Glycosylated Aα Chains in Chicken Fibrinogen†

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ABSTRACT: Fibrinogen immunoprecipitated from cultured chick embryo hepatocytes was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and compared with fibrinogen from chicken plasma. The character and relatedness of the constituent polypeptide bands were established on the basis of enzymatic treatment, peptide analysis, metabolic labeling with [^{14}C]glucosamine, and inhibition of glycosylation with tunicamycin. Hepatocyte-derived fibrinogen resolved into five polypeptides that, in order of decreasing apparent molecular weight, were identified as glycosylated Aα, nonglycosylated Aα, Bβ, γ′, and γ. Fibrinogen immunoprecipitated directly from chicken plasma yielded an identical profile except for an additional smaller Aα chain. This small Aα chain appears to be the product of partial proteolysis in the circulation and was the only Aα band found in purified plasma fibrinogen (fraction I-2). The observation of glycosylated Aα chains is novel. The γ/γ′ chain heterogeneity appears to be due to an amino acid extension similar to that observed in mammalian fibrinogens. Fibrinogen from cells exposed to fetal bovine serum, a potent stimulator of fibrinogen production, was enriched in glycosylated Aα chains, which constituted approximately one-third of the Aα chain population. Serum did not affect the γ/γ′ chain distribution.

The fibrinogen molecule from many species, including chicken, has been shown to be a glycoprotein dimer consisting of three different disulfide-bridged subunits chains: (Aα, Bβ, γ)$_2$ (Blombäck, 1972; Doolittle, 1983). The Bβ and γ polypeptides are glycosylated; no carbohydrate has been previously detected in the Aα chains (Blombäck et al., 1973; Töpfer-Petersen et al., 1979; Watt et al., 1979; Townsend et al., 1982). In the circulation, fibrinogen undergoes varying degrees of catabolic proteolytic attack to yield a heterogeneous population (Mosesson, 1983). Study of fibrinogen in hepatocyte culture minimizes these postsecretory modifications, allowing investigations of the molecule's original structure (Wangh et al., 1983). The well-defined and easily manipulated cultures further enable study of how fibrinogen structure may be influenced by factors in the hepatocellular environment.

The primary monolayer cultures of chick embryo hepatocytes used in this study have served as a model system for probing the hormonal control of fibrinogen production [for a review, see Grieninger et al. (1983)]. The cells secrete a fully assembled fibrinogen molecule, which closely resembles mammalian fibrinogen in size (Amrani et al., 1983). The effects of added agents can be clearly discerned in these cultures, which possess the unusual ability to sustain a low, but steady, rate of fibrinogen production for several days in the absence of hormone, serum, or other macromolecular supplement to the medium.

Chicken plasma fibrinogen has been shown to be structurally and functionally similar to mammalian fibrinogen (Pindyck et al., 1977; Murano et al., 1977). Using different electrophoretic conditions, we have reexamined fibrinogen from chicken plasma and compared its subunit composition with that of fibrinogen newly synthesized by hepatocytes in culture. We report that chicken fibrinogen contains both glycosylated and nonglycosylated Aα chains as well as two γ chain species. We present evidence that, while the ratio of the two γ chain species remains constant during serum stimulation of fibrinogen production in culture, the ratio of glycosylated to nonglycosylated Aα chains increases.

Materials and Methods

[^{35}S]Methionine (sp act. approximately 1000 Ci/mmol) and [^{14}C]glucosamine (sp act. 54.2 mCi/mmol) were obtained from New England Nuclear. Plasma from individual chickens (age 3.5 months) was obtained essentially as described (Beuving & Vonder, 1977). Fetal bovine serum was purchased from Grand Island Biological Co., Grand Island, NY.

Primary monolayer cultures of hepatocytes from 16-day-old chicken embryos were prepared as described (Liang & Grieninger, 1981; Grieninger, 1983). They were plated in chemically defined medium, free of added hormones and macromolecules (referred to as "basal condition"). Heparin sodium salt (Fisher Scientific Co., Fairlawn, NJ; 156 units/mg) was added at a concentration of 15 μg/mL at the first medium change, 4–5 h after plating, at which time fetal bovine serum (2%, if not otherwise indicated) was also added.

