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ISOLATION OF THE MEMBRANE GLYCOPROTEINS OF HUMAN BLOOD PLATELETS BY LECTIN AFFINITY CHROMATOGRAPHY

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Summary

The major platelet membrane glycoproteins have been solubilized in 1.0% sodium deoxycholate and subjected to affinity chromatography on the lectins from *Lens culinaris*, wheat germ and *Abrus precatorius*. Polyacrylamide gel electrophoresis in the presence and absence of a reducing agent together with the differential binding of the lectins to the glycoproteins permitted the distinction of at least seven separate glycoprotein entities. A new nomenclature for the glycoproteins is proposed to accomodate the additional data.

Using combinations of lectin columns, glycoproteins Ia and Ib could be prepared in a pure state and IIb and IIIa could be greatly purified. The binding of lectins to glycoprotein Ib has been strongly implicated as a necessary step in the aggregation response of platelets to lectins.

Introduction

The adhesive properties of platelets to surfaces and to each other are important in the specific functions of these cells. Electron microscopy has shown that platelets possess a pronounced glycocalyx [1] and after labelling the surface proteins by reagents thought not to penetrate the membrane the bulk of the label has been found in components identified as glycoproteins [2—4].

In two inherited bleeding disorders, Bernard-Soulier syndrome and Glanzmann's thrombasthenia in which platelets are defective, the expression and surface exposure of glycoproteins have been shown to be altered [5—8]. Thrombin, a potent inducer of platelet aggregation, has been shown to affect one of

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the membrane glycoproteins [9]. These findings imply that glycoproteins may play a major role in the interactions of platelets with their environment. Thus the isolation and study of these glycoproteins seem important steps in the understanding of their role in platelet function.

The basic problem in the isolation of membrane glycoproteins is their solubilization from the membrane. This was first approached by digestion of platelets with proteolytic enzymes giving water-soluble products [10]. This method has the disadvantage in that, apart from chymotrypsin, the other commonly used proteolytic enzymes, trypsin, pronase and papain are all known to induce aggregation and release of the contents of intracellular granules. When this release reaction is induced by proteolytic enzymes it has been shown that a glycoprotein is among the components which are released [11—13] and that it comes from the granules and is not a normal surface component. This, coupled with the difficulty of identifying the membrane components from which fragments are derived, makes the isolation of intact glycoproteins by a method which does not induce release an attractive goal. In order to avoid the interference of the release reaction, most later work has been done with isolated platelet membranes [2,3,14]. Analysis of platelet membranes by sodium dodecyl sulphate-polyacrylamide gel electrophoresis followed by staining for carbohydrate and for protein [2,3] shows an apparently simple distribution of glycoprotein with three major bands, which were termed I, II and III [3]. More recently a fourth band in the II region has been described [4,5] and the current nomenclature is I, IIa, IIb and III in order of decreasing molecular weight.

Nachman et al. [15] solubilized platelet membranes with lithium diiodosalicylate and attempted to isolate the glycoproteins by affinity chromatography on concanavalin A-Sepharose 4B. Elution of the affinity column with methyl-α-mannopyranoside released only glycoprotein III. Concanavalin A is, however, known to have a high affinity for membrane glycoproteins and it has been reported [5] that complete release of bound material is not always achieved.

The binding of some lectins to platelets and the modification of this binding by thrombin have been examined [16]. After thrombin treatment the binding of *Lens culinaris* lectin was greatly increased and the increase was shown to be due to exposure of binding sites in the central part of the surface-connected canalicular system [17]. Greenberg and Jamieson [18] have examined the response of platelets to a range of lectins and have shown that it varied considerably and appeared to be related to the specificity of the lectin. In particular wheat germ agglutinin (*N*-acetylglucosamine specificity) and *Ricinus communis* lectin (galactose specificity) caused platelets to aggregate at concentrations of 50 and 20 μg/ml, respectively, whereas *L. culinaris* lectin (glucose, mannose specificity) did not induce aggregation at a concentration of 100 μg/ml.

