Platelet adhesion to collagen

Factors affecting Mg^{2+} -dependent and bivalent-cation-independent adhesion

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Platelet adhesion to collagens immobilized on plastic has been measured, with the following results. (1) Human, but not rabbit, platelets adhered readily to pepsin-extracted monomeric collagens in an Mg^{2+} -dependent manner. (2) Rabbit platelets adhered to a monomeric collagen extracted without pepsin by a process that was cation-independent; human platelet adhesion to this collagen exhibited a cation-independent element. (3) Human platelet adhesion to polymeric collagens, including intact native fibres and those reconstituted from pepsin-extracted monomeric collagens, exhibited appreciable cation-independence; adhesion of rabbit platelets to these collagens occurred only by a cation-independent process; pepsin treatment of the intact fibres caused a reduction in cation-independent binding. Two mechanisms of adhesion can therefore be distinguished, one Mg^{2+} -dependent, expressed by human, but not rabbit, platelets, the other cation-independent and exhibited by platelets of both species. Mg^{2+} -dependent and cation-independent adhesion sites are located within the triple helix of collagen, but the latter sites are only expressed in collagen in polymeric form. In neither case is the helical conformation of the sites essential for their binding activity. Cation-independent adhesion sites are also located in the pepsin-sensitive non-helical telopeptides of collagen and can be expressed in both monomeric and polymeric collagens. Chemical modification of collagen lysine residues indicates that specific lysine residues may be involved in Mg^{2+} -dependent adhesion. Adhesion using human citrated platelet-rich plasma is Mg^{2+} -independent. Plasma contains factors, conceivably the adhesive proteins fibronectin and von Willebrand factor, that promote the Mg^{2+} -independent mechanism.

INTRODUCTION

The aggregation of blood platelets by collagens may be an important cause of thrombosis, especially that arising in diseased arteries after rupture of the atherosclerotic plaque [1–4], an event leading, in our view, to contact of collagens in the plaque [5,6] with platelets and subsequent deposition of a thrombus at the site of rupture.

The initial step in collagen–platelet interaction is the adhesion of platelets to the collagen surface. A highly specific Mg^{2+} -dependent adhesion has been described that is mediated by the platelet surface glycoprotein Ia/IIa, a protein identical with the integrin-type receptor known as VLA-2 (very late antigen 2) [7–10]. In accord with this, we sometimes observed in our previous work [11] a marked stimulation of platelet adhesion to collagen (bovine type I) by Mg^{2+} . We also noted, however, that under some circumstances appreciable adhesion occurred in the absence of bivalent cations. There are, indeed, many instances reported where platelet adhesion to collagen (fibres) has been recorded in the presence of EDTA (to remove Ca^{2+} to prevent aggregation) and hence it follows in the absence of bivalent cations (see, e.g., [12–21]). In the present study we have attempted to define more precisely those factors governing adhesion to collagen that may sometimes lead to a requirement for Mg^{2+} , whereas in other situations bivalent cations are not necessary. Our data suggest that the two processes may represent separate mechanisms displaying different collagen structural requirements and perhaps utilizing different platelet receptors.

MATERIALS AND METHODS

^{51}Cr as sodium chromate was obtained from Amersham International. BSA (Cohn fraction V) and crystalline pepsin

were purchased from Sigma Chemical Co. Falcon 1008 35 mm-diameter polystyrene Petri dishes were obtained from Becton Dickinson, Plymouth, U.K.

Collagens

Monomeric bovine collagen type I was prepared from calf skin, as described previously [22–24], both by extraction with a solution of sodium citrate [25] and also by digestion of the tissue with pepsin [26]. Human monomeric collagens types I and III were purified from a pepsin digest of placental tissue, as described in our previous studies [27,28].

A dispersion of native-type fibres of collagen type I from bovine tendon [27] was generously provided by Ethicon, Somerville, NJ, U.S.A. Before use, the dispersion was diluted to the required concentration with 0.01 M-acetic acid and dialysed against the same solution.