Labeling of the cultures was performed approximately 24 h after initiation of culture, using HEPES[1]-buffered, methionine-free medium for short pulses and methionine-containing medium for long-term labeling, as described previously (Amrani et al., 1983; Plant et al., 1983). Treatment of monolayers with tunicamycin (Calbiochem) was initiated 4 h before radioactive labeling by adding the inhibitor to the medium (5 μg/mL) and maintaining it in all subsequent labeling, chase, and wash media.

Fibrinogen, isolated from pooled chicken plasma to yield fraction I-2 as described (Pindyck et al., 1977), was further purified by Sepharose CL-6B (Pharmacia) column chromatography (Amrani et al., 1983). For electrophoretic analysis, fraction I-2 fibrinogen was generally dissolved in lysis buffer (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM Na_2EDTA, 0.5% Triton X-100, 0.1% SDS, 50 KIU/mL Trasylol, 1 mM PMSF, and 0.1 mM TPCK) (Amrani et al., 1983; Plant et al., 1983); fibrinogen subjected to enzymatic treatment was dissolved directly in double-strength enzyme buffer (see below) containing 4 M urea.

Fibrinogen as well as albumin and transferrin was immunoprecipitated from cell extracts and spent medium in the

† From the Lindsley F. Kimball Research Institute of The New York Blood Center, New York, New York 10021. *Received February 15, 1984; revised manuscript received May 30, 1984.* This work was supported by NIH Grants HL-28444 and HL-09011. P.W.P. was supported by Institutional Grant NRSA 5 T32 HL-07331 from the NIH. G.G. is an Established Investigator of the American Heart Association.

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[1] Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; KIU, kalikrein inhibitor unit; PMSF, phenylmethanesulfonyl fluoride; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; gAα, glycosylated Aα; gα, glycosylated α; dAα, partially degraded Aα.

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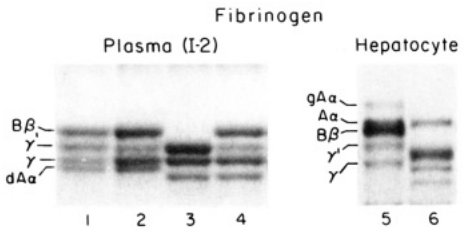

FIGURE 1: Electrophoretic analysis of fibrinogen purified from plasma (fraction I-2) and immunoprecipitated from hepatocytes. All samples were separated by electrophoresis on 7.5% SDS–polyacrylamide gels. (Lanes 1–4) Plasma fibrinogen (fraction I-2), stained with Coomassie blue. (Lane 1) 35 μg dissolved in lysis buffer. (Lanes 2–4) 50 μg dissolved in urea-containing enzyme buffer and incubated with either no enzyme (lane 2), 1.7 units of thrombin (lane 3), or 6.6 units of batroxobin (lane 4). (Lanes 5 and 6) Hepatocyte fibrinogen, autoradiographed. Monolayers were pulse-labeled for 5 min with 400 μCi of [^{35}S]methionine/mL in the absence (land 5) or presence (lane 6) of tunicamycin, after which fibrinogen was immunoprecipitated from cell extracts. In chicken fibrinogen the methionine content of the Bβ chain is approximately twice that of the Aα and γ chains; hence, it is more intensely labeled (Murano et al., 1977). Lanes 1–4 and lanes 5 and 6 are derived from separate gels. When analyzed on the same gel, the Bβ and γ subunits of hepatocyte fibrinogen comigrate with those of fraction I-2 fibrinogen.

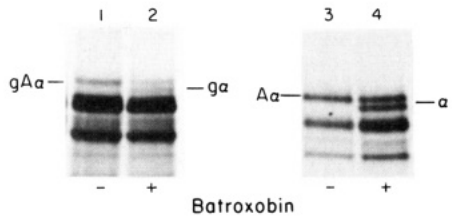

FIGURE 2: Batroxobin treatment of glycosylated and nonglycosylated hepatocyte fibrinogen. Fibrinogen was derived from the same hepatocyte cultures described in Figure 1, pulse-labeled either in the absence (lanes 1 and 2) or in the presence (lanes 3 and 4) of tunicamycin. Immunoprecipitates were treated either without enzyme (lanes 1 and 3) or with 1.7 units of batroxobin/sample (lanes 2 and 4). Autoradiographs are shown of the samples analyzed on 7.5% SDS–polyacrylamide gels. For the autoradiograph of lanes 1 and 2, the film was overexposed to enhance visibility of the gAα and gα band. Since the enzyme-catalyzed removal of fibrinopeptide A from Aα chains was not complete under the experimental conditions used, bands corresponding to the intact Aα chains as well as their respective digestion products appear in the digests.

presence of protease inhibitors and detergents by using monospecific antibodies and protein A–Sepharose CL-4B (Pharmacia) as the adsorbent as detailed previously (Amrani et al., 1983; Plant et al., 1983).