Because of these latter results it seemed of interest to see if there were any similarities between the glycoprotein receptors of a lectin with *N*-acetylglucosamine specificity and one of galactose specificity and whether differences existed compared with the *L. culinaris* lectin. Solubilization of plasma membranes in detergents followed by affinity chromatography on lectins attached to insoluble supports has recently been extensively applied to isolation of membrane glycoproteins [15,19—22]. This paper deals with the solubilization of platelet membranes in sodium deoxycholate solution followed by affinity

chromatography on the lectins from *L. culinaris*, wheat germ and *Abrus precatorius*.

Materials and Methods

Human blood platelets. These were isolated within 20 h after collection from citrated blood collected for the Central Laboratory of Blood Transfusion Service of the Swiss Red Cross in Berne [23]. The buffy coats were syphoned into a buffered glucose solution to give platelet-rich plasma containing about 20 mM glucose, 12 mM phosphate buffer, pH 6.8, and about $4 \cdot 10^9$ platelets per ml [24].

Platelet membranes. These were prepared in batches from the platelet concentrates from 30 units of blood by the glycerol-lysis technique [25] which yielded on average 40 mg of membrane protein. On occasions the platelets from 100 units were used yielding 140 mg of membrane protein.

Solubilization of platelet membranes. Platelet membranes (2 ml, approx. 20 mg of protein) which had been stored at −70° C were quickly thawed and brought to 1% sodium deoxycholate by adding the solid salt. The mixture was vortexed until the sodium deoxycholate dissolved and was then left for 30 min at room temperature. The solution was centrifuged at 100 000 × *g* for 1 h and the supernatant was used in subsequent investigations.

Preparation of lectins. L. culinaris lectin was purified as described [26] with the inclusion of an ammonium sulphate fractionation step [27]. Wheat germ agglutinin was prepared by the method of Bloch and Burger [28] with the omission of the DEAE-cellulose step which was found to be unnecessary.

A. precatorius lectin was isolated from seeds (Pratrap Nursery and Seed Stores, Dehra Dun, India) by the method of Adair and Kornfeld [22].

Coupling of lectins to Sepharose 4B. The Sepharose 4B was activated with CNBr [29] and coupled with the lectin at a concentration of 1—2 mg lectin/ml Sepharose in the presence of 2% of the appropriate sugar, to protect the binding site of the lectin. The coupled material was extensively washed to remove unbound lectin and was then packed in a column (14 × 1.6 cm). When not in use, columns were stored at 4° C in buffer containing 0.05% NaN_3. The storage buffer for the *L. culinaris* lectin-Sepharose also contained 1 mM $CaCl_2$ and 1 mM $MnCl_2$ as this lectin is known to require Ca^{2+} and Mn^{2+} in order to express full activity.

Affinity chromatography. The solubilized membranes (approx. 2 ml) were applied to a column (14 × 1.6 cm) of *L. culinaris* lectin-Sepharose which had been equilibrated with 0.5% sodium deoxycholate solution. The column was washed with 0.5% sodium deoxycholate until the flow-through peak had eluted and the absorbance of the effluent at 280 nm had returned to the base line. The eluent was then changed to 2% (w/v) methyl-α-mannopyranoside in 0.5% sodium deoxycholate. The flow-through and the eluted fractions were separately pooled and dialysed against 0.1 M ammonium acetate which had been adjusted to pH 8 with NH_4OH. The pooled fractions were then freeze-dried.

Affinity chromatography on the other lectins bound to Sepharose 4B was carried out in a similar manner using the appropriate sugar for elution (*N*-acetyl-

glucosamine, 2.5% (w/v) for wheat germ agglutinin; galactose, 2% (w/v) for *A. precatorius* lectin).

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Samples were dissolved in either 2% sodium dodecyl sulphate (unreduced) or in 2% sodium dodecyl sulphate and 2% dithiothreitol by heating to 100° C for 5 min. Electrophoresis was carried out essentially as described by Lenard [30] but using 0.1% sodium dodecyl sulphate.

Gels were stained for protein with Coomassie blue or for carbohydrate with periodic acid-Schiff's reagent [31].

Samples of whole membranes, of sodium deoxycholate solubilized supernatant and pellet and of flow-through fractions used for gel electrophoresis contained 200 μg protein for Coomassie blue staining and 700 μg protein for periodic acid-Schiff's reagent staining. Other samples contained 100 μg protein for Coomassie blue staining and 200 μg protein for periodic acid-Schiff's reagent staining. These amounts are necessarily arbitrary for the glycoproteins due to their different carbohydrate contents.