Fibres of human collagens I and III were reconstituted from a solution of the appropriate monomeric (pepsin-extracted) collagen by prolonged dialysis (over several days) at 4 °C against sodium phosphate buffer (0.02; pH 7.6).

Pepsin-digested fibres were obtained by treatment of the bovine tendon fibres, dispersed in 0.5 M-acetic acid, with pepsin at an enzyme/substrate ratio of 1:20 (w/w) for 24 h at 4 °C. After digestion, the suspension was adjusted to pH 8.0, then dialysed against 0.5 M-acetic acid. Fibres were collected by centrifugation (12000 g for 1 h at 4 °C), washed with 0.5 M-acetic acid, resuspended at 500 µg/ml in 0.5 M-acetic acid and finally dialysed against 0.01 M-acetic acid.

Collagen was polymerized as necessary by random association of molecules in solution by cross-linking with glutaraldehyde, precisely as we described previously [22–24]. The cross-linked polymer was subsequently redispersed at a known concentration

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in 0.01 M-acetic acid with a glass/Teflon homogenizer before its immobilization on plastic.

Monomeric collagen in solution was denatured as required by heating to 60 °C for 30 min. Bovine tendon fibres in suspension, or the polymer obtained from cross-linking monomeric collagen with glutaraldehyde, were denatured by heating to 90 °C for 15 min.

Human collagens I and III in solution, either native or denatured, or a suspension of collagen fibres, were treated with trinitrobenzenesulphonic acid as described previously [24,29], the conditions of Wilner *et al.* [30] being employed.

Platelets

⁵¹Cr-labelled gel-filtered platelets were prepared from citrated platelet-rich plasma of human or rabbit origin as described previously [11,22–24]. Additions, to the final concentration specified, of MgCl₂, CaCl₂, EDTA, trisodium citrate or BSA, were made as required to appropriate portions of the platelet suspension in standard Tris/HCl buffer [11], before measurement of adhesion.

Platelet adhesion to collagen

Adhesion of radiolabelled platelets was measured to collagens immobilized on plastic essentially as we have described previously [11], but with the following modification of the washing procedure used to remove non-adherent platelets. Unattached platelets in suspension were removed with a plastic Pasteur pipette, after which dishes received five 'vigorous' manual washes, using 1 ml of the same buffer employed in adhesion, in which the washing fluid each time was forcefully expelled from a Pasteur pipette, ensuring that all parts of the dish were washed in the course of this procedure. The washing was repeated with a second 1 ml of buffer. In our previous work [11], washing had been achieved by two very gentle rinses in which the washing buffer was introduced very much more cautiously. Levels of adhesion to collagen were only slightly reduced by the more vigorous washing, but the 'background' level, as determined by measuring adhesion to albumin, previously relatively high, was now reduced to insignificant values, being in most instances less than 1 % of the total number of platelets applied.

After washing, adherent platelets were lysed with two extractions with 2 % (w/v) SDS, and the extent of adhesion was determined from the released radioactivity as described previously [11].

Assays were performed in triplicate. The platelet concentration was usually of the order of 1.5×10^8 /ml for human platelets and 2×10^8 /ml in the case of rabbit platelets. However, the precise number of platelets varied slightly from experiment to experiment. Adhesion is expressed as a percentage of the total platelets applied rather than the actual number attached, since we have observed that percentage adhesion is largely independent of platelet concentration (within the range used in these experiments) and therefore represents the appropriate value for comparison of results when platelet numbers vary. Values are given as means of triplicate determinations, which generally did not vary, one from another, by more than 10 %.

In some experiments adhesion was measured using platelet-rich plasma rather than gel-filtered platelets. Platelet-rich plasma was diluted with platelet-poor plasma to the desired concentration. Adhesion was determined from the radioactivity associated with adherent platelets compared with total platelet-associated radioactivity. The latter was measured by spinning a portion of ⁵¹Cr-labelled platelet-rich plasma [10 min at full speed (approx. 10000 g) in a micro-haematocrit centrifuge], washing the pellet twice with Tris/HCl buffer [11], and then counting the radioactivity in the final pellet.