For reaction with enzymes, immunoprecipitates were washed 4 times with lysis buffer followed by four washes with enzyme buffer (0.15 M Tris-HCl, pH 7.4, 0.075 M NaCl, Trasylol 150 KIU/mL) (Yu et al., 1980) and incubated in the same buffer now containing 4 M urea for 1 h at 37 °C to dissociate the immunocomplex. They were incubated for 3 h at 37 °C with either thrombin (human, 100 NIH units/mg; Sigma) or batroxobin (a snake venom enzyme from *Bothrops marajoensis*, 200 units/mg; Pentapharm, Basel, Switzerland), which were added in enzyme buffer, diluting the urea to 2 M. Fraction I-2 fibrinogen was digested directly in enzyme buffer containing 2 M urea.

SDS–polyacrylamide gel electrophoresis was performed under disulfide-reducing conditions on slab gels as described (Plant et al., 1983), using the buffer system of Laemmli (1970). Incorporation of radioactivity was determined by liquid scintillation counting of the appropriate bands excised and solubilized from the gels (Plant et al., 1983). Peptide mapping, involving limited proteolysis with *Staphylococcus aureus* V8 protease (Miles) and electrophoresis in SDS in a two-dimensional gel system, was performed as described (Cleveland et al., 1977; Bordier & Crettol-Järvinen, 1979).

Results and Discussion

Fibrinogen (fraction I-2) purified from chicken plasma was compared by SDS–polyacrylamide gel electrophoresis under disulfide-reducing conditions with fibrinogen immunoprecipitated from cultured chick embryo hepatocytes (Figure 1, lanes 1 and 5). Fraction I-2 fibrinogen yielded four distinct bands, ranging between 50 000 and 56 000 in apparent molecular weight, whereas hepatocyte fibrinogen gave five bands (in the 51 000–61 000 dalton range). The character and relatedness of these polypeptide bands were then established on the basis of enzymatic treatment, peptide analysis, metabolic labeling with [^{14}C]glucosamine, and inhibition of glycosylation with tunicamycin, as described below.

The Aα and Bβ chains of fibrinogen are characterized by differential sensitivity to digestion with the enzymes thrombin and batroxobin. While only the Aα chain is cleaved by batroxobin, both chains are cleaved by thrombin, which removes fibrinopeptides A and B from their respective amino termini. The effect of thrombin on fraction I-2 fibrinogen was to increase the mobility of both the highest and lowest molecular weight bands (Figure 1, compare lanes 2 and 3). Treatment with batroxobin shifted only the position of the lowest band (compare lanes 2 and 4), positively identifying it as an Aα chain. These results indicate that the band in fraction I-2 fibrinogen with the highest apparent molecular weight corresponds to Bβ, while the two middle bands, which were not sensitive to either enzyme treatment, are both γ chains.[2]

The five bands in the polypeptide profile of hepatocyte-derived fibrinogen were identified in a similar manner. Digestion of hepatocyte fibrinogen with thrombin increased the mobility of the three highest molecular weight bands (not shown). Upon treatment with batroxobin, only the two largest were shifted (Figure 2), identifying them directly as Aα chains and the smaller thrombin-sensitive band, by inference, as Bβ. The two lowest molecular weight bands, which were not affected by either enzyme treatment, represent γ chains.[2] Due to the close migration of the Aα and Bβ chains of hepatocyte fibrinogen, the effect of batroxobin on the smaller Aα chain could be better discerned by using fibrinogen from hepatocytes treated with tunicamycin (Figure 2, compare lanes 3 and 4).

