Abnormal Sialic Acid Content of the Dysfibrinogenemia Associated with Liver Disease

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ABSTRACT To evaluate the possibility that the carbohydrate composition of fibrinogen may be altered in the dysfibrinogenemia associated with liver disease, we studied the sialic acid content of purified fibrinogen from 12 patients with liver disease and its relationship to the prolongation of the thrombin time. Purified fibrinogen showed intact Aα-, Bβ-, and γ-chains when reduced and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and exhibited prolongation of the thrombin time similar to that of the plasma from which it was derived. Sialic acid content of the purified fibrinogen ranged from 12.7 to 71.4% higher in patient fibrinogens when compared to normal controls. A progressive delay in thrombin time was associated with increasing sialic acid content of the patient fibrinogen. Enzymatic removal of sialic acid from four of the abnormal fibrinogens resulted in a shortening of their thrombin times to the range of the desialylated normal control. Periodic acid-Schiff reagent stained only the Bβ- and γ-chains of the reduced patient fibrinogens after sodium dodecyl sulfate-polyacrylamide gel electrophoresis suggesting that the excess sialic acid is located on these two chains. These studies demonstrate a biochemical alteration of the functionally abnormal fibrinogen found in some patients with liver disease, and indicate that the excess sialic acid plays an important role in the functional defect of this protein.

INTRODUCTION

A dysfibrinogenemia functionally characterized by an abnormality of fibrin monomer polymerization has been described in some patients with liver disease (1, 2). Because of a suggestion that the carbohydrate composition of fibrinogen may be altered in liver disease (3), and because of previous work in which we demonstrated the influence of sialic acid on normal fibrin monomer aggregation (4), we studied the sialic acid content of the abnormal fibrinogen from 12 patients with liver disease and its relationship to the prolongation of the thrombin time.

METHODS

The patients chosen for study had plasma thrombin times at least 40% longer than normal controls. There was no clinical or laboratory evidence of disseminated intravascular coagulation and(or) fibrinolysis. Eight patients had alcoholic liver disease, three had postnecrotic cirrhosis of undetermined etiology, and one had acetaminophen-induced hepatic failure. All diagnoses were based on clinical and histological criteria. The functional characterization of the abnormal fibrinogen of five of these patients formed the basis of a recent report (1).

Normal fibrinogen was purified from the Anticoagulant Citrate Dextrose Solution USP (Fenwal Laboratories, Div. of Travenol Laboratories, Deerfield, Ill.) and plasmas of six healthy donors were purified by the glycine precipitation method of Kazal et al. (5) modified as previously described (1). Patient fibrinogen was purified from plasma collected in 3.8% sodium citrate by the same method. The recovery of fibrinogen varied between 55 and 75% and the fibrinogen was 96% clottable.

Total sialic acid content of the purified fibrinogen was measured after acid hydrolysis by the thiobarbituric acid method of Warren (6). Asialofibrinogen was prepared by incubating six normal and four patient fibrinogens with 50 U of *Vibrio cholerae* neuraminidase (Behringwerke A. G., Marburg/Lahn, West Germany) per mg of protein for 2.5 h as previously described (4). The released sialic acid was measured by the thiobarbituric acid assay, and the residual sialic acid of the protein was measured by the same method after acid hydrolysis (6).

The thrombin clotting times of the purified fibrinogen and asialofibrinogen were performed by adding to 0.1 ml of fibrinogen at a concentration of 2.0 mg/ml in 0.02 M sodium citrate, 0.15 M NaCl (pH 7.4), 0.2 ml of imidazole-buffered saline (0.15 M NaCl, 0.045 M imidazole, pH 7.4) followed by 0.1 ml of thrombin (10 U bovine thrombin [Parke, Davis & Co., Detroit, Mich.] per ml imidazole-buffered saline).

Sodium dodecyl sulfate (SDS)[1]-polyacrylamide gel electrophoresis of the purified fibrinogen and asialofibrinogen reduced with β-mercaptoethanol was performed using 7.5%

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[1] *Abbreviations used in this paper*: PAS, periodic acid-Schiff; SDS, sodium dodecyl sulfate.

gels according to the method of Weber and Osborn (7). After electrophoresis, the gels were stained with Coomassie Blue and densitometric scans were made using a Gilford gel scanner (Gilford Instrument Laboratories Inc., Oberlin, Ohio). In addition, the gels of the reduced fibrinogen were stained with periodic acid-Schiff reagent (PAS) (8).