RESULTS

Adhesion of human platelets

Human platelets, under the standard conditions employed here, utilizing Tris/HCl buffer containing 2 mM-Mg²⁺, adhered well to human monomeric collagens I and III isolated by pepsin digestion, and to bovine monomeric collagen I similarly isolated, when these three collagens were immobilized on plastic. Representative data are shown in Fig. 1 and Table 1. Adhesion occurred to both native and denatured species, usually to a similar extent (Fig. 1*b* and Table 1, *b*). Adhesion increased with time (Fig. 1*a*). The level of adhesion was independent of the concentration of collagen used for coating within the range tested of 1–250 µg/ml (Fig. 1*b*). These results are comparable with those we obtained previously [11] for human platelet adhesion to monomeric bovine collagen I extracted with acid citrate buffer (without the use of pepsin). In our previous study we had observed that the adhesion of human platelets to the latter collagen was improved in some measure by the presence of Mg²⁺. In the present study, adhesion to pepsin-digested collagen I of bovine or human origin was always very markedly stimulated by Mg²⁺, more often than not, in the case of the native collagen, being totally dependent on the presence of this cation, with no adhesion at all occurring when EDTA was present (see, e.g. Table 1, *a* and *b*, and Table 2). The dependence on Mg²⁺ was the same whatever the concentration of collagen employed for coating (cf. Table 1, *a* and *b*, with Table 1, *g*). In agreement with the findings of Santoro [7], not only was Ca²⁺ ineffective at supporting this adhesion, it was also strongly inhibitory of the Mg²⁺-dependent process (Table 2). Adhesion to collagen III also revealed a strong stimulation by Mg²⁺, although adhesion in the presence of EDTA was rarely zero (Table 1, *a* and *b*). On the other hand, adhesion to non-pepsin-treated collagen I extracted with citrate buffer regularly exhibited an appreciable level of bivalent-cation-independent attachment. This cation-independent element tended to increase as the concentration of protein employed in coating was increased, and at relatively high coating concentrations it could reach a value as much as one-quarter to one-half of the optimum level achieved in the presence of Mg²⁺ (Table 1, *f* and *g*).

Good adhesion of human platelets also occurred to bovine tendon (type I) fibres immobilized on plastic. The level of adhesion was independent of the concentration of fibres used for coating over the range, as tested, of 10–500 µg/ml (Fig. 1*d*). The adhesion to fibres exhibited a definite cation-independent element that was especially evident at the higher coating concentrations, where adhesion was sometimes almost as good in the absence as in the presence of Mg²⁺ (Table 1, *c*, and Table 2). Similar results were obtained with fibres that had been denatured (Table 1, *c*). As might be anticipated, Ca²⁺ did not exert any inhibitory effect on the cation-independent process (Table 2). Pepsin digestion of the tendon fibres caused a reduction in the cation-independent binding, whereas overall adhesion remained unaffected (Table 1, *d*).

Reconstituted fibres of collagen I prepared from the pepsin-extracted monomeric collagen also showed some cation-independent binding, similar to that of the pepsin-treated bovine tendon fibres (Table 1, *e*, and Table 3, *c*). Consistently, adhesion to fibres of collagen III reconstituted from the pepsin-extracted monomeric collagen showed a marked degree of cation-independence (Table 1, *e*, and Table 3, *c*). Collagen (type III) polymerized by cross-linking the pepsin-extracted monomer with glutaraldehyde was also able to support some cation-independent binding, tested in either native or denatured form (Table 1, *h*).

Treatment of monomeric collagens and collagen fibres in native or denatured form with trinitrobenzenesulphonic acid did

Fig. 1. Adhesion of human and rabbit platelets to collagen

(a) Time course of adhesion of human platelets to pepsin-extracted human monomeric collagen III (□) and to BSA (●), and of rabbit platelets to citrate-extracted bovine monomeric collagen I (○). Petri dishes (35 mm diam.) were coated with a solution of the collagen in question at a concentration of 10 μg/ml. Adhesion was measured with a 1 ml platelet suspension containing, in the case of human

not alter the overall level of adhesion. However, Mg^{2+} -dependent adhesion to the monomeric species was largely abolished, and the cation-independent adhesion to collagen fibres already noticeable before treatment was even more marked after treatment (Table 3).

