Three Types of Vitronectin in Human Blood

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ABSTRACT. Vitronectin is a cell-adhesive glycoprotein in serum and plasma, also termed serum spreading factor and complement S-protein. It consists of a mixture of a polypeptide of molecular weight 75 kilodalton (kDa) and its nicked product of 65 kDa plus 10 kDa. By a quantitative immunoblotting assay, human blood samples could be classified into three distinct vitronectin types; type I (58% of the population) was 75 kDa rich and 65 kDa poor, type II (35% of the population) contained approximately equal amounts of 75 kDa and 65 kDa, and type III (5% of the population) was 75 kDa poor and 65 kDa rich. The vitronectin type did not correlate with age, sex, or ABO blood type.

Vitronectin is a 75 kilodalton (kDa) glycoprotein in plasma and tissue (4), also termed serum spreading factor (2) and complement S-protein (7, 11). It promotes attachment and spreading of animal cells *in vitro* (2, 11), inhibits cytolysis by the complement C5b-9 complex (9), and modulates antithrombin III-thrombin action in blood coagulation (5, 6, 10, 12). The concentration of vitronectin in plasma and serum is 0.1–0.4 mg/ml (2, 4), and it does not correlate substantially with age or sex (13). The primary structure of vitronectin has been deduced from its cloned cDNA sequence (7, 14). Vitronectin is usually detected as a mixture of 75 kDa and 65 kDa polypeptides (2–4), in which the 65 kDa polypeptide is derived from the amino terminal portion of the 75 kDa polypeptide (15). This paper describes a very sensitive assay for individual 65 kDa and 75 kDa polypeptides and classifies human sera and plasma into three vitronectin types depending on the ratios of 65 kDa to 75 kDa polypeptides of vitronectin.

MATERIALS AND METHODS

Preparation of vitronectin and anti-vitronectin Antiserum. Human vitronectin and anti-human vitronectin rabbit antibody were prepared as described previously (1, 3) or by our recent procedure (17).

Human Serum and Plasma. Human blood was collected from 104 healthy human

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Abbreviation used: kDa, kilodalton.

volunteers, clotted at room temperature for 1 h followed by incubation at 4°C overnight, and centrifuged at $12,000 \times g$ for 2 min in a Beckman Microfuge B. The supernatant (serum) was stored at -80°C . Blood was also collected randomly from outpatients at Jichi Medical School and immediately anticoagulated with 1/10 volume of 3.18% trisodium citrate dihydrate. Plasma was harvested by centrifugation at $3,000 \times g$ for 30 min at room temperature and stored frozen until use.

Quantitative assay of the 75 kDa and 65 kDa vitronectin polypeptides. For quantitative assay of the 75 kDa and 65 kDa vitronectin polypeptides, the indicated amounts of human plasma and serum were subjected to SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (8) after chemical reduction with 0.1 M dithiothreitol. Immunoblotting was performed essentially according to Towbin *et al.* (16). The serum proteins separated in the SDS gel were transferred to a nitrocellulose sheet (Schleicher and Schuell, Inc., type BA-85, 0.45 μm pore size, Keene, New Hampshire) in 5 mM sodium tetraborate at 4°C for 90 min at 90 V in a Trans-blot cell (Bio-Rad Japan, Tokyo). The nitrocellulose sheet was blocked with 0.2% (w/v) skim milk (Yuki-jirushi Milk Co., Tokyo) in phosphate-buffered saline. Vitronectin on the sheet was allowed to react with rabbit anti-vitronectin antiserum at 1/3,000 dilution followed by the second antibody consisting of horseradish peroxidase-conjugated goat antibody against rabbit IgG at 1/1,500 dilution. Color for the bound peroxidase was developed with 34 $\mu\text{g}/\text{ml}$ *o*-dianisidine and 0.01% H_2O_2 in phosphate-buffered saline. Two-dimensional densitometry of the 75 kDa and 65 kDa bands on the nitrocellulose sheets was performed with a graphic analyzer Shonic GA (Showa Denko Co., Tokyo) equipped with a personal computer PC-9801F2 (NEC Co., Tokyo) utilizing a custom program termed Sumiko 1. Statistical analysis was performed using the Student's *t*-test.

