Platelet-reactive sites in collagens type I and type III

Evidence for separate adhesion and aggregatory sites

Laurence F. MORTON, Anthony R. PEACHEY and Michael J. BARNES*

Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 4RN, U.K.

The adhesion of human and rabbit platelets to collagens and collagen-derived fragments immobilized on plastic was investigated. Adhesion appeared to be independent of collagen conformation, since similar attachment occurred to collagen (type I) in monomeric form, as fibres or in denatured state. The adhesion of human platelets was stimulated to a variable degree by Mg^{2+} , but rabbit platelet adhesion showed little if any dependence on this cation. Collagens type I, III, V and VI were all able to support adhesion, although that to collagen type V (native) was lower than that to the other collagens. Adhesion to a series of peptides derived from collagens I and III was measured. Attachment did not require the presence of peptides in triple-helical configuration. The extent of adhesion ranged from relatively high, as good as to the intact parent collagen molecule, to little if any adhesive activity beyond the non-specific (background) level. The existence of very different degrees of activity suggests that platelet adhesion is associated with specific structural sites in the collagen molecule. Adhesion in many instances was essentially in accord with the known platelet-aggregatory activity of individual peptides. However, two peptides, $\alpha 1(I)CB3$ and $\alpha 1(III)CB1,8,10,2$, exhibited good adhesive activity although possessing little if any aggregatory activity. Of particular interest, despite its near-total lack of aggregatory activity, adhesion to peptide $\alpha 1(I)CB3$ was as good as that to the structurally homologous peptide $\alpha 1(III)CB4$, in which is located a highly reactive aggregatory site. This implies that platelet adhesion to collagen may involve sites in the collagen molecule distinct from those more directly associated with aggregation.

INTRODUCTION

Following injury to the blood-vessel wall, thrombogenic components, in particular the collagenous elements, in the vessel wall (and perivascular space) are brought into contact with the circulating blood. The resulting adhesion of blood platelets to the exposed collagenous surfaces, predominantly fibres of the two common interstitial collagen types I and III [1,2], is the first step in the haemostatic process leading subsequently to platelet aggregation and the formation of a haemostatic plug responsible for the arrest of bleeding. The interaction of platelets with collagen(s) may also be the cause in a number of instances of pathological platelet thrombus formation. For example, thrombosis is an extremely serious, often fatal, complication of atherosclerotic disease of arteries. It is thought to arise as a result of rupture of the atherosclerotic plaque [3,4]. This is likely to lead to the contact of collagens, known to be abundant in the plaque [1,2], with circulating platelets and hence deposition of a platelet thrombus on the damaged wall of the affected artery.

We have been attempting to define the structure of platelet-reactive sites in the collagen molecule with a view to obtaining inhibitors of collagen-platelet interaction that could be of use in the prevention of thrombosis. Our studies to date suggest that collagen type III may possess just a single, highly reactive, platelet-aggregatory site able to account basically for the total aggregatory activity of the type III molecule. In contrast, the platelet-aggregatory reactivity of collagen type I appears to be

distributed among several sites of moderate activity along the length of the molecule [5–7].

As an extension to these studies, in the present work we have investigated the location of platelet-adhesion sites in collagen types I and III in an attempt to establish whether or not the same sites are responsible for both the initial adhesion of platelets and their subsequent aggregation.

Platelet adhesion to isolated collagens has been studied in many laboratories by a variety of procedures ([8–14] and references cited therein). We have chosen in the present investigation to measure adhesion to collagen and collagen-derived fragments immobilized on plastic by adsorption on the plastic surface from solution. The basic characteristics of platelet adhesion to collagenous surfaces obtained in this way have been defined in detail by Santoro [13]. Our results suggest the presence in the collagen molecule of separate adhesion and aggregatory sites.

MATERIALS AND METHODS

^{51}Cr as sodium chromate was obtained from Amersham International. Bovine serum albumin (Cohn fraction V), potato apyrase (grade 1), hirudin from leeches (1000 units/mg of protein), prostaglandin E_1 and human thrombin (3000 units/mg of protein) were all purchased from Sigma Chemical Co. Prostacyclin was kindly given by Dr. J. Westwick, Department of Pharmacology, Royal College of Surgeons, London,

* To whom correspondence should be addressed.