Adhesion of rabbit platelets

Adhesion of rabbit platelets to collagen never reached the levels attained with human platelets, at best being one-third to one-half of the latter. Adhesion was also much more variable as between one rabbit and another or from one time to another with any one given animal. Representative data are given in Fig. 1 and Table 4.

Of particular interest, and in marked contrast with the excellent adhesion of human platelets, no significant adhesion of rabbit platelets was ever observed to any of the three monomeric, pepsin-digested collagens (human collagens I and III and bovine I), whether native or denatured, and irrespective of the presence of Mg^{2+} or EDTA; and yet the monomeric bovine collagen I species prepared by citrate extraction (without pepsin) was consistently able to support adhesion in native or denatured form [Fig. 1c; Table 4, a and b]. This adhesion increased with time (Fig. 1a), was independent of the concentration of collagen used for coating within the range tested of 10–500 μg/ml (Fig. 1c) and was essentially a cation-independent process (Table 4, b).

Adhesion also occurred to immobilized collagen fibres (bovine tendon). In this case, adhesion increased as the concentration of fibres used for coating increased (Fig. 1d). Again, the adhesion was cation-independent. Pepsin digestion caused a reduction in the level of adhesion (Table 4, c). Adhesion also occurred by a cation-independent mechanism to fibres reconstituted from pepsin-extracted monomeric collagens (Table 4, d).

Adhesion of platelets in platelet-rich plasma

Adhesion was also tested by using human platelet-rich plasma in place of gel-filtered platelets. Citrated platelet-rich plasma is widely used to measure collagen-induced platelet aggregation. In the present study, for optimal adhesion, gel-filtered platelets were normally suspended in a standard Tris/HCl buffer containing 0.5% BSA and to which was added 2 mM- Mg^{2+} . As indicated above, adhesion under these conditions to monomeric collagen was good, reaching values (at 90 min) as high as about 60%. When, however, gel-filtered platelets were suspended in Tris/HCl buffer containing 3.5% BSA, together with 1.3 mM- Ca^{2+} ,

platelets, 1.3×10^8 gel-filtered platelets and, in the case of rabbit platelets, 2.4×10^8 , suspended in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} . (b) Adhesion of human platelets to human monomeric collagens I and III (extracted with pepsin). Dishes were coated at various protein concentrations as indicated. Adhesion was measured, at 90 min, with a 1 ml platelet suspension containing 1.2×10^8 gel-filtered platelets in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} . Collagens were denatured as required by heating at 60 °C for 30 min. ■, Native type I; □, denatured type I; ○, native type III; ●, denatured type III. (c) Adhesion of rabbit platelets to citrate-extracted bovine monomeric collagen I (○) and to pepsin-extracted human monomeric collagen I (●). Dishes were coated at various protein concentrations as indicated. Adhesion was measured, at 90 min, with a 1 ml platelet suspension containing 2.3×10^8 gel-filtered platelets in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} . (d) Adhesion of human (□) and rabbit (●) and ○ represent separate experiments) platelets to bovine tendon collagen type I fibres. Dishes were coated using a fibre suspension at various concentrations as indicated. Adhesion was measured, at 90 min, with a 1 ml platelet suspension containing, in the case of human platelets, 1.4×10^8 gel-filtered platelets and, in the case of rabbit platelets, 2.4×10^8 , suspended in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} .

Table 1. Adhesion of human platelets to various collagens

Dishes were coated at the concentrations ($\mu\text{g/ml}$) indicated in parentheses. Adhesion was measured, at 90 min, with a 1 ml platelet suspension containing on average 1.5×10^8 gel-filtered platelets in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} , 2 mM- Ca^{2+} or 2 mM-EDTA as indicated. (a)–(h) represent separate experiments.