When N-linked glycosylation was inhibited with tunicamycin (Tkacz & Lampen, 1975), the cultures produced a fibrinogen molecule with only one batroxobin-sensitive band (Figure 2). The band migrated in the original position of the smaller of the two Aα bands (see also Figure 1, compare lanes 5 and 6), indicating that this polypeptide (Aα) is normally not glycosylated; the other fibrinogen subunits, including the larger batroxobin-sensitive chain (gAα), displayed increased mobility, indicating that under normal conditions they do contain carbohydrate residues. One of the two Aα chains of hepatocyte fibrinogen is, therefore, glycosylated while the other is not. Sensitivity of Aα chain glycosylation to tunicamycin indicates oligosaccharide attachment at an asparaginyl residue. Interestingly, recent DNA sequence analysis has revealed the presence of two potential N-glycosylation sites in the Aα chain of human fibrinogen, at Asn_{269} and at Asn_{400} (Rixon et al.,

[2] Incubation of plasma as well as hepatocyte fibrinogen in urea-containing enzyme buffer results in an apparent increase in the ratio of γ to γ' (Figure 1, compare lanes 1 and 2; Figures 1 and 2, compare lanes 5 and 1, respectively). This artifactual distortion of the apparently normal 50:50 ratio in chicken fibrinogen does not affect the conclusions drawn in this paper.

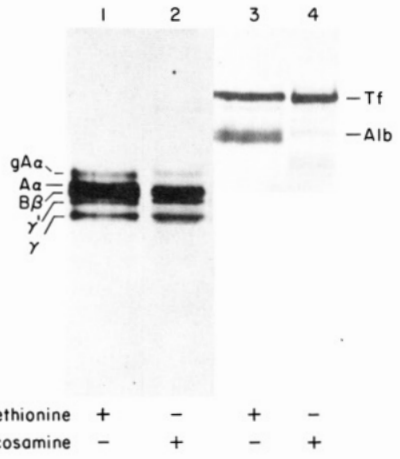

FIGURE 3: Comparison of plasma proteins labeled with [^{35}S]methionine and [^{14}C]glucosamine. Cells were cultured in medium supplemented with 6% fetal bovine serum. They were labeled for 4 h by the addition of either [^{35}S]methionine or [^{14}C]glucosamine. Fibrinogen (lanes 1 and 2) or albumin and transferrin (lanes 3 and 4) were immunoprecipitated from samples of spent culture media and subjected to electrophoresis on a 7.5–11.4% linear gradient SDS–polyacrylamide gel. An autoradiofluorogram (Bonner & Laskey, 1974) of the gel is shown.

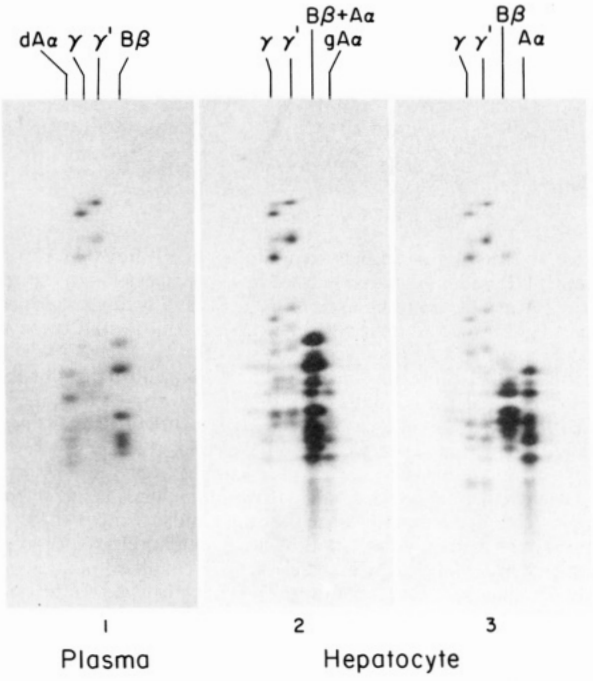

FIGURE 4: Peptide analysis of plasma (fraction I-2) and hepatocyte fibrinogens following limited proteolysis. All samples were first resolved by electrophoresis from right to left on the same 7.5% SDS–polyacrylamide gel. The unstained gel strips were cut out and layered in a perpendicular fashion atop a single second gel (6% stacking gel and 15% resolving gel) and then exposed to *S. aureus* V8 protease during electrophoresis in the second dimension (Cleveland et al., 1977; Bordier & Crettol-Järvinen, 1979). (Track 1) Fraction I-2 fibrinogen (100 μg) (Coomassie blue stain). (Tracks 2 and 3) Hepatocyte fibrinogen (autoradiograph). Cells were labeled for 90 min with [^{35}S]methionine in the absence (track 2) or presence (track 3) of tunicamycin; fibrinogen was immunoprecipitated from spent culture medium.