U.K. The collagen-type-III-derived peptide $\alpha 1(\text{III})\text{CB3A}$ was generously provided by Professor Dr. J. Rauterberg, Institut für Arterioskleroserecherche, Münster, Germany. Falcon 1008 35 mm polystyrene Petri dishes were obtained from Becton Dickinson, Plymouth, U.K.

Preparation of washed platelets

Washed platelets were prepared routinely essentially as described by Santoro [13]. Platelet-rich plasma was obtained either from rabbit blood or from human blood collected from healthy volunteers who had not received aspirin or any other anti-platelet drug in the previous 2 weeks. Blood was collected into polypropylene tubes containing $\frac{1}{9}$ vol. of acid/citrate/dextrose anticoagulant (39 mM-citric acid/75 mM-sodium citrate/135 mM-glucose). Platelet-rich plasma was labelled with ^{51}Cr (20 $\mu\text{Ci}/\text{ml}$) for 30 min at room temperature. Labelled platelets were then applied directly to a 2 cm $\times$ 25 cm column containing Sepharose 2B equilibrated with adhesion buffer (0.05 M-Tris/HCl buffer, pH 7.4, containing 0.14 M-NaCl, 5 mM-glucose and 0.5% bovine serum albumin). Platelets were eluted with the same buffer, normally containing 2 mM- MgCl_2 , except in those experiments designed specifically to examine the bivalent cation requirement for adhesion, when MgCl_2 or CaCl_2 or EDTA, each at 2 mM, was added to the suspension of platelets after their elution. In some instances prostaglandin E_1 (10 μM [13]), hirudin (0.06 unit/ml) and apyrase (100 $\mu\text{g}/\text{ml}$), added immediately before use, were included in the acid/citrate/dextrose anticoagulant and the adhesion buffer. Alternatively, prostacyclin (300 ng/ml) was sometimes included [15,16].

In some experiments washed platelets were obtained by a procedure involving centrifugation, normally in the presence of prostacyclin (300 ng/ml), which was included in the acid/citrate/dextrose anticoagulant as well as the adhesion buffer used for washing [15,16]. In order to assess the effect of activation on adhesion, washed platelets were exposed to thrombin (1 unit/ml) over the period of time adhesion was allowed to occur.

Each separate experiment involved the use of just the one platelet suspension prepared from a single blood sample taken from one donor or one individual animal.

Preparation of collagens and collagen-derived peptides

Collagen type I was obtained, as before [5-7], from calf skin by extraction with sodium citrate buffer, pH 3.7 [17]. Collagen type III was obtained from the same source by pepsin digestion and subsequent precipitation with NaCl at acid and then neutral pH [18], as in previous work [7]. Collagens types V and VI were isolated from human placenta by pepsin digestion and purified as we have described previously [19,20]. Collagen $\alpha 1(\text{I})$ - and $\alpha 1(\text{III})$ -chains for digestion with CNBr were isolated as before [7] from the respective collagens, by chromatography on CM-cellulose [21]. CNBr-cleavage peptides from the $\alpha 1(\text{I})$ - and $\alpha 1(\text{III})$ -chains were prepared by a combination of ion-exchange chromatography and gel filtration as in our previous work [6,7]. Homogeneity of peptide preparations was established by SDS/polyacrylamide-gel electrophoresis [22] in 12.5%-(w/v)-acrylamide gels. Peptides were renatured, as before [6,7],

by using the method of Tkocz & Kuhn [17]. Where necessary, collagens and peptides were denatured at 100 °C for 10 min before use.

A dispersion of native-type fibres of collagen type I from bovine tendon, details of which we have presented previously [19], was generously provided by Ethicon, Somerville, NJ, U.S.A. Before use, the dispersion was diluted to the desired concentration with 0.01 M-acetic acid and dialysed against the same.