Expt.	Collagen species	Adhesion (%)		
		Mg^{2+}	Ca^{2+}	EDTA
(a)	Human native monomeric, pepsin-extracted			
	Type I (10)	59	0	0
	Type III (10)	59	4	3
(b)	Human monomeric type I, pepsin-extracted			
	Native (10)	52	–	0
	Denatured (10)	49	–	4
	Human monomeric type III, pepsin-extracted			
	Native	55	–	4
	Denatured	50	–	10
(c)	Bovine tendon fibres			
	Native (10)	46	17	16
	Denatured (10)	45	29	22
	Native (500)	54	51	45
	Denatured (500)	52	36	40
(d)	Bovine tendon fibres, pepsin-treated			
	Control (500)	56	46	40
	Treated (500)	57	22	22
(e)	Reconstituted fibres			
	Human type I (500)	48	–	22
	Human type III (500)	52	–	41
	Human pepsin-extracted monomeric III (10)	47	–	–
(f)	Bovine monomeric type I, citrate-extracted			
	(10)	53	12	8
	(500)	48	27	27
(g)	Bovine monomeric type I			
	Pepsin-extracted (500)	61	–	1
	Citrate-extracted (10)	68	–	12
	Citrate-extracted (500)	68	–	17
(h)	Human monomeric type III pepsin-extracted, cross-linked with glutaraldehyde			
	Native (100)	52	–	15
	Denatured (100)	53	–	15

Table 2. Effect of Ca^{2+} on the adhesion of human platelets to collagen

Dishes were coated with pepsin-extracted monomeric bovine collagen I (at 10 $\mu\text{g/ml}$) or bovine tendon collagen type I fibres (at 500 $\mu\text{g/ml}$). Adhesion was measured at 90 min using gel-filtered platelets in a standard Tris/HCl buffer containing the addition indicated.

Addition	Adhesion (%)	
	Monomer	Fibre
2 mM- Mg^{2+}	55	62
2 mM- Ca^{2+}	1	52
2 mM- Mg^{2+} and 2 mM- Ca^{2+}	5	52
2 mM-EDTA	0	40

0.4 mM- Mg^{2+} and 0.38% trisodium citrate, concentrations calculated to mimic those in citrated platelet-rich plasma, then adhesion to monomeric collagen was zero, i.e. Mg^{2+} -dependent adhesion was totally abolished. When platelet-rich plasma itself

Table 3. Effect of modification of collagen lysine residues by treatment with trinitrobenzenesulphonic acid on platelet adhesion

(a)–(d) represent separate experiments. Monomeric collagens I and III were human pepsin-extracted collagens. Type I fibres were reconstituted from human pepsin-extracted monomeric collagen or were intact bovine tendon (collagen type I) fibres. Control samples were subjected to precisely the same experimental conditions as for treated samples, except for the omission of the modification reagent. Dishes were coated with monomeric collagen at 10 $\mu\text{g/ml}$ in (a) and (b) and with fibres at 250 $\mu\text{g/ml}$ in (c) and 500 $\mu\text{g/ml}$ in (d). Adhesion was measured at 90 min using human gel-filtered platelets in a standard Tris/HCl buffer containing 2 mM- Mg^{2+} in (a) or the addition as indicated in the case of (b)–(d).

Expt.	Collagen	Adhesion (%)	
		Control	Treated
(a)	Monomeric I		
	Native	54	53
	Denatured	47	48
	Monomeric III		
	Native	55	55
	Denatured	51	55
Expt.	Collagen	Adhesion (%)	
		2 mM- Mg^{2+}	2 mM-EDTA
(b)	Monomeric I		
	Control		
	Native	56	1
	Denatured	53	3
	Treated		
	Native	60	48
	Denatured	53	36
(c)	Reconstituted type I fibres		
	Control	56	22
	Treated	60	55
	Reconstituted type III fibres		
	Control	61	41
	Treated	58	53
(d)	Bovine tendon fibres		
	Control	50	32
	Treated	50	41

