Glomerular Basement Membrane

STUDIES ON ITS STRUCTURE AND INTERACTION WITH PLATELETS

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Vascular basement membrane, isolated from the capillary wall of the bovine renal glomerulus, induced the irreversible aggregation of sheep and human platelets in platelet-rich plasma. The aggregation was inhibited by 2-methylthio-AMP and apyrase and was associated with the release of \([^{14}C]\) serotonin from prelabeled platelets, suggesting that it was mediated primarily by ADP released from platelets. The noncollagenous and collagenous domains of the glomerular membrane were excised in soluble form by digestion with bacterial collagenase and pepsin, respectively, as an approach to investigating structural features which confer platelet-aggregating activity and to further examine the chemical and physical properties of these domains. A noncollagenous complex, composed of several polypeptides \((M_r = 100,000 \text{ to } 500,000)\) cross-linked by disulfide bonds, was isolated and found to induce irreversible platelet aggregation. The aggregation was not associated with the release of \([^{14}C]\) serotonin from prelabeled platelets, nor was the aggregation response inhibited by 2-methylthio-AMP or apyrase, suggesting that it was not mediated via the platelet release reaction. A collagenous complex, composed of several polypeptides \((M_r = 90,000 \text{ to } 300,000)\), certain of which are cross-linked by disulfide bonds, was isolated which induced irreversible platelet aggregation and was characterized by a long lag time to onset of aggregation. The lag time could be shortened by preincubating the complex under conditions which are conducive to collagen fibril formation. The aggregation response of the recombinant noncollagenous and collagenous complexes was not identical with that of the native basement membrane, but did indicate some interaction between the two complexes. These results suggest that the expression of platelet-aggregating activity by the native basement membrane is dependent upon the structural integrity of both the noncollagenous and collagenous domains and possibly upon an interaction between them.

A primary step in the arrest of bleeding following vascular injury and in the formation of mural thrombi involves the adhesion of platelets to exposed subendothelial connective tissues, in particular, collagen fibrils and basement membrane of the vessel wall (2-8). Platelet adhesion to the subendothelial results in the formation of prostaglandin endoperoxides and thromboxane \(A_2\) and in the release of platelet ADP, leading ultimately to the aggregation of platelets at the adhesion site (9-11). The mechanism by which collagen induces platelet adhesion and aggregation has been the subject of intensive investigation in recent years. Studies have concentrated on the role of the triple helical conformation (12-14), the quaternary structure (15-19), the primary structure (20-22), the amino groups (23), and the carbohydrate moiety (12, 13, 20, 21, 24-26) of collagen, using collagen from avascular sources.

The mechanism of the interaction of vascular basement membrane with platelets has not been investigated in detail. In vitro studies have shown that platelets adhere both to exposed arterial basement membrane, forming platelet aggregates (2, 8, 27-30), and to the exposed basement membrane of capillary vessels (30). Capillary basement membrane isolated from the renal glomerular capillaries was shown by electron microscopy to cause the in vitro aggregation of rat platelets in platelet-rich plasma (31). In recent years, a substantial amount of information has been obtained about the structure of this capillary basement membrane from bovine kidneys (32-40). This information provides a basis for investigating the mechanism of platelet interaction with vascular basement membrane.

The purpose of the studies reported here was to investigate the interaction of vascular basement membrane with platelets and to investigate the structural features of the membrane required for the interaction. The findings demonstrate that vascular basement membrane from renal capillaries induces platelet aggregation and that expression of platelet-aggregating activity is dependent upon the structural integrity of both noncollagenous and collagenous domains within the matrix of the basement membrane.

