogen isolated as previously reported (1) we have purified mRNAs of fibrinogen polypeptides. Fig. 1 shows the SDS-polyacrylamide gel electrophoresis of cell-free reaction products under direction of poly A containing RNAs prepared from immunoprecipitated polyribosomes (slot 2). As can be seen, the isolated RNAs had been purified to homogeneity and represent mRNAs of fibrinogen polypeptides. The purified plasma fibrinogen and the three polypeptide chains obtained after reduction of fibrinogen with 2-mercaptoethanol are illustrated in figure 2. There is a difference in banding pattern of the fibrinogen polypeptides on the electrophoresis according to Weber and Osborn (18) and on the SDS-polyacrylamide

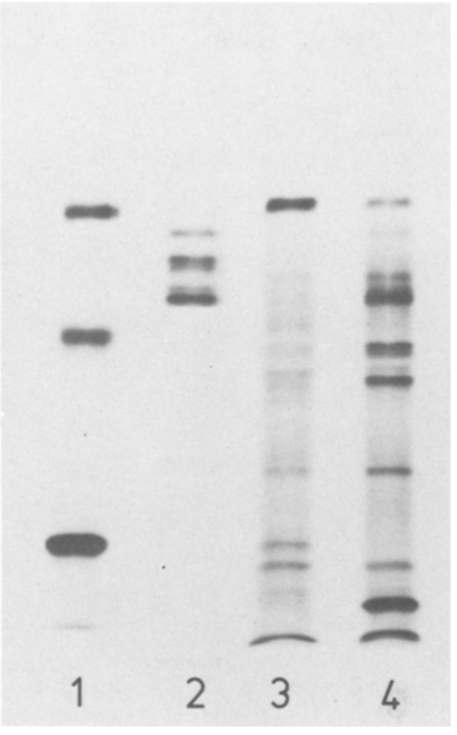

Figure 1. Autoradiograph of SDS-polyacrylamide gel electrphoresis of cell free (wheat germ) translation products under direction of various poly A containing liver RNA.
Slot 1 : marker proteins : albumin (Mt 68,000), ovalbumin (Mt 45,000), chymotrypsin (Mt 25,000).
Slot 2 : cell free translation products directed by poly A containing RNA isolated from immunoprecipitated polyribosomes using antibodies against fibrinogen polypeptides (fibrinogen polypeptide mRNAs).
Slot 3 : cell free translation products under direction of poly A containing RNA from livers of control rats.
Slot 4 : cell free translation products directed by poly A containing RNA from livers of rats, 24 hours after turpentine treatment.

slab gel electrophoresis according to Laemmli (16). Although two different rat fibrinogen γ chains have been reported recently by Franks et al (19), we have now clearly demonstrated that there are also two different Bβ chains present in rat plasma fibrinogen using SDS-polyacrylamide gel electrophoresis according to Laemmli. Since the plasma fibrinogen polypeptide Bβ and γ chains are almost completely glycosylated (1), these differences in banding patterns can not be the result of differences in glycosylic acid content. On the gel electrophoresis according to Weber and Osborn, these two Bβ and γchains were not observed (fig. 2) The cell free reaction products under the direction of poly A containing RNA prepared from immunoprecipitated polyribosomes (fig. 1, slot 2) show also identically two different preBβ and two pre γchains. The possibility of a

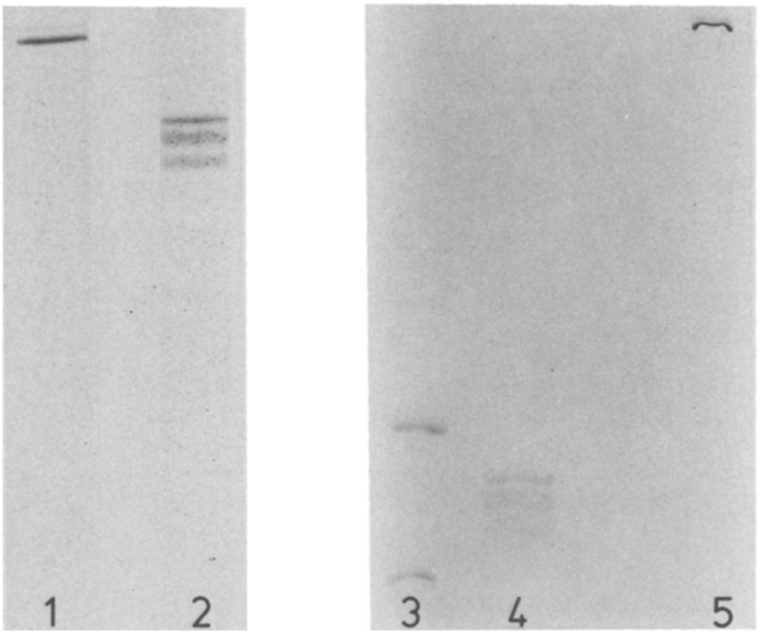

Figure 2. SDS-polyacrylamide gel electrophoresis of fibrinogen and fibrinogen polypeptides according to Weber and Osborn (left, slot 1 and 2) and according to Laemmli (right, slot 3-5).
Slot 1 : non reduced rat fibrinogen on SDS-polyacrylamide (5%) gel electrophoresis according to Weber and Osborn (18).
Slot 2 : reduced rat fibrinogen.
Slot 3 : marker proteins : -galactosidase (Mt 130,000), phosphorylase -b (Mt 92,000), bovine serum albumin (Mt 68,000), ovalbumin (Mt 45,000).
Slot 4 : reduced rat fibrinogen on SDS-polyacrylamide (7%) gel electrophoresis according to Laemmli (16).
Slot 5 : non reduced rat fibrinogen.