1983), each comprised of the tripeptide sequence Asn-X-Ser.

The presence of both glycosylated and nonglycosylated Aα chains was also evaluated by examining the incorporation of [^{14}C]glucosamine into the polypeptide chains of metabolically labeled hepatocellular fibrinogen (Figure 3). All the polypeptides, including the glycosylated Aα chain, incorporated this precursor, the only exception being the nonglycosylated Aα chain. Two other plasma proteins were immunoprecipitated in this experiment, one that is known to be glycosylated (transferrin) and one that is not (albumin); of these, only transferrin incorporated the labeled sugar.

Thus, hepatocyte fibrinogen contains glycosylated as well as nonglycosylated Aα chains, both of which are considerably larger in size than the single, nonglycosylated (Pindyck et al., 1977) Aα chain of fraction I-2 fibrinogen from plasma. Avian Aα chains are unusually sensitive to proteolytic degradation (Krajewski et al., 1980), and the relatively small Aα chain of fraction I-2 fibrinogen (dAα) apparently represents a catabolite. Generation of dAα from the higher molecular weight Aα chains, which would involve the loss of at least 7000 daltons, presumably occurs by proteolysis at the carboxy terminus since dAα was converted by both thrombin and batroxobin to a band of yet lower molecular weight in reactions requiring an intact amino terminus (Figure 1, lanes 3 and 4).

The chain assignments based on enzyme and inhibitor studies were corroborated and extended by two-dimensional peptide mapping following limited proteolysis (Figure 4). With this technique, each band of the original SDS–polyacrylamide gel profile yields a sequence-specific pattern of spots upon electrophoresis of peptide fragments in the second dimension. Structurally homologous subunit chains yield similar peptide patterns. In Figure 4, the chains comprising fraction I-2 and hepatocyte fibrinogens were analyzed and compared (tracks 1 and 2, respectively). To enhance separation of the Bβ and nonglycosylated Aα chains, a sample of fibrinogen from tunicamycin-treated cells was also analyzed (track 3).

The two γ chains in each sample gave rise to a closely parallel series of spots, particularly in the lower molecular weight region. The size difference between the two chains (ca. 2000 daltons) and the manner in which the larger peptide fragments are offset are consistent with the type of γ/γ′ heterogeneity observed in human fibrinogen. This heterogeneity arises from an amino acid extension at the carboxy terminus of the larger chain, γ′ (Wolfenstein-Todel & Mosesson, 1980; Francis et al., 1980). We have labeled the chicken γ chains accordingly. The mobilities of the γ and γ′ chains were not differentially affected by tunicamycin treatment (Figure 1, compare lanes 5 and 6). However, increased mobility of the smaller peptide fragments generated from both the γ and γ′ chains derived from the inhibitor-treated cells (Figure 4, compare tracks 2 and 3) suggests that they may correspond to sequences containing the amino terminus near which, in the γ chain of human fibrinogen, a single glycosylation fork has been characterized (Blombäck et al., 1973; Townsend et al., 1982; Mizuochi et al., 1982).

While the variant (γ′) accounts for only 10% of the γ chains in human fibrinogen (Wolfenstein-Todel & Mosesson, 1980; Francis et al., 1980) and 30% in rat fibrinogen (Legrele et al., 1982), the distribution of γ and γ′ subunits appears nearly equal in chicken fibrinogen, be it derived from plasma or hepatocyte culture (Figure 1, lanes 1 and 5).[2] This even distribution is also suggested by the chromatographic resolution of the γ chains of *S*-sulfo chicken fibrinogen into two similarly sized peaks (Pindyck et al., 1977). It is possible that most molecules of chicken fibrinogen contain one γ and one γ′ chain since elution of the unmodified protein from DEAE-cellulose yields a single clottable peak at the same position as human fibrinogen of the structure $(A\alpha)_2(B\beta)_2(\gamma)(\gamma')$ (Finlayson & Mosesson, 1964; Mosesson et al., 1972).