EXPERIMENTAL PROCEDURES AND RESULTS

Details of experimental procedures and results are presented in the miniprint supplement which follows.1

1 Portions of this paper, including "Experimental Procedures," "Results," "References," Figs. 1 to 14, and Tables I to III are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full-size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 78M-652, cite author(s), and include a check or money order for $4.95 per set of photocopies.
DISCUSSION

These studies demonstrate that vascular basement membrane isolated from the capillary wall of bovine renal glomerulus induces the in vitro aggregation of sheep and human platelets and that the rate and extent of platelet aggregation depends upon the concentration of basement membrane. Basement membrane-induced platelet aggregation was inhibited by 2-methylthio-AMP and apyrase which are potent inhibitors of the ADP-induced aggregation of sheep and human platelets. Moreover, the membrane induced the release of $[^3H]$serotonin from platelets which had been prelabeled with $[^3H]$serotonin. These findings indicate that glomerular basement membrane mediates platelet aggregation primarily by inducing the release of intraplatelet ADP. Two different vascular basement membranes, bovine lens capsule and Ascaris suum intestinal basement membrane, did not cause platelet aggregation, observations which may indicate that platelet-aggregating activity is unique to basement membranes of vascular origin.

It was of interest to identify the structural components which confer platelet-aggregating activity to the glomerular basement membrane. The membrane consists of multiple polypeptides that are cross-linked by disulfide bonds (32). These polypeptides, which are solubilized by reduction in SDS, range in molecular weight from 30,000 to greater than 700,000 (32, 36) and have amino acid compositions of varying degrees of relatedness to collagen (33, 35). Both collagenous and noncollagenous domains are present within this complex structure, as demonstrated by partial characterization of fragments solubilized on degradation of the membrane with pepsin (38, 39) and collagenase (37), respectively, but their structural relationship to the intact polypeptides remains undefined. The investigation to determine which particular components of the basement membrane possess platelet-aggregating activity could focus on either the intact polypeptides obtained by reduction or on the noncollagenous and collagenous domains obtained by proteolytic digestion. The present study focused on the latter because the methods for isolating the intact polypeptides require the use of rigorous denaturing conditions (34, 35) which would likely destroy their platelet-aggregating activity. This also permitted a further examination of the chemical and physical properties of the noncollagenous and collagenous domains of the membrane.

The noncollagenous domain was excised in soluble form by extensive digestion with bacterial collagenase and was found to have platelet-aggregating activity. Analysis of the solubilized components by SDS-polyacrylamide gel electrophoresis revealed the presence of several large polypeptides, which are solubilized by reduction in SDS, range in molecular weight from 30,000 to greater than 700,000 (32, 36) and have amino acid compositions of varying degrees of relatedness to collagen (33, 35). Both collagenous and noncollagenous domains are present within this complex structure, as demonstrated by partial characterization of fragments solubilized on degradation of the membrane with pepsin (38, 39) and collagenase (37), respectively, but their structural relationship to the intact polypeptides remains undefined. The investigation to determine which particular components of the basement membrane possess platelet-aggregating activity could focus on either the intact polypeptides obtained by reduction or on the noncollagenous and collagenous domains obtained by proteolytic digestion. The present study focused on the latter because the methods for isolating the intact polypeptides require the use of rigorous denaturing conditions (34, 35) which would likely destroy their platelet-aggregating activity. This also permitted a further examination of the chemical and physical properties of the noncollagenous and collagenous domains of the membrane.

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The aggregation response of human and sheep platelets induced by this noncollagenous complex was substantially different from that observed for the native basement membrane. Unlike that of the membrane, the complex induced aggregation without an initial platelet shape change or a lag phase to the onset of aggregation, and the platelet aggregation was not mediated by the platelet release reaction, as indicated by the absence of $[^3H]$serotonin release and the absence of inhibition of aggregation by 2-methylthio-AMP and apyrase, possibly indicating platelet agglutination. The platelet response to the complex is completely abolished upon limited pepsin digestion and upon disulfide cleavage. These results indicate that the expression of platelet-aggregating activity by the native basement membrane is dependent, in part, upon the structural integrity of a noncollagenous domain, consisting of several large polypeptides cross-linked by disulfide bonds.

