Structural and immunological differences between human platelet and endothelial thrombospondins

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The structural and immunological properties of human thrombospondins isolated from platelets and from endothelial cells were compared. Both thrombospondins were digested with either trypsin or thermolysin, in the presence or absence of calcium, then injected onto a Superose 12 gel filtration column. The isolated thermolysin-generated fragments of thrombospondins were identified by radioimmunoassays using either different monoclonal antibodies or a polyclonal antibody directed against platelet thrombospondin. The results show that platelet and endothelial thrombospondins are both partially protected from trypsin digestion in the presence of calcium but have different trypsin and thermolysin fragmentation patterns. The thermolysin-generated fragments from platelet and endothelial thrombospondins are recognized differently by a monoclonal antibody whereas all of them are identified by a polyclonal antibody.

Thrombospondin (Human endothelial cell, Platelet) Gel filtration chromatography Monoclonal antibody Radioimmunoassay

1. INTRODUCTION

Thrombospondin, like fibronectin [1], is secreted by different cells including platelets and endothelial cells [2–5], and is composed of multiple domains which differ in their biological activities [4,6–9].

All thrombospondins have been found to be immunologically indistinguishable [2,3,5] and have similar amino acid compositions [5]. Similar results were obtained with fibronectins isolated from plasma and from cells in culture [1]. However, it is now apparent that fibronectins differ in several respects [1] including carbohydrate structure [1], domain structure [10] and immunological properties [10].

Because of the structural and immunological differences between fibronectins taken together with the apparent homology between fibronectin and thrombospondin, we have compared the structural and immunological properties of human thrombospondins isolated from platelets and from endothelial cells.

2. MATERIALS AND METHODS

Human endothelial cells from untraumatised umbilical cord veins were cultured using methods and materials described by Hunter et al. [11]. Cell supernatants were harvested at confluence. Endothelial and platelet thrombospondins were purified as in [12,13].

For the enzymatic digestion, platelet and endothelial thrombospondins were treated at a ratio of 1:10 (w/w) with trypsin-TPCK (Millipore), for 2 min at room temperature or with thermolysin (Sigma) at a ratio of 1:20 (w/w) for 90 min at 37°C. Digestion was terminated by addition of

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soybean trypsin inhibitor (Sigma) in a 3-fold (w/w) excess (trypsin) or by addition of 10 mM EDTA (thermolysin). In some experiments thrombospondins were incubated overnight at room temperature with 10 mM EDTA before trypsin digestion.

A Superose 12 HR 10/30 gel filtration column connected to an FPLC system (Pharmacia) was used to compare the products of trypsin and thermolysin digestion of platelet and endothelial thrombospondins.

For the immunodetection of isolated proteolytic fragments of thrombospondins a solid-phase radioimmunoassay [13] and a competitive radioimmunoassay [14] were used.

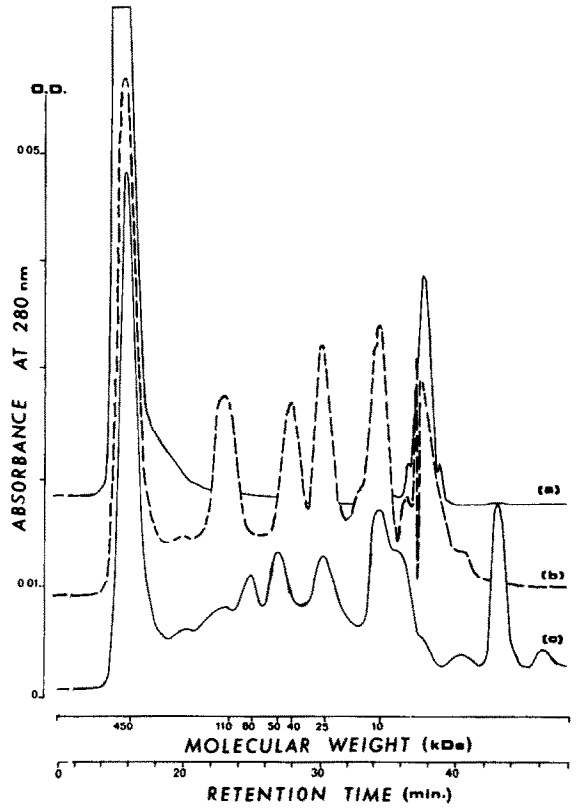

Fig.1. Separation of tryptic fragments from human platelet and endothelial thrombospondins using Superose 12 gel filtration chromatography. (a) Purified thrombospondin in the presence of 10 mM EDTA. (b) EDTA-treated platelet thrombospondin digested for 2 min at room temperature with trypsin-TPCK. (c) EDTA-treated endothelial thrombospondin under the same conditions as (b). The Superose 12 column was equilibrated and eluted with 20 mM triethanolamine buffer (pH 7.4) containing 0.35 M NaCl. The flow rate was 0.5 ml/min.