Direct comparison of the peptide fragments produced by the glycosylated and nonglycosylated Aα chains of hepatocyte-derived fibrinogen (Figure 4, track 2) is complicated by

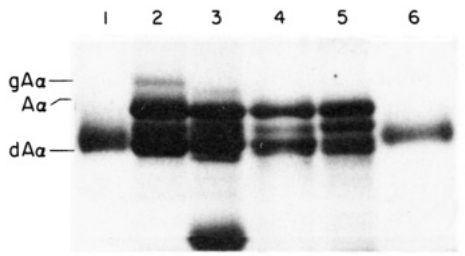

FIGURE 5: Enzymatic characterization of fibrinogen immunoprecipitated from chicken plasma and comparison of its polypeptide profile with that of fraction I-2 fibrinogen. Fibrinogen was immunoprecipitated from single-donor chicken plasma by using anti-fibrinogen IgG coupled to Sepharose 4B (Amrani et al., 1983). The samples were separated on 7.5% SDS–polyacrylmide gels and proteins stained with Coomassie blue; (Lane 1) Mock immunoprecipitation (no plamsa added) revealing some anti-fibrinogen IgG leaking from the resin (only the heavy chain is shown). (Lane 2) Immunoprecipitated fibrinogen (no enzyme), 70 μg. (Lane 3) Immunoprecipitated fibrinogen (70 μg) treated with 10 units of batroxobin (the enzyme is visible at the bottom of this lane). (Lane 4) Fraction I-2 fibrinogen (50 μg) dissolved in enzyme buffer containing urea.[2] (Lane 5) Fraction I-2 fibrinogen (70 μg) dissolved in lysis buffer. (Lane 6) 7 μg of anti-fibrinogen IgG, not coupled. Samples were overloaded to enhance visibility of gAα and gα bands in lanes 2 and 3, respectively. The gAα and Aα bands of hepatocyte-derived fibrinogen (not displayed) migrate in the positions indicated on the left.

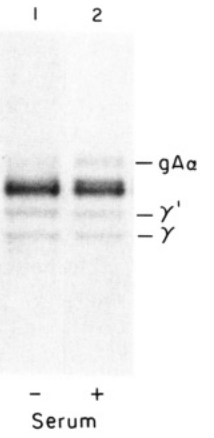

FIGURE 6: Polypeptide profile of fibrinogen produced in the presence and absence of serum. After 3 h of culture, cells were preincubated for 21 h with or without 2% fetal bovine serum. They were pulse-labeled for 5 min in the presence and absence of dialyzed serum by using 0.2 and 1 mCi of [^{35}S]methionine/mL of medium, respectively. They were chased for 2 h in the continued presence or absence of serum. Fibrinogen was immunoprecipitated from the spent media and analyzed by electrophoresis on 7.5% SDS–polyacrylamide gels. Autoradiographs of the gel are shown. (Lane 1) No serum (film exposed for 7 days). (Lane 2) Serum (film exposed for 1 day). The faint band below the γ chain in lane 1 is not of Aα origin, based on peptide maps, analogous to those of Figure 4, analyzing fibrinogen synthesized and secreted under basal conditions (unpublished results).

overlapping of the spots generated by the Bβ chain with those of the nonglycosylated Aα chain. In the resultant composite, however, the fragments attributable to Bβ alone can be derived by comparison with those clearly visible in the analysis of Bβ in fraction I-2 fibrinogen (track 1). The remainder, presumably derived from nonglycosylated Aα (compare track 2 with track 3), form a pattern of spots that largely comigrate with those derived from the glycosylated Aα chain. The low molecular weight Aα chain of fraction I-2 fibrinogen (dAα) yielded a pattern of spots similar to but sparser than that of the hepatocyte Aα chain (compare tracks 1 and 3).

Our electrophoretic profile of plasma fibrinogen (fraction I-2) deviates, in certain respects, from that described by Pindyck et al. (1977), presumably due to the use of a different buffer system that provides greater resolution of the fibrinogen bands. In particular, the Aα band (dAα), which in our system migrates more anodally than the others, migrates under their conditions between the Bβ and γ chains. These buffer-dependent differences were not fully appreciated in a recent report from our laboratory and resulted in mislabeling of the Aα chain (Amrani et al., 1983; Grieninger et al., 1983).