Sugars. All sugars used were of the D-configuration.

Results

The distribution of glycoproteins and proteins of the platelet membranes was examined by sodium dodecyl sulphate gel electrophoresis and compared to published data [2,3]. Since the distribution of the glycoproteins is known to vary, depending whether or not a reducing agent is present [4,5], membranes were solubilized in sodium dodecyl sulphate either in the presence or absence of dithiothreitol. These results are shown in Fig. 1 together with the sodium dodecyl sulphate-polyacrylamide gel electrophoresis of the supernatant from the solubilization of platelet membranes with 1.0% sodium deoxycholate. As can be seen, the solubilized material contains all of the glycoproteins present in the membranes and the pellet contains virtually none of the glycoproteins. The bands in the lower part of Fig. 1B (k,l) have not stained with periodic acid-Schiff's reagent but show up in the photograph due to their opalescence. The glycoproteins I, IIa, IIb and III have been marked following current notations [3—5]. Some differences from previously published work were noted. A band which stained for carbohydrate was seen at a somewhat higher molecular weight than glycoprotein I with unreduced samples which was not visible with reduced samples. The intensity of this band varied between batches of membrane and with some it was not visible. In addition to the changes in the distribution of glycoproteins IIa and IIb between the reduced and unreduced states it can be seen that glycoprotein III splits into two components in the reduced state, one of which runs at an apparently higher molecular weight. Several faint bands staining for carbohydrate can be detected at lower molecular weights and can be seen, although they are not described, in densitometer scannings previously published [2,4].

Table I presents a comparison of the old and new nomenclature of the glycoproteins and a summary of the data obtained by lectin binding studies which will now be reported in detail.

TABLE I

BINDING OF PLATELET GLYCOPROTEINS BY VARIOUS LECTINS

	Glycoproteins						
Previous nomenclature	I		IIa	IIa + IIb	III		
Proposed nomenclature	Ia	Ib	IIa	IIb	IIIa	IIIb	IV
L. culinaris lectin	+			+	±		
Wheat germ agglutinin	+	+					
A. precatorius lectin	±	±	+	±		±	

+, all or most of glycoprotein bound by lectin.
±, part of glycoprotein bound by lectin.

Binding of platelet membrane glycoproteins to L. culinaris lectin

The distribution of protein in affinity chromatography of sodium deoxycholate-solubilized platelet membranes on a *L. culinaris* lectin-Sepharose 4B column is shown in Fig. 2. Fractions were pooled as indicated and after dialysis and freeze-drying samples were solubilized and electrophoresed (in Fig. 3 are shown densitometer scannings of the gels of the different fractions). The peak in the flow-through before pool 1 consisted of lipid and glycolipid and gave an absorbance because of the opalescence of this material. Gels c and g are of material, bound by *L. culinaris* and eluted by methyl-α-mannopyranoside (Fig. 2, pool 2), electrophoresed in the unreduced state and stained for protein and carbohydrate, respectively. Two major bands and a faint band all staining for carbohydrate can be seen. The highest molecular weight band corresponds to the band of higher molecular weight than glycoprotein I seen in the gels run on membranes (Fig. 1, gel g). The second band corresponds to glycoprotein IIa and the third band to glycoprotein III. It can be seen that whereas IIa and III stain heavily for protein, the higher molecular weight glycoprotein stains only faintly. Gels d and h show the same material electrophoresed in the reduced state. The higher molecular weight band has disappeared and has been replaced by a band in the position of glycoprotein I, glycoprotein IIa has moved to the position of IIb and III has moved to a somewhat higher molecular weight. Gels a, b, e and f show the material which did not bind to *L. culinaris* lectin. Although there is virtually no difference between these and gels run on whole membranes this is not due to overloading of the affinity column. When the unbound material was recycled (data not shown), no additional material could be bound. It should be noted, however, on gel e that there is none of the glycoprotein present in the position higher than glycoprotein I. Although this higher molecular weight glycoprotein electrophoreses in the same position as glycoprotein I in the reduced state, it is apparently a distinct entity since it is bound by *L. culinaris* lectin whereas glycoprotein I is not. We propose that the higher molecular weight glycoprotein I be termed Ia and the one which does not change on reduction Ib.