3. RESULTS

When human platelet or endothelial thrombospondin, either calcium treated or incubated with 10 mM EDTA, was injected on a Superose 12 gel filtration column, a single peak was observed at 450 kDa (fig.1a). Digestion of EDTA-treated platelet thrombospondin with trypsin generated 4 major tryptic fragments (110, 40, 28 and 10 kDa) (fig.1b) having apparent molecular masses different from those observed with EDTA-treated endothelial thrombospondin (120, 82, 54, 29 and 11 kDa) (fig.1c). Trypsin digestion performed with human platelet or endothelial thrombospondin purified in the presence of 2 mM calcium showed that the 110 and 11 kDa tryptic fragments of EDTA-treated platelet and endothelial thrombospondins were not observed in the presence of calcium (table 1). Digestion of calcium-treated

Table 1

Trypsin-generated fragments of platelet and endothelial thrombospondins obtained in the presence or absence of calcium

		Molecular mass of fragment (kDa)
Platelet thrombospondin	−	450
+ EDTA[ac]	+	450 110 40 28 10
Platelet thrombospondin	−	450
+ Ca^{2+} [bc]	+	450 40 28 10
Endothelial thrombospondin	−	450
+ EDTA[ac]	+	450 120 82 54 29 11
Endothelial thrombospondin	−	450
+ Ca^{2+} [bc]	+	450 120 82 54 29

[a] Platelet and endothelial thrombospondins, incubated overnight at room temperature in the presence of 10 mM EDTA, were injected on a Superose 12 gel filtration column with (+) or without (−) prior treatment with trypsin-TPCK

[b] Platelet and endothelial thrombospondins, purified in the presence of 2 mM Ca^{2+}, were injected on a Superose 12 gel filtration column with (+) or without (−) prior treatment with trypsin-TPCK

[c] The trypsin to thrombospondin ratio was 1:10 (w/w) at room temperature for 2 min

platelet thrombospondin with thermolysin generated 5 main fragments (110, 55, 30, 12 and 10 kDa) (fig.2b) having apparent molecular masses different from those obtained with endothelial thrombospondin (120, 80, 45, 25 and 10 kDa) (fig.2c).

Table 2

Solid-phase radioimmunoassay (SPRIA) of thermolysin-generated fragments from platelet thrombospondin using different monoclonal antibodies (P9, P10, P11, P12) directed against whole platelet thrombospondin

		Thermolysin-generated fragments[a] (kDa)					
		450	110	55	30	12	10
SPRIA[b]	P9	+	+	+	−	−	−
	P10	+	+	+	−	+	+
	P11	+	+	+	−	+/−	−
	P12	+	+	+	−	−	−

[a] The digestion of thrombospondin with thermolysin was performed in the presence of 2 mM Ca^{2+}. The protease to thrombospondin ratio was 1:20 (w/w) at 37°C for 90 min

[b] Symbols indicate a positive (+), a slightly positive (+/−) or a negative (−) binding of antibody compared to control

Table 3

Radioimmunoassay (RIA) of thermolysin-generated fragments from endothelial thrombospondin using a polyclonal and a monoclonal antibody (P10) directed against whole platelet thrombospondin

		Thermolysin-generated fragments[a] (kDa)					
		450	120	80	45	25	10
RIA[b]	polyclonal antibody	+	+	+	+	+	+
	monoclonal antibody	+	−	−	+/−	+/−	+

[a] The digestion of thrombospondin with thermolysin was performed in the presence of 2 mM Ca^{2+}. The protease to thrombospondin ratio was 1:20 (w/w) at 37°C for 90 min

[b] Symbols indicate a positive (+), a slightly positive (+/−) or a negative (−) binding of antibodies compared to control

Isolated thermolysin-generated fragments of platelet thrombospondin were immunodetected in a solid-phase radioimmunoassay with 4 ^{125}I-labelled monoclonal antibodies (P9, P10, P11, P12) directed against platelet thrombospondin (table 2).