Determination of collagen-platelet adhesion

Platelet adhesion was normally measured essentially as described by Santoro [13]. Routinely, collagens and collagen-derived peptides were adsorbed on 35 mm-diameter polystyrene dishes (Falcon 1008), by incubating the dishes containing 1 ml of a solution of the species in question at known concentration (generally 10 $\mu\text{g}/\text{ml}$) in 0.01 M-acetic acid for 2 h at room temperature, or sometimes (as specified) overnight at 4 °C. Coating with fibres (from bovine tendon) was achieved by the same means with the fibre suspension at a concentration of 500 $\mu\text{g}/\text{ml}$ [13]. Dishes then received two washes with the adhesion buffer containing 0.5% bovine serum albumin to block non-specific binding sites. In some experiments with denatured collagens, dishes were incubated with a solution of the freshly denatured protein in a neutral buffer (sodium phosphate buffer, pH 7.6 and 1/0.2) at 37 °C, to avoid any possibility of renaturation during the coating procedure. An alternative coating procedure sometimes employed was to allow the solution in dilute acetic acid to air-dry. The dried film was then rehydrated by washing with the bovine-serum-albumin-containing buffer as above. Controls were prepared by coating dishes with bovine serum albumin, with the use of adhesion buffer for this purpose.

For the adhesion assay, a sample (routinely 1 ml) of the platelet suspension (containing 2×10^8 – 4×10^8 platelets/ml as estimated by counting the number of platelets with a Coulter model ZF counter) was added to each dish and incubated normally at room temperature (sometimes, as specified, at a lower temperature of 15 °C) for a period of 2 h unless otherwise stated. Unattached platelets were removed by aspiration and the dishes were washed twice with adhesion buffer. Adherent platelets were lysed with two sequential washes of 2% (w/v) SDS, which were pooled. Determination of the amount of radioactivity in both the aspirate plus washes and the lysate provided a measure of platelet adhesion, which was expressed as a percentage of the total number of platelets originally added to the dish. Each assay was performed in triplicate. Results are given as the means for the three, which in most instances did not differ from one another by more than 10%.

In some experiments the extent of platelet adhesion was determined by counting non-adherent platelets with the Coulter counter.

In some cases adherent platelets were observed directly by phase-contrast microscopy, with a Wild M40 inverted microscope.

RESULTS

Both human and rabbit platelets were found to adhere to dishes coated with monomeric type I collagen (see Figs. 1 and 2), in agreement with the findings made by others with human platelets [13,14,23-25]. The extent of

adhesion was similar with collagen solutions for coating ranging in concentration from 2 to 250 $\mu\text{g/ml}$, as found by Santoro [13]. Routinely, a concentration of 10 $\mu\text{g/ml}$ was employed. Furthermore, adhesion was the same whether coating was achieved by the standard procedure involving direct adsorption from solution (in dilute acetic acid) or by allowing a solution to air-dry (results not shown). The extent of adhesion increased with time, as shown in Figs. 1 and 2, essentially as described by Santoro [13], although we observed a lower rate of adhesion than reported by this author, and for this reason we generally measured adhesion at 1½–2 h rather than (or as well as) 30 min. We also recorded a higher extent of adhesion to bovine serum albumin than observed by Santoro [13]. Irrespective of the precise extent of this adhesion, that to collagen was consistently 30–40% higher (Figs. 1 and 2 and Tables 1–3).

Measurement of the adhesion of platelets to collagen by determination of the radioactivity associated with adherent and non-adherent ⁵¹Cr-labelled platelets gave the same results as obtained by counting directly the non-adherent fraction with a Coulter counter. Furthermore, similar extents of adhesion were obtained with either gel-filtered platelets or platelets washed by a procedure involving their collection by centrifugation (results not shown). Routinely, adhesion was measured with ⁵¹Cr-labelled gel-filtered platelets.

Examination of attached platelets by phase-contrast light microscopy revealed them to be discrete cells showing little platelet-platelet association, typical of resting platelets. Adhesion characteristics were precisely the same when platelets were isolated in the presence of various inhibitors (to minimize platelet activation), including a mixture of prostaglandin E₁, apyrase and hirudin or, added alone, prostacyclin. Furthermore, exactly the same time course of adhesion was observed whether in the presence or in the absence of thrombin (to activate platelets) included in the adhesion buffer (at 1 unit/ml) during the period of adhesion (results not shown). We conclude therefore, in agreement with Santoro [13], that the adhesion process is independent of platelet activation.