posttranslational modification for these differences in electrophoretic mobilities is therefore not very likely. Although the fibrinogen polypeptides are the only translation products observed in the cell free system under the direction of this RNA fraction, the intensities on autoradiograph are not equally distributed between these chains. This finding may be due to the different amount of individual mRNAs present in this RNA fraction, as a result of immunoprecipitation of polyribosomes or due to a differential translation activity of these mRNAs in wheat germ cell free system. Since the individual fibrinogen polypeptides contain approximately an equal amount of leucine (6,8%; 1) the obtained result is not due to the differences in ^{3}H leucine incorporation as a consequence of different leucine contents.
The sequence complexity analysis of the purified RNA fraction with the complementary DNAs transcribed from this RNA fraction is given in figure 3A. Using this cDNA probe, the purification step of fibrinogen polypeptides synthesizing

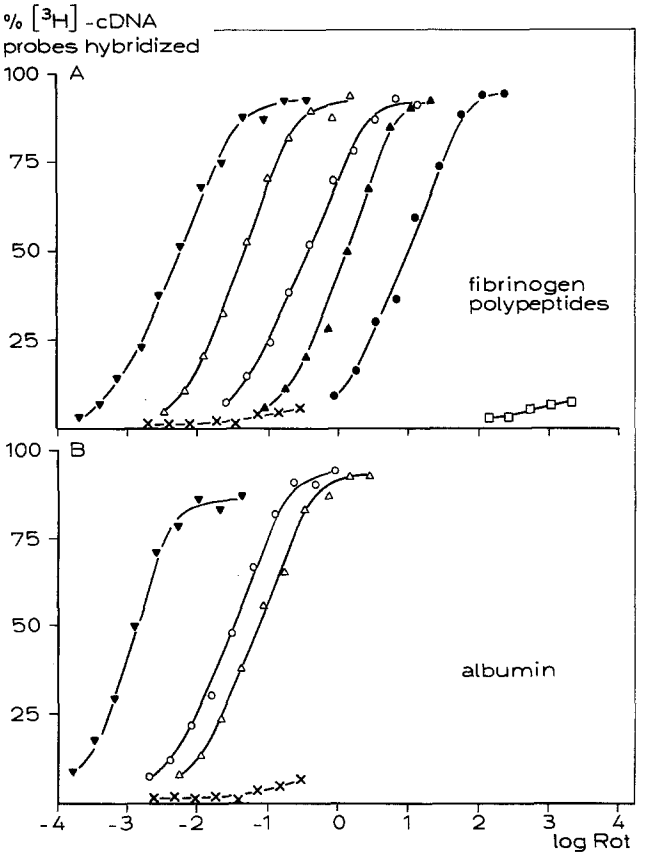

Figure 3. Hybridization kinetics of different RNA fractions prepared from rat liver using ^{3}H DNA complementary to fibrinogen polypeptide mRNAs and ^{3}H DNA complementary to albumin mRNA.
A. ^{3}H DNAs complementary to fibrinogen polypeptide mRNAs were hybridized to purified fibrinogen polypeptide mRNAs (▼—▼); to purified albumin mRNA (x—x); to poly A containing polysomal RNA prepared from livers of rats, 24 hours after turpentine injection (Δ—Δ); to poly A containing RNA of control rat livers (o—o); to polysomal liver RNA of rats treated with turpentine (▲—▲); to polysomal RNA of control rat livers (●—●); and to polysomal rat kidney RNA (□—□).
B. ^{3}H DNA complementary to albumin mRNA was hybridized to purified albumin mRNA (▼—▼); to purified fibrinogen polypeptide mRNAs (x—x); to poly A containing RNA of control rat livers (o—o) and to poly A containing RNA isolated from livers of turpentine treated rats (Δ—Δ).

polyribosomes by immunoprecipitation has been determined. The $Rot_{\frac{1}{2}}$ value of poly A containing RNA prepared from immunoprecipitated polyribosomes is 4.47×10^{-3} mole-sec/liter. The $Rot_{\frac{1}{2}}$ value of poly A containing polyribosomal RNA from control rats is 2.82×10^{-1} mole-sec/liter. These findings suggest that fibrinogen polypeptides synthesizing polyribosomes represent at least 1.6% of total polyribosomes prepared from control rats.

The specificity of fibrinogen polypeptide cDNA is also demonstrated in this figure, as can be seen no annealing was found when these cDNA probes were hybridized to purified albumin mRNA or to rat kidney polysomal RNA.
From numerous studies it has been shown that fibrinogen synthesis in the liver can be stimulated by a number of nonspecific agents causing tissue injury or inflammation (4-8). In this study, we have measured the concentration of fibrinogen polypeptide mRNAs directly by molecular hybridization technique using radiolabelled cDNA probe in the total liver polyribosomal RNA fraction isolated from control rats and from animals 24 hours after injection of turpentine. As shown in fig. 3A, the concentration of mRNA sequences for fibrinogen polypeptides in stimulated liver is dramatically increased (7 fold). In contrast, the albumin mRNA content decreased in liver of rats 24 hours after turpentine treatment (approximately 50% of the original value; fig. 3B). These results are consistent with the findings of cell free translation product under the direction of poly A containing RNA prepared from liver of control and turpentine treated rats as demonstrated in fig. 1 (slot 3 and 4). Although purified DNA complementary to individual mRNAs for different fibrinogen polypeptides has not been used in this study to quantitate the mRNA content, our present findings of increased levels of mRNAs for fibrinogen polypeptides and the decreased content of albumin mRNA in rat liver during the acute phase response suggest that the fibrinogen and albumin synthesis is reciprocally regulated at the transcriptional level. The studies on the regulation of fibrinogen polypeptide mRNA synthesis as well as the purification of individual mRNA for Aα, Bβ and γ-fibrinogen polypeptide respectively are in progress.

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