was used, however, adhesion was approx. 20%, indicating the presence of factor(s) in plasma promoting Mg^{2+} -independent adhesion. Likewise, adhesion of gel-filtered platelets in the presence of albumin, Mg^{2+} , Ca^{2+} and citrate at the concentrations shown above, to collagen fibres (bovine tendon, coated at 500 $\mu\text{g/ml}$) was reduced from a value of around 60% (at 90 min) when measured in buffer containing 0.5% albumin and 2 mM- Mg^{2+} to about 15–20%. With platelet-rich plasma, adhesion was in the region of 40–50%, again indicative of a stimulation in platelet-rich plasma of Mg^{2+} -independent adhesion.

DISCUSSION

Our studies utilizing collagens immobilized on plastic have served to emphasize the existence of at least two processes of adhesion of human platelets to collagen: one that exhibits a requirement for Mg^{2+} , and a second that is bivalent-cation-independent. Rabbit platelets appear to recognize only the cation-independent mechanism.

Exposure of collagen to pepsin causes the loss of the short non-helical telopeptides located at each end of the molecule, leaving the helical structure intact. The lack of any significant

Table 4. Adhesion of rabbit platelets to various collagens

Dishes were coated with either monomeric collagen (at 10 µg/ml) or a suspension of fibres (at 500 µg/ml). Adhesion, at 90 min, was measured with a 1 ml platelet suspension containing, on average, 2×10^8 of gel-filtered platelets suspended in a standard Tris/HCl buffer containing 2 mM-Mg²⁺ or 2 mM-EDTA as indicated. (a)–(d) represent separate experiments.

Expt.	Collagen species	Adhesion (%)	
		Mg ²⁺	EDTA
(a)	Bovine type I, monomeric		
	Extracted with citrate		
	Native	18	–
	Denatured	20	–
	Extracted with pepsin		
	Native	0	–
(b)	Bovine type I, monomeric, extracted with citrate		
	Native	15	15
	Denatured	13	11
	Bovine type I, monomeric, citrate-extracted	27	–
(c)	Bovine tendon (type I) fibres	23	22
	Bovine fibres, pepsin-treated	8	6
	Bovine type I, monomeric, citrate-extracted	21	–
(d)	Reconstituted human (pepsin-extracted) type I fibres	15	13

cation-independent binding of human platelets to pepsin-extracted monomeric collagen, the absence of any real adhesion at all of rabbit platelets to this collagen in contrast with their cation-independent adhesion to citrate-extracted collagen and the reduction in cation-independent platelet adhesion to intact bovine tendon collagen (type I) fibres after their treatment with pepsin, all point to the involvement of the telopeptides of collagen in the cation-independent adhesion process. However, since fibres reconstituted from pepsin-treated collagens, especially type III, also supported some cation-independent adhesion, cation-independent binding sites must exist in the triple-helical region of the molecule as well. Mazurov *et al.* [31] have recently reported that the binding of fibrillar type III (pepsin-extracted) collagen in suspension to platelets is unaffected by EDTA, implying a cation-independent adhesion.

Our bovine tendon fibres, as expected, remained intact after their exposure to pepsin. They could be recovered by centrifugation with little loss of material (less than 5% of the original total). Electron microscopy confirmed the presence of fibres which appeared, however, rather more swollen and less obviously striated than those not treated with enzyme. Although our data strongly suggest a role for collagen telopeptides in cation-independent platelet adhesion, the possibility must be considered that the effect of the pepsin could be due, at least in part, to altered polymer organization or to the removal by the enzyme of other proteins present in minor amount that play an intermediary role in platelet adhesion to collagen.

Although we have concluded that both cation-dependent and -independent adhesion sites occur within the triple-helical region of the collagen molecule, our results indicate that the triple-helical configuration is not essential for either form of binding. In this respect we are at variance with those who have reported little if any binding of platelets to denatured collagen [7,32–34]. However, other studies have described the adhesion of other types of cells to the denatured protein as well as to native collagen [35–37], and we have ourselves noted the binding of smooth muscle and endothelial cells equally well to the denatured as to

the native protein (A. R. Peachey, L. F. Morton & M. J. Barnes, unpublished work).