Effect of collagen conformation

In agreement with others [13,14], we detected platelet adhesion to both monomeric and fibrillar collagen immobilized on plastic (Figs. 1c, 1d, 2b and 2c). However,

Fig. 1. Adhesion of human platelets to bovine collagen type I

(a) Adhesion to native monomeric collagen in the presence of 2 mM-Mg²⁺ or 2 mM-EDTA. (b) Adhesion to native and denatured monomeric collagen in the presence of 2 mM-Mg²⁺ or 2 mM-EDTA. (c) Adhesion to native monomeric collagen in the presence of 2 mM-Mg²⁺, 2 mM-Ca²⁺ or 2 mM-EDTA. (d) Adhesion to collagen fibres in the presence of 2 mM-Mg²⁺, 2 mM-Ca²⁺ or 2 mM-EDTA; (c) and (d) represent a single experiment. Adhesion was measured with ⁵¹Cr-labelled gel-filtered platelets. Falcon 1008 35 mm Petri dishes were coated with native monomeric collagen (10 $\mu\text{g/ml}$) or fibres (500 $\mu\text{g/ml}$) in 0.01 M-acetic acid or with bovine serum albumin (BSA) (0.5%) in adhesion buffer, for 2 h at room temperature, or were coated with denatured monomeric collagen (250 $\mu\text{g/ml}$) in sodium phosphate buffer, pH 7.6 and 10.2, for 2 h at 37 °C. Each point represents the mean of three determinations.

Fig. 2. Adhesion of rabbit platelets to bovine collagen type I
 (a) Adhesion to native and denatured monomeric collagen in the presence of 2 mM-Mg²⁺. (b) Adhesion to native monomeric collagen in the presence of 2 mM-Mg²⁺, 2 mM-Ca²⁺ or 2 mM-EDTA. (c) Adhesion to collagen fibres in the presence of 2 mM-Mg²⁺, 2 mM-Ca²⁺ or 2 mM-EDTA; (b) and (c) represent a single experiment. Experimental conditions were as given in the legend to Fig. 1.

we also observed an adhesion of both human and rabbit platelets to denatured collagen (monomeric) that was consistently as good as that to the native monomeric collagen (Figs. 1b and 2a). As for native collagen, the

Table 1. Comparison of platelet adhesion to different collagen types

Dishes were coated with denatured collagens, dissolved at 10 µg/ml in 0.01 M-acetic acid, for 2 h at 37 °C. Adhesion was recorded at 90 min. Experimental details otherwise were as given in the legend to Fig. 1.

Species	Adhesion (%)
Collagen type I	
Native	63
Denatured	60
Collagen type III	
Native	62
Denatured	57
Collagen type V	
Native	46
Denatured	56
Collagen type VI	
Native	60
Denatured	59
Bovine serum albumin	22

Table 2. Platelet adhesion to CNBr-cleavage peptides from collagens I and III

Adhesion was recorded at 90 min. Experimental details otherwise were as given in the legend to Fig. 1. Peptides are listed in the order in which they occur in the respective collagen molecule, beginning at the N-terminal end.

Species	Adhesion (%)
Native monomeric collagen (type I)	55
Peptide α1(I)CB8	40
Peptide α1(I)CB3	53
Peptide α1(I)CB7	42
Peptide α1(I)CB6b	30
Peptide α1(III)CB3A	55
Peptide α1(III)CB1,8,10,2	47
Peptide α1(III)CB4	55
Peptide α1(III)CB5	28
Peptide α1(III)CB9A	24
Bovine serum albumin	24

extent of adhesion to denatured collagen was independent of the concentration of protein used for coating within the range tested, 2–250 µg/ml. Adhesion was similar irrespective of the actual coating procedure adopted, either by adsorption from acid or neutral solution at room temperature or 37 °C, or by air-drying of a solution in dilute acetic acid.

