Radioimmunological Identification of Polysomes Synthesizing Fibrinogen Polypeptide Chains†

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ABSTRACT: Hepatocytes of rats stimulated by turpentine into a hyperfibrinogenemic state produce sufficient quantities of fibrinogen to permit unequivocal identification of specific polysomal complexes involved in the synthesis of this molecule. Monospecific antibodies directed against intact fibrinogen and one of its subunits, the γ-chain, have shown two size classes of polysomes. Furthermore, it seems possible that polypeptide chain assembly may occur by having completed nascent chains bind to partially completed chains that are still attached to the polysome.

Vertebrate fibrinogen is a large molecular weight plasma protein that is capable of undergoing a rapid polymerization following a limited proteolytic cleavage by the enzyme thrombin. The fibrinogen molecule has a mass of ca. 340000 daltons and is composed of three nonidentical pairs of polypeptide chains held together by a complex series of disulfide bridges. Considerable information is available on the chemical and physical properties of vertebrate fibrinogen (see reviews by Doolittle, 1973; Murano, 1974).

It is well established that fibrinogen is synthesized in hepatic parenchymal cells (Barnhart and Forman, 1963; Hamashima et al., 1964) but no definitive information relative to the regulation of its synthesis nor the processes of polypeptide assembly are presently known. In part, such studies have been hampered by the low constitutive rates of fibrinogen biosynthesis in vivo as well as in hepatocyte tissue culture systems. It has been shown, however, that hepatocytes producing fibrinogen as well as several other exportable hepatic proteins can be stimulated into a hypersynthetic state following exposure to a number of nonspecific agents causing tissue injury or inflammation (Koj, 1970; Reeve and Franks, 1974; Jamieson et al., 1975). This response is called the acute-phase reaction and involves the hypersynthesis of liver-synthesized plasma glycoproteins over the course of several days during which the plasma concentrations of these acute phase reactants increase several fold. While the specific mechanism of the acute-phase reaction of the liver is not understood, it does facilitate the study of some processes of fibrinogen synthesis.

The use of antibodies to isolate polysomes engaged in the synthesis of a specific protein was reported as early as 14 years ago for β-galactosidase (Cowie et al., 1961). Since then, several studies using different systems have shown that antibodies can bind to nascent chains on polysomes (Schubert and Cohn, 1968; Palacios et al., 1972; Delovitch et al., 1972; Sarker and Moscona, 1973; Taylor and Schimke, 1974; Schecter, 1974). This approach, together with the finding that poly(A) is present in most mRNAs (Brawerman, 1974), has greatly facilitated the isolation and characterization of mRNAs coding for specific proteins. If polysomes synthesizing individual subunits can be identified and isolated, then the assembly of proteins with more than one polypeptide chain subunit can be studied in more detail (Pawlowski et al., 1975).

In this paper, we demonstrate the use of monospecific ^{125}I-tagged antibodies directed against intact fibrinogen as well as fibrinogen γ-chains to identify classes of polysomes involved in the synthesis of this multichained protein. Finally, some possible modes of polypeptide assembly are proposed for this unique protein.

Materials and Methods

Purified rat fibrinogen and its subunits were prepared as previously described (Bouma and Fuller, 1975). Fibrinogen levels of normal and acute-phase rats were measured with a Fibrometer (Bioquest Division; Becton, Dickinson and Co.) on plasma samples which were anticoagulated with 0.10 *M* sodium citrate (1:9 plasma).

Rats were induced into an acute-phase response, i.e., a hyperfibrinogenemic state by a 1.0-ml subdermal injection of commercial turpentine to increase the yield of fibrinogen isolated from the plasma (Weimer and Humelbaugh, 1967; Koj, 1970). Liver polysomes were prepared from acute phase rats 24 hr after induction to the hyperfibrinogenemic state.

Antibody Purification. Monospecific antibodies to hen ovalbumin, rat fibrinogen, and rat fibrinogen γ-chain were obtained from the sera of rabbits immunized by three 3-mg subdermal injections of electrophoretically homogeneous antigens at weekly intervals. Relatively high antibody titres facilitated the purification of approximately 3–10 mg of antibody per 30 ml of serum. Purification of specific antibodies was accomplished using immunoadsorbent columns to which the purified antigen had been covalently coupled (Bouma and Fuller, 1975) except for anti-rat fibrinogen γ-chain which was purified on a rat fibrinogen immunoadsorbent column. The monospecificities of these antibodies were established on Ouchterlony double-immunodiffusion and immunoelectrophoresis tests.