RESULTS AND DISCUSSION

Immunoblotting indicated that the patterns of vitronectin polypeptides in human serum could be readily classified into three types characterized by high, medium, or low ratios of 65 kDa to 75 kDa polypeptides (Fig. 1). The two bands migrated at 80 kDa and 72 kDa in our system, but the terms 75 kDa and 65 kDa are retained for consistency with the previous nomenclature. Individual 75 kDa and 65 kDa polypeptides of vitronectin stained by anti-vitronectin antiserum were quantitated by a two-dimensional densitometry of the polypeptide bands on nitrocellulose blots. Figure 2 shows a standard curve expressed as the sum of the 75 kDa and 65 kDa vitronectin polypeptides; the standard deviation was approximately $\pm 10\%$. The densitometric value increased linearly with the amount of plasma in the range of 25–175 nl, roughly corresponding to 5–35 ng vitronectin. Since staining intensity of the band slightly varied from blot to blot, an internal vitronectin standard of 20 ng was included in every blot in the following experiments.

Systematic quantitative analyses were performed with aliquots of 100 nl of human sera from 104 healthy volunteers as well as 111 nl of human plasma from 100 outpatients. Samples of 202 out of 204 contained detectable amounts of the 65 kDa and/or 75 kDa polypeptides. They were readily classified into three distinct groups according to 65 kDa/(65 kDa + 75 kDa) ratios: ≥ 0 and < 0.35 (type I), ≥ 0.35 and < 0.85 (type II), and ≥ 0.85 and ≤ 1.0 (type III) comprising 58%, 35%, and 5% of the samples tested, respectively (Figs. 1 and 3). This population substantially differed neither between serum and plasma nor between healthy volunteers and outpatients. One of the remaining 2 lots of blood did not contain the 65 kDa or 75 kDa vitronectin, but rather 60 kDa and 52 kDa polypeptides stained by anti-

Fig. 1. Immunoblotting of human serum with anti-vitronectin antiserum. Different lots of human sera were immunoblotted and stained with anti-vitronectin antiserum. Samples were chemically reduced with 0.1 M dithiothreitol; typical type I (lane 2), type II (lane 3), and type III (lane 4). Lane 1 shows a typical serum protein pattern stained by Coomassie blue using the same amount of serum protein as for immunoblotting. Size markers (in kilodaltons) are shown at the left.

vitronectin antibody. The other lot of blood contained an approximately 25-fold lower concentration of vitronectin, and the vitronectin pattern was similar to type III. The three vitronectin types did not correlate with age (subjects were between 21 and 65 years), sex, or ABO blood type.

The total concentration of vitronectin in serum and plasma was estimated as the summation of the amounts of 75 kDa and 65 kDa polypeptides. The concentration differed neither between serum and plasma nor between healthy volunteers and outpatients. The means and standard deviations for vitronectin concentrations were 0.206 ± 0.079 mg/ml for type I, 0.196 ± 0.069 mg/ml for type II, and 0.176 ± 0.060 mg/ml for type III, respectively (Fig. 4). However, statistical analysis using the Student's t-test indicated that the difference in the three concentrations was not significant at $p < 0.1$.

The biological importance of the vitronectin type in human serum and plasma is not known, and no functional differences between 75 kDa and 65 kDa polypeptides have been reported. Since the 65 kDa polypeptide appears to be a post-translational cleavage product of the 75 kDa polypeptide (14, 15), it is assumed that the 75 kDa vitronectin is degraded to 65 kDa by some protease in the blood. The ratio of

Fig. 2. Standard curve for the vitronectin assay. The indicated amounts of human plasma were immunoblotted. Bands stained by anti-human vitronectin antibody were measured by two-dimensional densitometry and expressed as a summation of the 75 kDa and 65 kDa polypeptides.

Fig. 3. The three vitronectin blood types. The ratios of 65 kDa/(65 kDa + 75 kDa) polypeptides of vitronectin were measured for 104 healthy human sera and 100 outpatient human plasma samples.

Fig. 4. Total vitronectin concentrations in each of the three blood types. Vitronectin concentrations were determined as a summation of the 65 kDa and 75 kDa polypeptides.

65 kDa/(65 kDa + 75 kDa) polypeptides did not, however, change during storage of serum at 4°C for 3 days, nor at -20°C for at least 6 months with 6 cycles of freezing and thawing.

The vitronectin blood type may reflect the activity of a blood protease cleaving vitronectin 75 kDa to 65 kDa, or the presence of two nonidentical 75 kDa polypeptides, *i.e.*, protease-susceptible and nonsusceptible populations. Future biochemical and biological studies of vitronectin should take into account this potential source of variability.

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