Since most of the thermolysin-generated fragments of platelet thrombospondin were recognized by monoclonal antibody P10 (see table 2), we chose to use this antibody to immunodetect the thermolysin-generated fragments of endothelial thrombospondin. The fragments of endothelial thrombospondin were tested in a com-

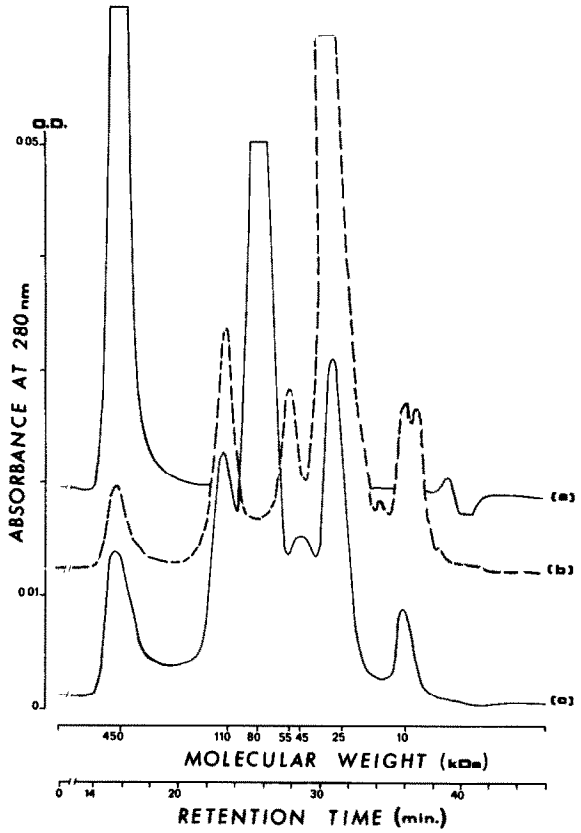

Fig.2. Separation of thermolysin-generated fragments from human platelet and endothelial thrombospondins using Superose 12 gel filtration chromatography. (a) Purified thrombospondin in the presence of 2 mM calcium. (b) Calcium-treated platelet thrombospondin digested for 90 min at 37°C with thermolysin. (c) Calcium-treated endothelial thrombospondin under the same conditions as (b). Experimental conditions of chromatography were similar to fig.1.

petitive radioimmunoassay using both ^{125}I-labelled whole platelet thrombospondin and either a polyclonal or a monoclonal antibody (P10) directed against platelet thrombospondin. All the proteolytic fragments of endothelial thrombospondin were recognized by the polyclonal antibody (table 3) whereas monoclonal antibody P10 only recognized 3 fragments (45, 25 and 10 kDa) (table 3).

4. DISCUSSION

The results demonstrate that platelet and endothelial thrombospondins are partially protected from limited proteolysis by trypsin in the presence of calcium. Removal of calcium from platelet thrombospondin with EDTA was shown to induce a partial denaturation of the thrombospondin nodular domains and an increased sensitivity to proteolysis by trypsin [4]. Such changes may also explain the increased sensitivity of endothelial thrombospondin to proteolysis in the presence of EDTA.

A clear difference in the fragmentation patterns of platelet and endothelial thrombospondins by trypsin and thermolysin was observed in this study. In agreement with previous studies [2,3,5], platelet and endothelial thrombospondins, in their native state, were immunologically indistinguishable since a polyclonal antibody and a monoclonal antibody (P10) recognized both forms of thrombospondin. In addition, the use of a polyclonal antibody which recognized all the proteolytic fragments of platelet and endothelial thrombospondins indicates that these two forms of thrombospondin share common determinants. These results are consistent with the fact that platelet and endothelial thrombospondins have similar amino acid compositions [5]. However, the pattern of detection of proteolytic fragments by a monoclonal antibody emphasises the different cleavage patterns of platelet and endothelial thrombospondins. The structural and immunological bases for the differences observed between platelet and endothelial thrombospondins are unknown. However, since changes in the glycosylation of glycoproteins take place in human endothelial cells as they approach confluence in culture [15], it is conceivable that an altered glycosylation pattern is found in thrombospondin isolated from culture supernatants of confluent endothelial cells. The carbohydrate moiety of human endothelial thrombospondin does appear to protect partially the glycoprotein against proteolysis [16]. Such changes in the carbohydrate moiety of endothelial thrombospondin could therefore affect the enzymatic proteolysis of this glycoprotein and thus account for the structural differences observed between the two forms of thrombospondins. Further work is in progress to clarify the role of carbohydrate structure in the polymorphism of platelet and endothelial thrombospondins.

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