Iodination of Antibodies. Fractions of monospecific antibodies were radioactively labeled with Na^{125}I (New England Nuclear; ~2.1 mCi/μmol) essentially after established procedures (Palacios et al., 1972). The reaction was

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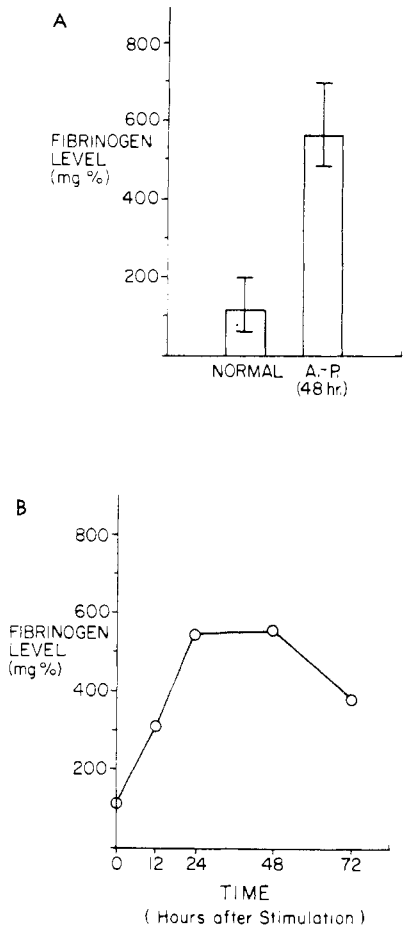

FIGURE 1: Plasma fibrinogen levels following acute-phase stimulation: (A) normal (control) vs. 48-hr acute-phase plasma fibrinogen levels in rats; (B) acute-phase reaction time course determined from different rats at 12, 24, 48, and 72 hr after acute-phase stimulation. Since maximum plasma levels are at 24–48 hr after stimulation, it appears that maximum synthesis occurs at 24 hr.

carried out at room temperature for 30 min and then stopped by the addition of an equal volume of cold, saturated $(NH_4)_2SO_4$ which precipitated the antibodies. The antibody pellets were suspended in a minimum volume of the phosphate buffer and dialyzed overnight in the cold against the same buffer. Antibody concentration was determined using the extinction coefficient ($E_{280}^{1\%} = 14.5$), and the radioactivity was determined on a Nuclear-Chicago gamma counter. Specific activities ranged from 10000 to 80000 cpm/μg. All antibodies, both labeled and unlabeled, were made RNase free by passing them over an ion-exchange column containing 1.0 cm of carboxymethylcellulose and 1.0 cm of DEAE-cellulose (0.5 cm × 2 cm for a sample size of 15 mg or less). This procedure is essentially identical with that described by Palacios et al. (1972).

Preparation of Polysomes. Polysome buffers consisted of 50 m*M* Tris, 25 m*M* NaCl, and 5 m*M* $MgCl_2$ (pH 7.6), and specified concentrations of sucrose. In experiments with EDTA the same buffer solution minus $MgCl_2$ was used.

Liver polysomes (including both free and membrane bound) were isolated from normal and 24-hr acute-phase male rats after a modification of procedures previously described (Kwan and Webb, 1967; Palacios et al., 1972). Rats were exsanguinated from the descending aorta under light ether anesthesia and the liver was perfused with cold 0.25 *M* sucrose polysome buffer, immediately removed, and chilled with 0.25 *M* sucrose polysome buffer. All subsequent steps were performed at 0°C. The liver was weighed, minced, and homogenized in 3 vol of 0.25 *M* sucrose–polysome buffer/g of tissue with 10 strokes of a tight fitting glass–Teflon homogenizer. The homogenate was filtered through two layers of surgical gauze sponges, then centrifuged at 10000 rpm ($12000g_{max}$) for 10 min in a Beckman J-21 preparative centrifuge. The postmitochondrial supernatant was saved, and the pellet was rehomogenized with two strokes in 1 vol of 0.25 *M* sucrose polysome buffer and centrifuged as before. The supernatants were pooled and recentrifuged to ensure complete removal of nuclei and mitochondria. This time only the uppermost two-thirds of the supernatant was saved. To the postmitochondrial supernatant was added Triton X-100 and deoxycholate to a final concentration of 1% each. Eighteen milliliters of the preparation was layered over a discontinuous sucrose gradient of 2 ml of 0.5 *M* sucrose, 14 ml of 1.0 *M* sucrose, and 4 ml of 2.5 *M* sucrose made in polysome buffer. These gradients were centrifuged at 26000 rpm ($120000g_{max}$) for 3.5 hr at 4°C in a Beckman L2-65B preparative ultracentrifuge using an SW27 rotor. The polysomes (collected as an opaque band in the 2.5 *M* sucrose layer) were dialyzed overnight against polysome buffer. Following dialysis, the dialysis tubing containing polysomes was covered with powdered Ficoll (Sigma) for concentrating. The volume was reduced about fourfold. The final concentration of purified polysomes was recorded as A_{260} units, and the polysomes were stored under liquid nitrogen with no evidence of breakdown.