RESULTS

Fibrinogen purified from six normal subjects had a mean of 6.0 sialic acid residues per molecule with a range of 5.7–6.3. In contrast, all 12 patient fibrinogens exhibited increased sialic acid content ranging from 7.1 to 10.8 residues per molecule. The relationship between the sialic acid content and the prolongation of the thrombin time is shown in Fig. 1. The normal control thrombin time ranged between 19 and 23 s with a mean of 22 s, whereas the thrombin times of the patient fibrinogens ranged from 31 s to 50 s. The prolongation of the thrombin time of the purified protein was similar to that of the respective patient's plasma. The 12 abnormal fibrinogens exhibited a progressive delay in the thrombin time with increasing sialic acid content. There was a linear relationship between the prolongation of the thrombin time and increasing sialic acid content with a correlation coefficient of +0.91 and $P < 0.001$ (9). The most abnormal fibrinogen was found in a patient with acute toxic hepatitis with liver failure induced by acetaminophen overdose. This fibrinogen had a sialic acid content of 10.8 residues and a thrombin time of 50 s.

Incubation of normal and patient fibrinogens with *Vibrio cholerae* neuraminidase resulted in the production of desialylated derivatives with <1.0 residue of sialic acid per molecule remaining except in the case of patient 4. This patient's fibrinogen contained 1.5 residues after treatment. Enzymatic hydrolysis of >80% of the sialic acid from each of the patient fibrinogens resulted in shortening of its thrombin time to the range of the desialylated normal control (Fig. 2).

SDS-polyacrylamide gel electrophoresis of each of the 12 reduced patient fibrinogens and of the 4 reduced patient asialofibrinogens demonstrated normal mobility and amounts of Aα-, Bβ-, and γ-chains with no evidence of proteolysis (Fig. 3). The distribution of carbohydrate on the constituent chains of normal and patient fibrinogens was studied by staining the SDS-polyacrylamide gels of the reduced protein with PAS reagent (Fig. 3). Only the Bβ-, and γ-chains stained with the PAS reagent.

DISCUSSION

Abnormal fibrin monomer polymerization is the most common functional defect reported both in the congenital dysfibrinogenemias (10) and in the acquired dysfibrinogenemias associated with liver disease (1, 2) and with hepatoma (11, 12). However, in neither the acquired nor the congenital dysfibrinogenemias characterized exclusively by an abnormality of fibrin monomer polymerization has the biochemical basis for this defect been defined. Abnormalities of the carbohydrate moiety of the fibrinogen molecule have been

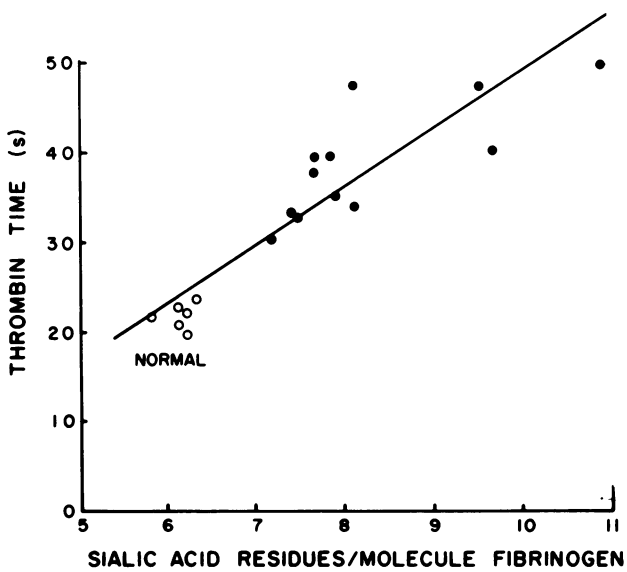

FIGURE 1 Comparison of sialic acid content vs. thrombin time of normal (○) and 12 patient (●) purified fibrinogens. Linear regression analysis of the data revealed a correlation coefficient of +0.91. The equation of the thrombin time vs. sialic acid content is $Y = 6.51x - 15.32$.

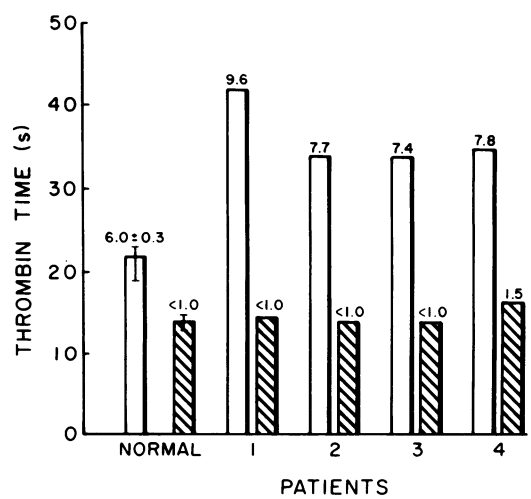

FIGURE 2 Thrombin times of normal and four patient fibrinogens before (open bars) and after (crosshatched bars) desialylation with neuraminidase. The normal control thrombin times are expressed as the mean ±1 SD. Numbers above the bars represent sialic acid residues per molecule of fibrinogen.