The noncollagenous domain of the membrane was excised in soluble form by controlled pepsin digestion and was found also to have platelet-aggregating activity. Analysis of the solubilized components by SDS-polyacrylamide gel electrophoresis revealed the presence of several large polypeptides (M, > 500,000) with the majority of the material existing as a high molecular weight aggregate (M, > 500,000) which is composed of at least four polypeptides (M, = 100,000 to 200,000) cross-linked by disulfide bonds, findings which are in agreement with those reported by Daniels and Chu (39). These polypeptides, accounting for about 10% of the membrane by weight, are collagen based on amino acid composition and exist in a triple helical conformation having a thermal stability of >37°C based on circular dichroism measurements.

The aggregation response of platelets induced by these collagenous polypeptides as a mixture were characterized by long lag times before the onset of aggregation, unlike that of the native basement membrane. The lag period could be shortened by preincubating the collagen fraction under conditions which are typically conducive to collagen fibril formation. The aggregation was mediated primarily by the release of intraplatelet ADP, as evoked by the inhibition of platelet aggregation by apyrase. These results suggest that the collagenous domain, in the form excised from the membrane, has platelet aggregating activity upon association of triple helical components to form multimers, analogous to that observed with types I, II, and III collagens (15–18). Whether such multimers are related in quaternary structure to those which may exist in the native membrane is unknown at the present time. Nonetheless, the expression of platelet aggregating activity by the native membrane is dependent, in

1 The abbreviations used are: SDS, sodium dodecyl sulfate; GBM, glomerular basement membrane; PPR, platelet-rich plasma; PPF, platelet-poor plasma; 2-MeS-AMP, 2-methylthioadenosine 5'-phosphate; MalNEt, N-ethylmaleimide.
part, upon the structural integrity of the collagenous domain since degradation, of this entity with collagenase markedly alters the characteristics of the platelet aggregation response curves.

Since both noncollagenous and collagenous domains have platelet-aggregating activity after excision from the membrane, and since each induces a platelet aggregation response different from each other and from that induced by the whole membrane, it would appear that the expression of activity by the native basement membrane is dependent upon the coexistence of both domains and possibly upon an interaction between them. Recombination of these domains, however, did not regenerate a platelet response characteristic of that induced by the native membrane, although some degree of interaction was observed. The failure to regenerate full activity is not surprising because the noncollagenous and collagenous domains, as isolated, are unique membrane fragments derived upon proteolytic degradation of the membrane. Conceivably, these are derived from a larger highly organized network of polypeptides, with certain large polypeptides containing both noncollagenous and collagenous domains. The presence of both types of domains within a single polypeptide was previously postulated to account for the varying degrees of relatedness to collagen exhibited by intact polypeptides isolated without the use of proteases (33, 34).

In conclusion, these studies demonstrate that basement membrane of the capillary wall of bovine renal glomerulus causes the in vitro aggregation of sheep and human platelets. The findings are consistent with the observations that platelets adhere to exposed vascular basement membrane in vivo forming mural thrombi (7, 28, 29) and suggest that vascular basement membrane-induced platelet aggregation plays a role in hemostasis and thrombosis. The structural findings confirm the reports of others that glomerular basement membrane yields noncollagenous and collagenous components upon degradation with collagenase and pepsin, respectively (37, 39). The present studies further show that both types of proteolytic products are composed of dissimilar polypeptide chains held together by disulfide bonds and that the collagen polypeptides exist in a triple helical conformation.

Acknowledgments—We wish to express our thanks to Dr. D. N. Fass for assaying for bovine factor VIII/von Willebrand factor. The excellent technical assistance provided by Mrs. P. Todd and Mrs. A. De is appreciated.

REFERENCES

References are found on p. 9074.
Basement Membrane-induced Platelet Aggregation

Introduction

All experiments were performed on bovine platelets obtained from non-fasted donors. Platelet-rich plasma (PRP) was ab- stained from the blood of healthy donors and used within 2 hours of collection. PRP was prepared by mixing equal volumes of blood and 0.32 M sodium citrate (pH 7.4), centrifuging at 100 g for 10 minutes, and removing the supernatant. Platelets were then counted by a Sedgewick-Rafter counting chamber. Platelets were used within 1 hour of collection, and all experiments were performed between 1 and 4 hours after collection.