Brain polysomes from 15-day chick embryos were prepared by similar procedures described for rat liver polysomes with the exception that heparin (100 μg/ml) and cycloheximide (20 μg/ml) were added prior to homogenization to prevent polysome breakdown. The polysome buffer used for dialysis and discontinuous sucrose gradients was also supplemented with heparin (100 μg/ml).

Zone Sedimentation Analysis of Polysomes. Purfied polysome preparations were analyzed on 12 ml of a 0.5 to 1.5 *M* continuous sucrose gradient (in polysome buffer) prepared with a Beckman Density Gradient Former. Usually 10 A_{260} units of polysomes were allowed to react with labeled antibody at 0°C for 30 min and in some cases for an additional 30 min with another antibody or 4 μg of RNase (bovine pancreatic RNase, Calbiochem) in a total volume of approximately 0.75 ml. This labeled antibody–polysome mixture was centrifuged through the above gradient at 4°C for 90 min at 40000 rpm ($280000g_{max}$) in the SW40 rotor. In other experiments, polysomes were dissociated into subunits with 30 m*M* EDTA for 30 min either before or after antibody incubation. These polysome profiles were established on 0.5 to 1.0 *M* continuous sucrose gradients in polysome buffer without $MgCl_2$ and centrifuged under the same conditions.

Polysome profiles were obtained by pumping the gradients from the bottom through an LKB Uvicord I, A_{254} monitor connected to a Simpson recorder. Eighteen drop (ca. 0.5 ml) fractions were collected and subsequently counted in a two-channel gamma counter.

Results

Plasma Fibrinogen Levels and Stimulation of Fibrinogen Biosynthesis. A threefold increase in the plasma level of fibrinogen was observed following a 48-hr stimulation with turpentine (Figure 1). Polysomes were routinely harvested

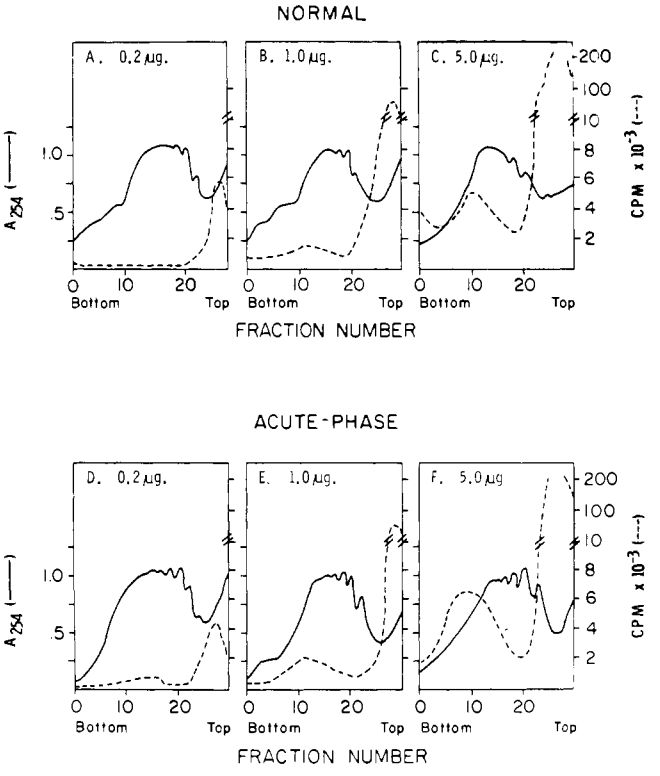

FIGURE 2: Optimal binding of ^{125}I-labeled anti-fibrinogen to normal (A–C) and acute-phase (D–F) rat liver polysomes. Polysomes (10 A_{260} units in 0.75 ml) were incubated at 0°C with 0.2 μg (A, D), 1.0 μg (B, E), and 5.0 μg (C, F) of ^{125}I-labeled anti-fibrinogen for 30 min. After incubation, polysomes were layered over a 0.5–1.5 *M* continuous sucrose gradient and centrifuged as described in the text.