The reaction of collagen lysine residues with trinitrobenzenesulphonic acid, although not affecting the level of adhesion, did render the process largely cation-independent. This could indicate the participation of specific lysine residues in the Mg²⁺-dependent adhesion mechanism. We have already remarked upon their likely involvement in collagen-induced platelet aggregation, since reaction with trinitrobenzenesulphonic acid strongly inhibits the aggregatory activity of fibres of collagens I and III [24,30], and we have proposed, for example, that the two lysine residues located at positions 479 and 486 in collagen III represent together the platelet-reactive site occurring in the highly reactive aggregatory peptide $\alpha 1(\text{III})\text{CB4}$ derived from this collagen [24]. It should be borne in mind, though, that both this peptide and the equivalent type I peptide $\alpha 1(\text{I})\text{CB3}$, which lacks aggregatory activity and in which lysine residue 486 is replaced by a hydroxyproline residue [24], support adhesion equally well and as well as the parent collagen; the adhesion in both cases, whether to the free peptide [11] or to the renatured cross-linked species tested for aggregatory activity (L. S. Zijenah & M. J. Barnes, unpublished work) shows the same degree of Mg²⁺-dependence. This may make it seem less likely that the same lysine residues are involved in Mg²⁺-dependent adhesion and aggregation.

The actual reduction in the overall level of adhesion of rabbit platelets to bovine tendon fibres after their exposure to pepsin supports the evidence that rabbit platelets possess only a cation-independent mechanism of adhesion. In the case of human platelets, overall adhesion to fibres was not reduced after pepsin, because of the availability of the alternative Mg²⁺-dependent process. The same argument can be advanced to explain why adhesion of rabbit platelets to fibres increased with increasing fibre-coating concentration, whereas the level of adhesion of human platelets was unaffected. The existence of only the one process of adhesion in rabbit platelets leads us to believe that the two mechanisms are distinct entities operating through different receptors. Mg²⁺-dependent adhesion of human platelets has been shown to involve the receptor platelet glycoprotein Ia/IIa [7–10]. However, evidence has been presented that the interaction of rabbit platelets with collagen involves a protein, 80 kDa in size, that is not an integrin [38]. Recently, Tandon *et al.* [34] have presented evidence that human platelet glycoprotein IV, M_r 88000, can mediate collagen–platelet interaction, and it is tempting to speculate that this protein may be involved in cation-independent adhesion.

Adhesion of gel-filtered human platelets to pepsin-extracted monomeric collagen was completely abolished when concentrations of albumin, Mg²⁺, Ca²⁺ and citrate designed to reproduce those in citrated plasma were used. This was due in part to an increased albumin level, since we have noted that albumin at plasma levels causes a reduction in the adhesion of gel-filtered platelets of some 50% or so (L. F. Morton & M. J. Barnes, unpublished work). It must also be attributable to the removal of Mg²⁺ and, conceivably, an inhibitory effect of residual free Ca²⁺. Whatever the precise mechanism, the overall effect is to eliminate Mg²⁺-dependent binding. This suggests that adhesion with citrated platelet-rich plasma, and presumably aggregation induced by collagen fibres in suspension in stirred platelet-rich plasma, must proceed without the utilization of Mg²⁺. This may cast some doubt on the essential role of glycoprotein Ia/IIa in collagen–platelet interaction.

The promotion of a cation-independent platelet adhesion to collagen by plasma may reflect the participation of adhesive proteins such as fibronectin and von Willebrand factor in the adhesion mechanism. Both have been shown to play a role in

adhesion of platelets to the injured vessel wall under conditions of flow [39] and are thought to serve as a 'bridge' between the platelet and collagen fibre surface. Both are known to be able to interact with the platelet plasma membrane ([40,41] and references therein) and to bind to specific sites in the collagen molecule [29,42-47]. Our data suggest that these proteins may play a role in collagen-platelet interaction under static conditions as well as those of flow.

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