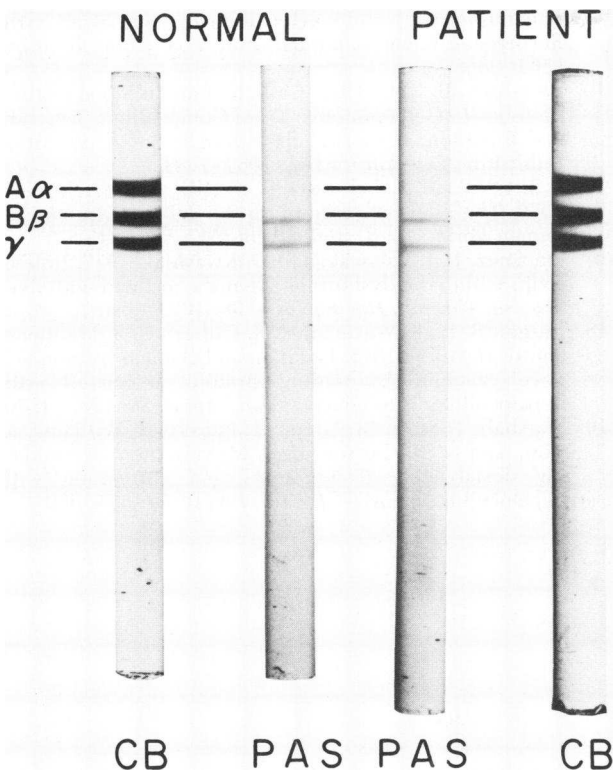

FIGURE 3 Electrophoresis in 7.5% SDS-polyacrylamide gels of reduced normal and patient fibrinogens. Protein was stained with Coomassie Blue (CB) and carbohydrate was stained with periodic acid-Schiff reagent (PAS).

described in several of the genetic dysfibrinogenemias, including fibrinogens Nancy and Paris 11 (10). Both fibrinogens Nancy and Paris 11 are reported to have an increased sialic acid content and both are functionally characterized by an abnormality of fibrin monomer polymerization (13, 14).

We have studied the purified fibrinogen of 12 patients with the dysfibrinogenemia associated with liver disease, and have demonstrated that this abnormal fibrinogen has an increased sialic acid content ranging from 12.7 to 71.4% higher than the normal control. The prolongation of the thrombin time of these fibrinogens strongly correlates with the increased sialic acid content.

Removal of sialic acid from normal fibrinogen results in an asialoderivative which exhibits a thrombin time shorter than the normal control due to the enhanced polymerization of the asialofibrin monomer (4). Enzymatic removal of >80% of the sialic acid from the abnormal fibrinogen resulted in a desialylated derivative whose thrombin time was similar to that of the asialoderivative of normal fibrinogen. The slight prolongation of the thrombin time of patient 4 fibrinogen after treatment with neuraminidase may reflect the incomplete removal of its sialic acid complement.

The distribution of carbohydrate on the constituent chains of the normal and patient fibrinogens was also investigated. The Aα-chain of normal fibrinogen contains little or no carbohydrate whereas significant quantities are present on its Bβ- and γ-chains (15, 16). Only the Bβ- and γ-chains of the reduced patient protein run in SDS-polyacrylamide gels stained with PAS reagent suggesting that the excess sialic acid is also located on these chains.

Our studies demonstrate that the excess sialic acid complement of this abnormal fibrinogen plays an important role in its functional defect. Studies involving the enzymatic removal of only excess sialic acid only and the effects of this partial desialylation on fibrin monomer polymerization are currently in progress (17).

Altered carbohydrate content of plasma glycoproteins has been described in patients with a variety of liver diseases. An R-type vitamin B_{12}-binding protein and a thyroxine-binding globulin have been reported to have abnormal sialic acid content in certain patients with liver disease (18, 19). Our studies taken together with these reports suggest that patients with a variety of liver diseases may exhibit a disturbance of the carbohydrate content of several of the plasma glycoproteins synthesized by this organ, and this alteration of the carbohydrate moiety may, in some cases, be responsible for a functional defect of the protein.

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