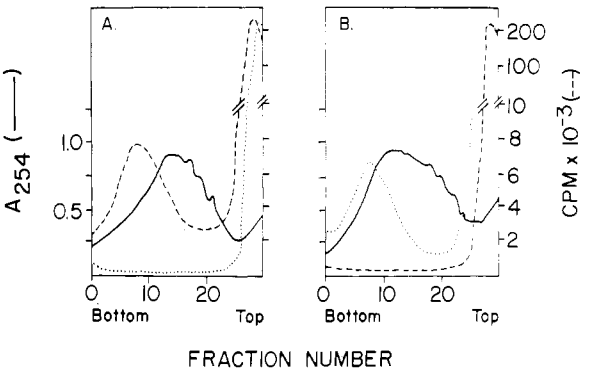

FIGURE 3: Antibody specificity of binding to rat liver polysomes. Rat liver polysomes (10 A_{260} units in 0.75 ml) were incubated at 0°C for 30 min with (A) 5 μg of ^{125}I-labeled anti-fibrinogen (- - -) or 5 μg of ^{125}I-labeled anti-ovalbumin (···) and (B) 100 μg of unlabeled anti-fibrinogen followed by 5 μg of ^{125}I-labeled anti-fibrinogen (- - -) for another 30 min, and 100 μg of unlabeled anti-ovalbumin followed by 5 μg of ^{125}I-labeled anti-fibrinogen (···) for an additional 30 min.

at 24 hr since there was no difference in the amount of fibrinogen seen between 24 and 48 hr following stimulation. Fibrinogen purified from hyperfibrinogenemic rats was identical with that from normal rats in all structural aspects examined (Bouma and Fuller, 1975).

Specificity of Antibodies. Antibodies purified by the methods described were tested by Ouchterlony double immunodiffusion and immunoelectrophoresis. Antifibrinogen exhibited single cross-reactivity with rat plasma on both tests demonstrating its monospecificity. Anti-ovalbumin demonstrated similar cross-reactivity when tested against crude ovalbumin preparations. Antibodies specific for the γ-chain of fibrinogen reacted against intact fibrinogen by the two criteria previously stated. Only double immunodiffusion (Ouchterlony's) could be used when testing the antibody against the isolated γ-chain due to insolubility and aggregation of the isolated polypeptide. Partial solubilization of the γ-chain was achieved by the method of Gollwitzer et al. (1972). The antibodies used in this report were purified to monospecificity by the methods previously described (Bouma and Fuller, 1975).

Determination of Optimum Antibody Binding in Polysomes of Normal and Acute-Phase Rats. Ten A_{260} units of polysomes from normal and 24-hr acute-phase rats were incubated at 0°C with 0.2, 1.0, and 5.0 μg of ^{125}I-labeled anti-fibrinogen (Figure 2). With these fivefold antibody increases there were threefold increases in the antibody-bound polysome peak. Subsequent fivefold increases above 5 μg resulted in only a twofold enhancement of antibody binding to polysomes. Since the 5.0 μg of ^{125}I-labeled anti-fibrinogen was optimum, this level was used throughout these experiments. In subsequent experiments, 5 μg of ^{125}I-labeled anti-ovalbumin was also used since this antibody exhibited a similar specific activity. For ^{125}I-labeled anti-fibrinogen γ-chain, however, a 10-μg level was used as this antibody had only 0.05 the specific activity of the other antibodies. It should be noted that the radioactivity does not follow the absorbance profile but is associated with the region of the relatively heavy polysomes. The radioactivity near the top of the gradient represents unreacted antibodies.

In the stimulated animals, a 40% increase in antibody binding to polysomes was observed as compared to the normal controls (Figure 2), and since their polysomal profiles were qualitatively similar, all subsequent experiments were performed with polysomes from the acute-phase rats.