Binding of platelet membrane glycoproteins to wheat germ agglutinin

The distribution of protein in affinity chromatography of sodium deoxycholate-solubilized platelet membranes on wheat germ agglutinin-Sepharose 4B

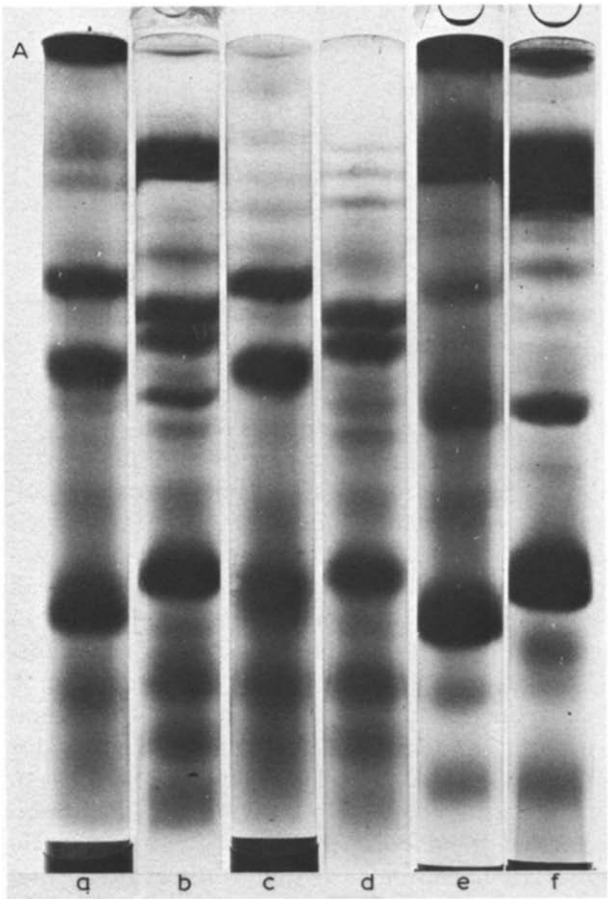

Fig. 1. For legend see opposite page.

is essentially the same as that shown in Fig. 2 for *L. culinaris* lectin-Sepharose. Fractions were pooled and treated similarly. In Fig. 4 are shown densitometer scannings of the gels of the different fractions. Gels c and g are of material bound by wheat germ agglutinin and eluted with 2.5% (w/v) *N*-acetylglucosamine, electrophoresed in the absence of reducing agent. Two glycoprotein bands can be seen, corresponding to Ia and Ib. On reduction (gels d and h), as before, Ia disappears and band I becomes more pronounced. Examination of the material which did not bind to wheat germ agglutinin shows the complete absence of any bands in the position of Ia and Ib (gel e, unreduced). In gel f (reduced) band I is also completely absent. The remaining glycoproteins behave as described above.

Binding of platelet membrane glycoproteins to A. precatorius lectin

Affinity chromatography on *A. precatorius* lectin-Sepharose 4B was carried out in a similar way to the other lectin columns and after elution with 2% (w/v) galactose the fractions were similarly treated. Fig. 5 shows gels run on the bound and flow-through fractions. In this case it can be seen that in general the