The presence of all three types of Aα chains in the circulation was revealed by direct immunoprecipitation of fibrinogen from chicken plasma (Figure 5). The most apparent Aα band in the SDS–polyacrylamide gel profile of immunoprecipitated plasma fibrinogen was the batroxobin-sensitive band comigrating with, and presumably identical with, the glycosylated Aα chain of hepatocyte fibrinogen. A band, comigrating with nonglycosylated Aα and barely visible above the Bβ chain, is more conspicuous by its absence following treatment with batroxobin as well as by its absence in the profile of fraction I-2 fibrinogen (compare lane 2 with lanes 3–5); it is distinctly visible following electrophoresis on 7.5–11.4% linear gradient SDS–polyacrylamide gels (not shown). Although the degraded Aα chain (dAα) of immunoprecipitated plasma fibrinogen is obscured by the more prominent γ chains, it generates a more visible, faster migrating band upon treatment with batroxobin.

Thus, the electrophoretic profile of immunoprecipitated plasma fibrinogen indicates that glycosylation of the Aα chain is neither an artifact of culture nor a phenomenon peculiar to embryonic liver. To the best of our knowledge, our report is the first to describe glycosylation of the Aα chain of fibrinogen. Glycosylated and nonglycosylated Aα chains have been detected previously, although not identified as such, in fibrinogen from chick embryo hepatocyte cultures (Amrani et al., 1983) but not in fraction I-2 fibrinogen purified from adult chicken plasma (Pindyck et al., 1977; Amrani et al., 1983). The absence of the high molecular weight Aα bands in fraction I-2 fibrinogen probably reflects the selective purification of molecules containing only degraded Aα chains. This is supported by the fact that differences in Aα chain composition of heterogeneous forms of human fibrinogen correlate with differential solubilities (Mosesson, 1983).

Production of both glycosylated and nonglycosylated forms of the same polypeptide by a single cell type is not common and is an intriguing phenomenon. Our results suggest it is so for Aα chains in chicken hepatic parenchymal cells. It may also be the case with bovine pancreatic ribonuclease, which has been isolated in glycosylated and nonglycosylated forms that share the same primary amino acid sequence (Plummer & Hirs, 1964). Precisely how production of both forms is regulated in these instances remains a subject for further investigation.

The ability of factors in the hepatocellular environment to influence the ratio of glycosylated to nonglycosylated Aα chains is indicated by comparative electrophoretic analysis of fibrinogen produced under basal and stimulating conditions in culture (Figure 6). Cells exposed to fetal bovine serum, a potent stimulator of fibrinogen production (Plant et al., 1981), secreted a molecule enriched in glycosylated Aα chains as compared to that secreted in the absence of hormones or other macromolecular supplement. We observed, in several different experiments, that under serum-stimulated conditions 30–40% of the hepatocyte Aα chains became glycosylated whereas under basal conditions gAα chains comprised only 10–20% of the Aα chain population. In contrast, stimulation did not affect the γ/γ′ chain distribution. A specific serum-induced increase in the level of glycoslylated Aα chains was also found in an experiment carried out in medium containing Trasylol (50 units/mL) to circumvent extracellular proteolysis,

which could be a factor in the effect of serum (unpublished results). In that experiment, in which the cells were labeled to steady state with [^{35}S]methionine, a larger percentage of gAα was found in not only secreted but also cellular fibrinogen. We are currently investigating the mechanism of this change in Aα chain glycosylation and its possible involvement in the overall control of fibrinogen production.

Acknowledgments

We acknowledge the skilled technical assistance of Mary Ann Chiasson and Peter Golikov. We thank Dr. G. Beuving of the Spelderholt State Institute for Poultry Research, Beekbergen, The Netherlands, who generously provided the single-donor chicken plasma used in this study. We are grateful to Drs. D. Amrani and M. W. Mosesson of the Mt. Sinai Medical Center (Milwaukee, WI) for Cibacron-blue column chromatography of anti-fibrinogen antiserum and for supplying fraction I-2 fibrinogen and antifibrinogen IgG coupled to Sepharose 4B. We also thank Kathe M. Hertzberg and Drs. B. Blombäck, B. Kudryk, C. Redman, and J. Pindyck for helpful discussions.

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