Specificity of ^{125}I-Labeled Antibody Binding. In order to demonstrate that the antibody binding was specific for nascent fibrinogen chains, the following controls were carried out. On one gradient, polysomes were allowed to react with only ^{125}I-labeled anti-fibrinogen and on a second with only ^{125}I-labeled anti-ovalbumin; on a third and fourth gradient, polysomes were first incubated for 30 min at 0°C with either 100 μg of unlabeled anti-fibrinogen or 100 μg of unlabeled anti-ovalbumin, followed by a 30-min incubation with ^{125}I-labeled anti-fibrinogen at 0°C (Figure 3). Only the antibody (anti-fibrinogen) specific for the liver-synthesized protein fibrinogen bound to the rat liver polysomes, whereas anti-ovalbumin specific for a hen oviduct protein showed no binding to the hepatocyte polysomes. Moreover, preincubation with unlabeled anti-ovalbumin does not affect binding of ^{125}I-labeled anti-fibrinogen (Figure 3A). The binding of labeled anti-fibrinogen is inhibited only by prior incubation with excess unlabeled anti-fibrinogen. Polysomes isolated from embryonic chick brain tissue were incubated with ^{125}I-labeled anti-fibrinogen followed by sucrose gradient centrifugation (Figure 4B). No antibody binding was observed demonstrating that anti-fibrinogen does not possess a nonantigen–antibody affinity for these polysomes.

To establish that the antibody was not binding to nonpolysome-bound fibrinogen from the hepatocyte cytoplasm, the pooled supernatant layers of the discontinuous sucrose gradient (which would contain cytoplasmic proteins) were allowed to react with ^{125}I-labeled anti-fibrinogen and ^{125}I-labeled anti-fibrinogen γ-chain (Figures 4C and 4D). Whereas the former antibody showed no binding, the latter

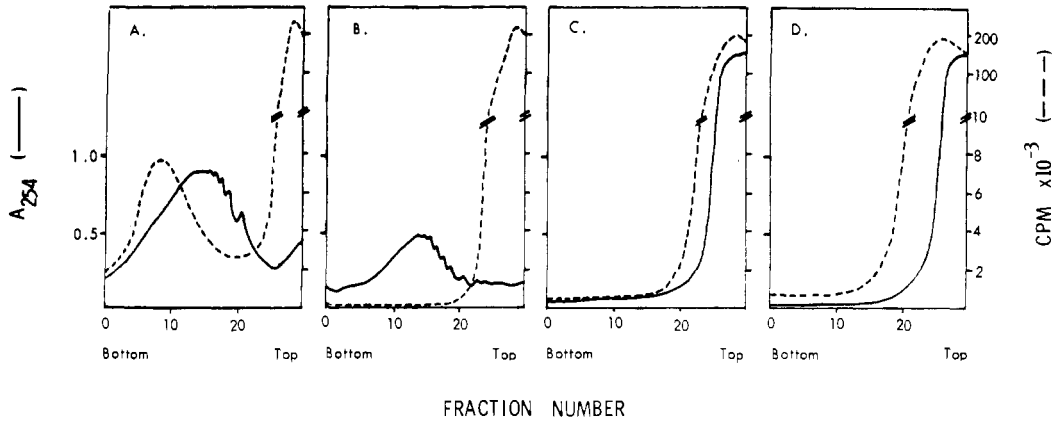

FIGURE 4: Specificity of antibody binding to nascent fibrinogen chains. ^{125}I-Labeled anti-fibrinogen (5 μg) was incubated for 30 min with (A) 10 A_{260} units of rat liver polysomes; (B) 4 A_{260} units of embryonic chick brain polysomes; and (C) 0.75 ml of rat hepatocyte cytoplasmic proteins (from the supernatant layers of the preparative discontinuous sucrose gradient). ^{125}I-Labeled anti-fibrinogen γ-chain (10 μg) was incubated with 0.75 ml of rat hepatocyte cytoplasmic proteins (D). (The ordinate axis cpm should be only 10^{-2} for D since the specific activity of this antibody was $\frac{1}{20}$ that of anti-fibrinogen.)

did migrate slightly further into the gradient, though insufficiently to account for the binding seen with polysomes allowed to react with either antibody.

A final set of control experiments was performed to further show that antibody binding to the polysome complex was not due to some nonspecific fibrinogen association with the polysomes. Liver polysomes were incubated with 10 μg of fibrinogen and 10 μg of labeled antibody for 30 min at 0°C. The mixture was then separated on a sucrose gradient by the usual procedure. An antigen–antibody complex was detected near the bottom of the tube. In addition, a second radioactive peak was seen at the usual polysomal region demonstrating nascent chain binding with the antibodies. Similarly, chick brain polysomes were incubated with fibrinogen followed by ^{125}I-labeled anti-fibrinogen and centrifuged in a linear gradient as before. An antigen–antibody complex was detected near the bottom of the tube; however, no second radioactive peak was seen in the polysomal region. These two experiments demonstrate in still another way that the polysomal labeling is the result of an antibody binding to specific nascent polypeptide chains still attached to the polyribosomal complex, and rule out the possibility that the labeled polysome peak is due to small amounts of fibrinogen being associated in some way with the polysomes (data not shown).