Methods

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Basement Membrane-induced Platelet Aggregation

Bovine glomerular basement membrane was obtained from a bovine kidney, and basement membrane preparation was performed as described elsewhere (41). Bovine glomerular basement membrane was then suspended in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 0.05 M ethylenediamine tetraacetic acid (EDTA) and 0.05 M sodium azide, and filtered through a 0.22-μm Millipore filter. The basement membrane preparation was then dialyzed against 0.9% saline and stored at 4°C. Platelet aggregation was induced by adding 0.1% of the final volume of basement membrane preparation to PRP. Platelet aggregation was monitored by measuring the percentage of platelets that were aggregates at 60 seconds. The aggregation was stopped by adding 100 μM of EDTA to the PRP.

Results

Aggregation of platelets induced by basement membrane was measured by the percentage of platelets aggregated at 1 minute after the addition of basement membrane. The percentage of platelets aggregated was determined by counting the platelets in a Sedgewick-Rafter counting chamber. The aggregation was considered to be significant when the percentage of platelets aggregated was greater than 50%.

Discussion

In conclusion, our results suggest that basement membrane is an effective agonist for platelet aggregation. The aggregation induced by basement membrane is not dependent on the presence of calcium or the release of ADP from platelets. These results are consistent with previous findings that basement membrane induces platelet aggregation in a calcium-dependent manner (41). Further studies are needed to elucidate the mechanism of platelet aggregation induced by basement membrane.

Footnotes

1 MalNNe was used to inhibit residual sulfhydryl protease activity of the purified collagenase preparation (41).

2 Bovine glomerular basement membrane also induces the aggregation of bovine platelets; J. L. Davis, B. C. Hudson, and K. L. Carraway, unpublished data.

References


The term "noncollagen" is used in this paper as a convenient expression to describe those components which have amino acid compositions greatly different from that of classical collagen; namely, much lower amounts of glycine, hydroxyproline, and hydroxylysine. Such components may, however, contain short segments of collagen-like amino acid sequences, consisting of the few hydroxyproline and hydroxylysine residues, which are resistant to collagenase cleavage. Alternatively, these few residues may be remnants of the collagenase cleavage of the collagen components or be positioned in the polypeptide chain in sequences unrelated to collagen.

5 Calculation based on 3 mannose residues/heteropolysaccharide unit and 1 glucose residue/disaccharide unit (57).
Basement Membrane-induced Platelet Aggregation

Table I

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>% of Total Amino Acid</th>
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<tr>
<td>Arginine</td>
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<td>Histidine</td>
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<td>Glycine</td>
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<tr>
<td>Asparatine</td>
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*Source: From Smith and colleagues.*

Figure 1(a): Aggregation of platelets by basement membrane. Platelets were incubated with basement membrane from glomeruli of normal rats and the aggregation was measured using a light transmittance aggregometer. The aggregation was measured at 37°C and was expressed as a percentage of the maximum aggregation. The results are shown as the mean ± SEM of three experiments. *P<0.05 compared to control.*

Figure 1(b): Effect of various concentrations of collagen on platelet aggregation. Platelets were incubated with collagen at different concentrations and the aggregation was measured using a light transmittance aggregometer. The aggregation was measured at 37°C and was expressed as a percentage of the maximum aggregation. The results are shown as the mean ± SEM of three experiments. *P<0.05 compared to control.*

Figure 1(c): Aggregation of platelets by basement membrane from glomeruli of normal rats in the presence of aspirin. Platelets were incubated with basement membrane from glomeruli of normal rats in the presence of aspirin at different concentrations and the aggregation was measured using a light transmittance aggregometer. The aggregation was measured at 37°C and was expressed as a percentage of the maximum aggregation. The results are shown as the mean ± SEM of three experiments. *P<0.05 compared to control.*

REFERENCES

Glomerular basement membrane. Studies on its structure and interaction with platelets.
J W Freytag, P N Dalrymple, M H Maguire, D K Strickland, K L Carraway and B G Hudson