Bivalent cation requirement

Adhesion of human platelets to monomeric type I collagen was enhanced in the presence of Mg²⁺, sometimes markedly so (Fig. 1a). However, the precise extent of this effect appeared to vary between donors, and in some cases there was relatively high adhesion in the absence of bivalent cations (as recorded in the presence of 2 mM-EDTA) (see Figs. 1b and 1c). Adhesion

Table 3. Comparison of platelet adhesion to renatured (triple-helical) and denatured CNBr cleavage peptides from collagens I and III

Dishes were coated with native (renatured) peptides overnight at 4 °C. Adhesion (at 2 h) was measured at 15 °C (to avoid any possibility of denaturation at room temperature of renatured peptides). Experimental details otherwise were as given in the legend to Fig. 1. A–D represent four separate experiments.

Species	Adhesion (%)			
	A	B	C	D
Native monomeric collagen (type I)	55	47	50	39
Peptide $\alpha 1(I)CB3$				
Renatured	57			
Denatured	50			
Peptide $\alpha 1(I)CB7$				
Renatured		30		
Denatured		21		
Peptide $\alpha 1(III)CB4$				
Renatured			54	
Denatured			53	
Peptide $\alpha 1(III)CB5$				
Renatured				15
Denatured				15
Bovine serum albumin	18	16	13	13

in the presence of Ca^{2+} (2 mM) was actually lower than in the presence of EDTA (Fig. 1c), and in this sense we found Ca^{2+} to exhibit an inhibitory effect on adhesion, as reported by Santoro [13]. Adhesion to monomeric collagen either in native state or denatured invariably exhibited the same degree of dependence on Mg^{2+} , whether this was relatively high or, as on some occasions, comparatively slight (Figs. 1b). The adhesion to fibrillar collagen appeared to be rather less affected by Mg^{2+} than that to monomeric collagen (native or denatured) (see Fig. 1).

In the case of rabbit platelets, adhesion was always relatively high in the absence of any bivalent cation and there appeared to be little effect if any of Mg^{2+} or Ca^{2+} on the adhesion process (see Figs. 2b and 2c). Since in these experiments the same collagen substratum was utilized as in the above studies (bovine collagen type I), the difference in Mg^{2+} -dependence must reflect a species difference in platelets.

Adhesion to different collagen types

A comparison of platelet adhesion to collagen types I, III, V and VI is shown in Table 1. Adhesion to native collagen type V was relatively low, but adhesion to the denatured species was higher, more akin to that occurring to the other collagens, where adhesion was similar whether the native or denatured species was used.

Adhesion to collagen-derived fragments

Adhesion of human platelets to a number of CNBr-cleavage peptides obtained from collagens types I and III was detected, as shown in Table 2. For any given peptide, the extent of adhesion did not alter significantly whatever

the concentration used for coating, tested in the range 1–100 $\mu g/ml$. Table 2 shows typical values for the complete range of peptides examined, tested in denatured (non-helical) form. The peptides investigated are those previously tested for platelet-aggregatory activity [6,7], plus the additional peptide $\alpha 1(III)CB3A$. The four collagen-type-I-derived peptides represent together around 90 % of the total length of the $\alpha 1(I)$ -chain, the five type-III-derived peptides around 75 % of the $\alpha 1(III)$ -chain. Although some variation in the extent of adhesion was encountered from experiment to experiment, it was found that the peptides could be divided into three main groups: those that routinely showed activity approaching and occasionally exceeding that of the intact parent collagen [peptides $\alpha 1(I)CB3$, $\alpha 1(III)CB3A$ and $\alpha 1(III)CB4$]; those that usually showed only slightly more activity than the control bovine-serum-albumin-coated plates [peptides $\alpha 1(I)CB6b$, $\alpha 1(III)CB5$ and $\alpha 1(III)CB9A$]; thirdly, those that exhibited a medium, but more variable, range of reactivity [peptides $\alpha 1(I)CB7$, $\alpha 1(I)CB8$ and $\alpha 1(III)CB1,8,10,2$].

A comparison of denatured peptides with their equivalent native species indicated (Table 3) a slight increase in platelet binding on renaturation in the case of those peptides that normally exhibited medium to high reactivity in the denatured state. Denatured peptides with little or no reactivity (beyond the extent of binding to bovine serum albumin) remained of low activity when tested in the native state.