Effect of RNase and EDTA on Polysomes. As is expected, the addition of RNase degrades the polysomes to monosomes. However, in the case of the polysomes identified by anti-fibrinogen, the radioactivity, instead of migrating with the absorbance, is shifted only two–three fractions toward the lighter region, regardless of whether the RNase was added either before or after the formation of the ^{125}I-labeled anti-fibrinogen–polysome complex (Figure 5B). This result is consistent even when higher levels of RNase were used. On the other hand, a distinct shift in the labeling pattern is seen following ribosomal disruption by EDTA (Figure 5C); however, the radioactivity peak still sediments faster than the 30S and 50S subunits. At present, we do not have an unequivocal interpretation for this observation; however, a potential significance of this finding will be discussed.

To determine if a unique size of polysome synthesizes the fibrinogen γ-chain (since this chain is approximately 10% smaller than the α and β chains), ^{125}I-labeled anti-fibrinogen γ-chain was allowed to react with intact polysomes and with polysomes treated with 4 μg of RNase or 30 m*M* EDTA before and after antibody incubation (Figure 5D–F). This antibody identified polysomes which are slightly smaller than those identified by anti-rat fibrinogen. The radioactivity peak of these polysomes shifted to the expected regions of the gradient following treatment with either RNase or EDTA.

Discussion

It has been firmly established that circulating levels of fibrinogen are greatly increased in the course of an acute-phase reaction (Weimer and Humelbaugh, 1967; Koj, 1970). Although the reaction is incompletely understood, this hyperfibrinogenemic state could be attained by an extended half-life of fibrinogen (retarded degradation), or by increased biosynthesis. It has been shown (Davies et al., 1966) that there is a decrease in the half-life of fibrinogen during the acute-phase reaction. Because of the significantly higher levels of plasma fibrinogen, it seems apparent that the second possibility, i.e., enhanced biosynthesis, gives rise to the huge increase in circulating fibrinogen levels. The maximum circulating level of fibrinogen is attained 24–48 hr following acute-phase stimulation. We have examined polysomes at 24 hr after stimulation considering this time to be representative of maximum biosynthesis of fibrinogen. The comparison of normal vs. acute-phase polysomes revealed a 40% increase in the biosynthesis of fibrinogen during this stage of the acute-phase reaction. Since the radioactivity peak has not shifted to a heavier polysome complex (more ribosomes/mRNA), this enhanced synthesis suggests the translation of more fibrinogen mRNA molecules.

The identification of polysomes specifically involved in fibrinogen biosynthesis was demonstrated using monospecific antibodies. The observations that anti-ovalbumin did not bind to the polysomes, nor could it inhibit the binding of anti-fibrinogen, and labeled anti-fibrinogen binding could be inhibited only by prior incubation with excess unlabeled anti-fibrinogen all support the observation that polysomal-bound nascent chains have been identified. This latter result also demonstrates that the binding can be saturated. Labeled anti-rat fibrinogen did not bind to chick brain polysomes, nor form a sedimenting complex with any proteins from the hepatocyte cytoplasm found in the post-mitochondrial supernatant. The labeled anti-fibrinogen γ-chain exhibited similar specificity, although the supernatant radio-

activity sedimented slightly heavier than anti-fibrinogen when allowed to react with the hepatocyte cytoplasmic proteins. In addition, the binding was specifically shown to be to nascent fibrinogen chains on polysomes as demonstrated by the effect of ribosome dissociation by EDTA on the radioactivity peak of both anti-fibrinogen and anti-fibrinogen γ-chain.