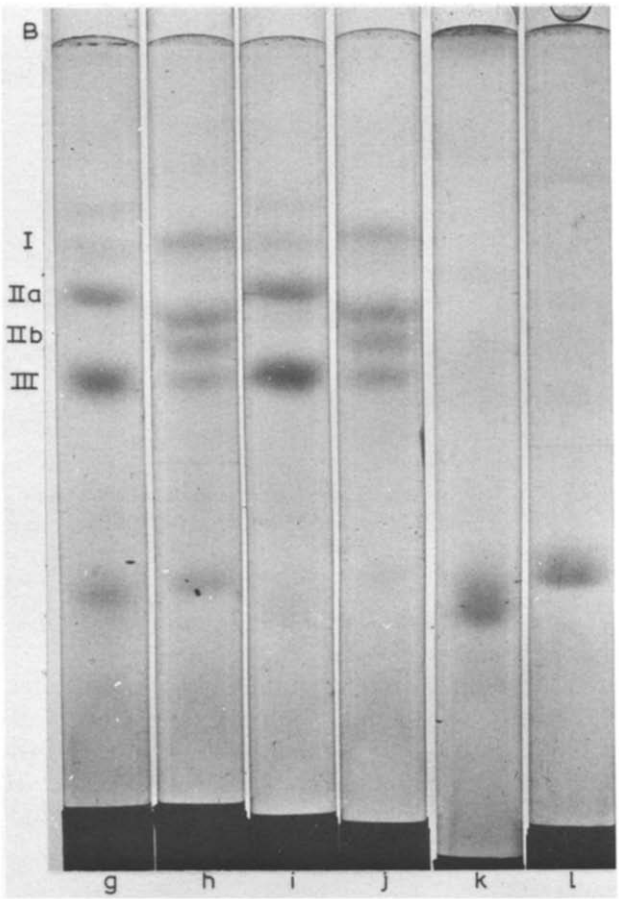

Fig. 1. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of platelet membranes and of supernatant and sediment from sodium deoxycholate solubilization of platelet membranes, stained for protein with Coomassie brilliant blue and for carbohydrate with periodic acid-Schiff's reagent. (A) a, whole membranes, protein distribution, unreduced sample; b, reduced sample; c, supernatant from sodium deoxycholate solubilization, protein distribution, unreduced sample; d, reduced sample; e, sediment from sodium deoxycholate solubilization, protein distribution, unreduced sample; f, reduced sample. (B) g—l. As a—f but stained for carbohydrate.

A. precatorius lectin appears to have bound a part of all of the glycoproteins normally present from Ia to III. The only notable difference is that on reduction of the bound material not all of the IIa band has moved to the IIb position. In the flow-through material the distinction between IIa and (after reduction) IIb is much sharper than in the whole membrane. Comparison of microdensitometer scans of these gels (Fig. 6) shows that there is indeed a faint glycoprotein band in reduced samples of whole membranes in the position of glycoprotein IIa and that it is this glycoprotein which is being totally removed from the solubilized membrane preparation by affinity chromatography on *A. precatorius* lectin. The part of glycoprotein III which is bound does not appear to be affected by reduction.

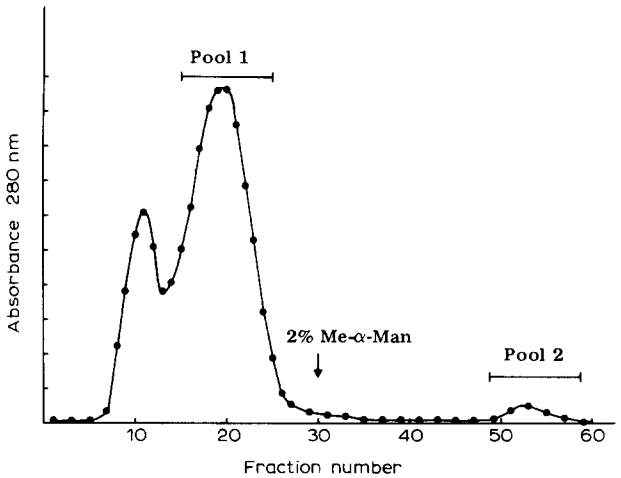

Fig. 2. Affinity chromatography of the supernatant from sodium deoxycholate-solubilized platelet membranes on *L. culinaris* lectin-Sepharose 4B. Me-α-Man, methyl-α-mannopyranoside.

Sequential use of lectin affinity chromatography columns: L. culinaris-Sepharose 4B and wheat germ agglutinin-Sepharose 4B columns

Since these columns appeared to bind different components of platelet membranes they were used sequentially to obtain an improved separation of the glycoproteins. This coupled-column technique has been previously described for other cell types [19—21]. Solubilized membranes were applied to a *L. culinaris* lectin-Sepharose column and eluted as previously described. When elution