Platelet binding to CNBr-cleavage peptides (over and above the 'background' extent to bovine serum albumin) could be stimulated by Mg^{2+} in essentially the same fashion as adhesion to the intact collagen molecule. For example, in one experiment binding to the peptide $\alpha 1(III)CB4$ (recorded at 90 min) was 51 % in the presence of 2 mM- Mg^{2+} but 36 % in the presence of 2 mM-EDTA. Equivalent values for native monomeric collagen in the same experiment were 56 % and 36 %.

DISCUSSION

Platelet adhesion to collagen and collagen-derived fragments (immobilized on plastic) has been examined under conditions, as described by Santoro [13], designed to minimize the possibility of platelet–platelet interaction (platelet aggregation), which could artificially enhance the extent of adhesion observed. These conditions include the use of Tris/HCl buffer in platelet isolation and the adhesion assay, the measurement of adhesion under static conditions at room temperature and, generally, the absence of Ca^{2+} ions. Examination of adherent platelets by phase-contrast microscopy confirmed the absence of aggregates of platelets. Furthermore, the lack of effect of inhibitors of platelet activation and, conversely, of the activation of platelets by thrombin is in accord with the view presented by Santoro [13] that adhesion as recorded represents a direct interaction between the platelet and the collagenous surface, independent of platelet activation.

Our results differ in a number of respects from those reported by Santoro [13]. Although Mg^{2+} , sometimes quite strongly, stimulated the adhesion of human platelets, we also frequently observed appreciable adhesion in the absence of bivalent cations (as recorded in the presence of EDTA). Others have reported a

substantial adhesion of platelets to collagen (fibres) when measured in the presence of EDTA in order to remove Ca^{2+} and thus prevent platelet aggregation [8,9,11,26–30]. We found that the extent of adhesion was somewhat lower in the presence of Ca^{2+} than in the absence of any bivalent cations. Santoro [13] observed an adhesion in the presence of Ca^{2+} that was higher than that when EDTA was present; adhesion in the absence of bivalent cations was always only slight. However, Ca^{2+} was inhibitory in the sense that it decreased the adhesion induced by the presence of Mg^{2+} . Using rabbit platelets we observed little dependence of adhesion, if any at all, on the presence of Mg^{2+} . It is not possible to conclude as yet whether two quite separate mechanisms of adhesion are involved, one Mg^{2+} -dependent, the other able to proceed in the absence of bivalent cations.

In contrast with the findings obtained by Santoro [13], we consistently observed adhesion to denatured collagen immobilized on plastic that was as good as that to the native immobilized molecule, suggesting that adhesion, unlike aggregation, did not require the presence of collagen in triple-helical configuration. It seems unlikely that our results can be attributed to renaturation occurring during the coating procedure, since adhesion was similar to both collagen and gelatin at the same concentration of protein used for coating, over a wide range of concentrations tested (from 2 to 250 $\mu\text{g}/\text{ml}$). Furthermore, adhesion to native and denatured forms was similar whether we coated with a dilute solution in weak acetic acid precisely as described by Santoro [13] or using a solution of gelatin at neutral pH and at 37 °C. Renaturation under the latter conditions seems even more improbable than under Santoro's conditions [13]. Others have noted [23], like Santoro [13], an adhesion of platelets to monomeric collagen when immobilized on plastic that is Mg^{2+} -dependent and requires collagen in native form. However, in these studies the collagen used was chick skin collagen, and yet this particular collagen has been reported to be able to cause platelet aggregation in denatured form [31] and must then, it follows, be able to support platelet adhesion in denatured state. Meyer & Weisman [32] reported that platelets did not adhere to gelatin absorbed on glass. However, they found that pre-exposure of platelets to gelatin in solution caused an inhibition of their adhesion to native collagen. This inhibition was just as effective as that caused by pre-incubation of platelets with soluble native collagen. Since in our studies adhesion to native and denatured collagens invariably showed the same degree of stimulation by Mg^{2+} , it would seem that the same mechanism or mechanisms are involved for adhesion to native and denatured forms.