Several conclusions can be drawn from these experiments. First, the specificity of the antibody binding is unequivocal, and, therefore, we have identified the population of polysomes synthesizing rat fibrinogen. Secondly, the size region of polysomes radioimmunologically identified corresponds in sedimentation size to the region of polysomes synthesizing rat albumin (Taylor and Schimke, 1974) and conalbumin and ovalbumin (Gonzalez et al., 1974). Since these proteins have molecular weights of 65000, 73000, and 43000, respectively, and the size of the polysome complexes synthesizing rat fibrinogen appears to be similar to these, we suggest that separate mRNAs are involved in the synthesis of fibrinogen. This conclusion is made by comparing to other polysomal systems whose mRNA and protein sizes have been identified. One cannot predict more than a general size range of the mRNA since the degree of ribosomal saturation on the message is not known. Polysomal aggregation would also influence the sedimentation position and would give erroneous estimations of the size of the template RNA. It would be surprising if aggregation played a key role in our conclusions since we are suggesting individual mRNAs over that of a larger polycistronic mRNA. The degrees of ribosome saturation and aggregation are important considerations for predicting mRNA sizes; however, the constancy of our findings under several experimental conditions appears to indicate that separate mRNAs exist for each of the polypeptide subunits of fibrinogen.

The unique behavior of the polysomes identified by the fibrinogen antibody when allowed to react with RNase may be explained by postulating some type of peptide assembly of nearly completed (nascent) chains still attached to the polyribosome with a small cytoplasmic pool of completed chains. It is conceivable that some completed subunits exist in cytoplasmic pools and that these unbound subunits could begin disulfide bridging (assembly) with NH_2-terminal alignment to a nascent polypeptide chain prior to its release from the polysome. This complex would be identified by the anti-rat fibrinogen antibody and could be sterically protected from RNase digestion. Since the ribosomes at the 5′ terminus of the mRNA would possess nascent chains too small to begin this assembly process, these would be cleaved by RNase, hence, our observed shift in the radioactivity profile of two–three fractions. This hypothesis of synthesis and assembly postulates the existence of a number of polypeptide chain intermediates. The isolation and identification of these intermediates can verify and expand this suggested mode of assembly.

These experiments demonstrate that the specific polysomes involved in the synthesis of the multi-chain molecule fibrinogen can be identified from a heterogenous population of polyribosomes. This identification suggests that separate mRNAs exist for each fibrinogen polypeptide chain which is in contrast to the tantalizing notion that a single mRNA codes for all three polypeptide chains (Doolittle, 1973).

Acknowledgment

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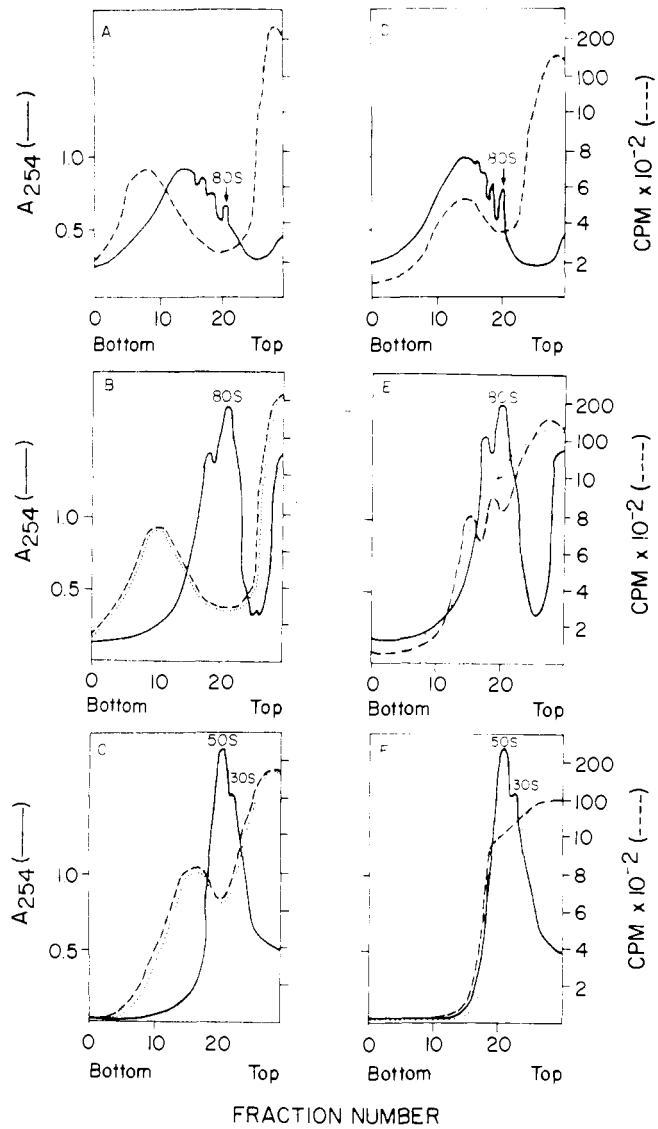