Our data on the adhesion of platelets to collagen-derived fragments point to some interesting conclusions. Thus the two peptides $\alpha 1(\text{I})\text{CB3}$ from type I collagen and $\alpha 1(\text{III})\text{CB4}$ from type III collagen are located at precisely the same position in the respective parent collagens (residues 403–551) and exhibit a high degree of structural homology. The latter peptide possesses a highly active platelet-aggregatory site, whereas the former exhibits little if any aggregatory activity [7]. In the present study both peptides were found to support adhesion equally well as each other and as well as the intact collagen. This implies the presence in collagen of separate sites for adhesion and aggregation and the possibility of interaction of collagen with two types of receptor on the

platelet surface, one associated with adhesion, the other more specifically with aggregation. This may explain in part the ambiguity that currently exists concerning the identity of the platelet membrane protein that serves as the collagen receptor [13,33–40]. The finding that the peptide $\alpha 1(\text{III})\text{CB1,8,10,2}$ has appreciable adhesive activity although it lacks aggregatory activity [7] supports the notion of separate adhesion and aggregation sites. The existence of peptides with very different adhesive activities implies that, as for aggregation, there must be structurally specific sites associated with adhesion.

Fauvel *et al.* [41] have also examined the adhesive activity of collagen-type-III-derived peptides and concluded that only the one peptide, $\alpha 1(\text{III})\text{CB4}$, exhibited activity. However, their experimental system appears to require platelet activation, during pre-incubation of peptide with platelets before gel filtration, for the detection of adhesion [29], and any adhesion to peptides unassociated with activation would have been overlooked. This may account for the apparent lack of activity in their study of peptides $\alpha 1(\text{III})\text{CB3A}$ and $\alpha 1(\text{III})\text{CB1,8,10,2}$. Alternatively, the use of peptides in the free state (rather than immobilized) may provide an explanation. Immobilization may serve in effect to provide a quaternary structure necessary for adhesion.

We do not know as yet whether the relatively high adhesive activity that we observed for peptide $\alpha 1(\text{III})\text{CB3A}$ is associated with a second so-far-unrecognized aggregatory site in collagen type III, since the aggregatory activity of this peptide has not yet been assessed. The adhesive activities of peptides $\alpha 1(\text{I})\text{CB6b}$, $\alpha 1(\text{I})\text{CB7}$, $\alpha 1(\text{I})\text{CB8}$, $\alpha 1(\text{III})\text{CB5}$ and $\alpha 1(\text{III})\text{CB9A}$ were essentially in accord with their aggregatory activities [6,7].

Others have remarked upon the lower, or even lack of, platelet reactivity of collagen type V relative to other collagens [12–14,24,42], and it has been regarded by some as an essentially non-thrombogenic species. As here, the collagen type V employed in all of these studies was of human origin, and it is possible that its lower reactivity might be attributable to this fact. However, we have found no difference in adhesive activity (using human platelets) between collagen types I and III of human or bovine origin (L. F. Morton, A. R. Peachey & M. J. Barnes, unpublished work), and we consider that the diminished platelet reactivity of collagen type V is a genuine property of the collagen independent of its species of origin. Although we found a diminished platelet adhesion to the native form, the extent of attachment was still appreciable. This is in accord with our previous conclusion [43] that platelet-reactive sites must be present in the type V collagen molecule; we and others have demonstrated that this collagen can cause the aggregation of platelets when in native (triple-helical) polymeric form, albeit less readily than other collagens [43,44]. In the present study we have also noted platelet adhesion to collagen type VI at an extent comparable with that to collagens I and III. We have previously demonstrated the ability of this collagen to induce platelet aggregation when exhibiting tertiary and quaternary structure [20]. Given the widespread distribution of this particular collagen throughout the vessel wall [45], it seems reasonable to consider that it may make an important contribution to the overall platelet reactivity of the injured vessel wall.

Although the amino acid sequence RGD has been reported to serve as a recognition site in collagen for cell-

binding [46], it would seem unlikely to fulfil this purpose in platelet adhesion to collagen since the sequence is absent from the peptides $\alpha 1(\text{I})\text{CB3}$, $\alpha 1(\text{III})\text{CB3A}$ and $\alpha 1(\text{III})\text{CB4}$, all exhibiting relatively high platelet-adhesive activity.

We gratefully acknowledge the support of the Medical Research Council, of which we are members of the External Staff. Thanks are also due to Dr. Jeremy Saklatvala for his willing help.

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Received 17 June 1988/30 August 1988; accepted 27 September 1988