FIGURE 5: Effects of polysome breakdown by RNase and EDTA on the binding of anti-fibrinogen and anti-fibrinogen γ-chain. Rat liver polysomes were incubated with (A–C) 5 μg of ^{125}I-labeled anti-fibrinogen or (D–F) 10 μg of ^{125}I-labeled anti-fibrinogen γ-chain and treated as follows: (A, D) no disruption; (B, E) enzymatic digestion with 4 μg of RNase for 30 min either before (···) or after (- - -) antibody incubation; and (C, F) chemical dissociation with 30 mM EDTA for 30 min either before (···) or after (- - -) antibody incubation. Polysome profiles A, B, D, and E were resolved on 0.5–1.5 M continuous sucrose gradients, while profiles C and F were resolved on 0.5–1.0 M continuous sucrose gradients. (The radioactivity ordinate axis of A–C should be cpm $\times 10^{-3}$.)

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Purification of Murine Thymus Leukemia Antigen (TL). A Quantitative Assessment of Limited Proteolysis†

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ABSTRACT: The murine thymus leukemia antigen (TL) has been solubilized from the tumor ASL1 and from an established cell line ASL1W, by papain digestion. When a 15-min digest was chromatographed on Sephadex G-200, two peaks of TL activity were eluted with apparent molecular weights of approximately 58,000 and 31,000. Chromatography of a 30-min digest under the same conditions resulted in elution of a single peak of activity with an apparent molecular weight of 58,000. Additional purification was carried out on the 58,000 molecular weight material by absorption to, and elution from DEAE-cellulose. The combination of gel filtration and ion exchange chromatography resulted in approximately a 150-fold purification.

Thymus leukemia antigen (TL)[1] has been described serologically by Boyse (Boyse et al., 1968). TL is found normally only on thymocytes of certain mouse strains. However, its expression has been noted on some leukemias of all strains tested, even those that are normally phenotypically TL$^-$. Biochemical studies using radioactive amino acids and sugars have shown TL to be a glycoprotein (Muramatsu et al., 1973). It is apparently made up of two chains. The heavier of the two chains contains the carbohydrate (Muramatsu et al., 1973) and the small chain mol wt 11000–12000 (Ostberg et al., 1975) has been shown to be antigenically cross-reactive with human β_2-microglobulin. These properties are similar to those of mouse transplantation antigens (H-2) (Vitetta et al., 1975; Nathenson and Cullen, 1974; Silver and Hood, 1974).

It has been suggested that TL (Vitetta et al., 1975), along with H-2 (Vitetta et al., 1975) and the human transplantation antigens, HL-A (Strominger et al., 1974), have overall structures similar to immunoglobulins. This suggestion is based on several observations. All of these cell surface antigens have a limited number of sites of papain cleavage, a property which is similar to immunoglobulin. The carbohydrate is found associated only with the heavy chain which is again suggestive of an immunoglobulin-like structure. Sequence analysis of the small chain, β_2-microglobulin, revealed significant homology with immunoglobulins (Cunningham et al., 1973). In addition, HL-A (Strominger et al., 1974; Cresswell, 1975) molecules have been shown to exist as dimers, with the heavy chains joined by disulfide bonds.

Most of the previous studies of TL structure have relied on immunoprecipitation to achieve purification (Muramatsu et al., 1973; Vitetta et al., 1972). Although this allows a quick method of obtaining relatively pure TL, the conditions of release of the TL from the antibody are generally quite severe, e.g., boiling in sodium dodecyl sulfate. This treatment does not leave the molecule in any condition for further biochemical and structural studies. Because of the small amount of TL expressed on cells, there are two principle requirements for further in-depth studies on its structure: (1) a larger source of cells than are available from animals and (2) a method of purification which will leave the TL in a more native state. This report describes the comparative purification of papain-solubilized TL taken from a tumor line (ASL1), and that taken from the established cell line (ASL1W) which has been adapted to spinner culture.

Materials and Methods

Mice. The congenic, A/TL$^-$ strain, was a generous gift from Dr. E. A. Boyse of Sloan Kettering Memorial Institute. Inbred strains, C57Bl/6 (TL$^-$), A/J (TL$^+$), and

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[1] Abbreviations used are: TL, thymus leukemia antigen; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.