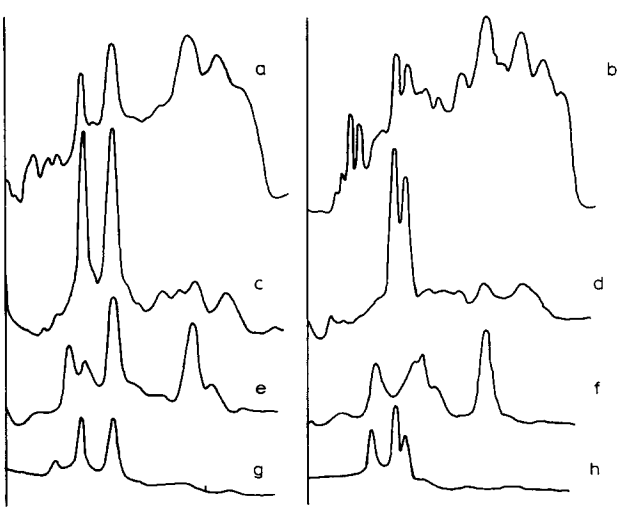

Fig. 3. Microdensitometer scans of sodium dodecyl sulphate-polyacrylamide gel electrophoresis of fractions of sodium deoxycholate-solubilized platelet mambranes from affinity chromatography on *L. culinaris* lectin. a, *L. culinaris* lectin column flow-through, protein distribution, unreduced; b, reduced; c, material bound to *L. culinaris* lectin, protein distribution, unreduced; d, reduced; e—h, as a—d but stained for carbohydrate.

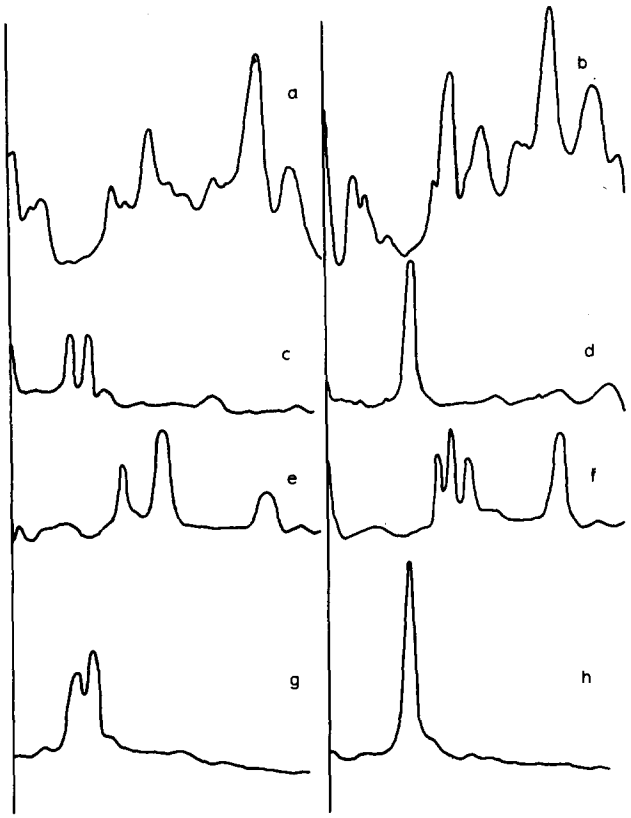

Fig. 4. Microdensitometer scans of sodium dodecyl sulphate-polyacrylamide gel electrophoresis of fractions of sodium deoxycholate solubilized platelet membranes from affinity chromatography on wheat germ agglutinin, a, wheat germ agglutinin column flow-through, protein distribution, unreduced; b, reduced; c, material bound to wheat germ agglutinin, protein distribution, unreduced; d, reduced; e—h, as a—d but stained for carbohydrate.

with methyl-α-mannopyranoside solution was begun, however, the effluent from the *L. culinaris* lectin-Sepharose column was connected to the top of a wheat germ agglutinin-Sepharose column. After the eluted material had passed through both columns the *L. culinaris* lectin-Sepharose column was detached and the wheat germ agglutinin-Sepharose column was eluted with *N*-acetyl-glucosamine solution as previously described. The fractions containing the flow-through from the *L. culinaris* lectin-Sepharose column, the eluted material which did not bind to wheat germ agglutinin and the eluted material which had bound to wheat germ agglutinin were separately pooled, dialysed and freeze-dried and were then examined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Fig. 7 shows the densitometer scannings of the gels which were run on these pooled fractions.

The glycoprotein distribution found is in complete agreement with that predicted from the results with the individual columns. Fig. 7A shows gels stained for protein and Fig. 7B gels stained for carbohydrate. In both, gels a and b are of the flow-through from *L. culinaris* lectin-Sepharose column and are similar to those in Fig. 3. Gels c and d show the material which bound to *L. culinaris*

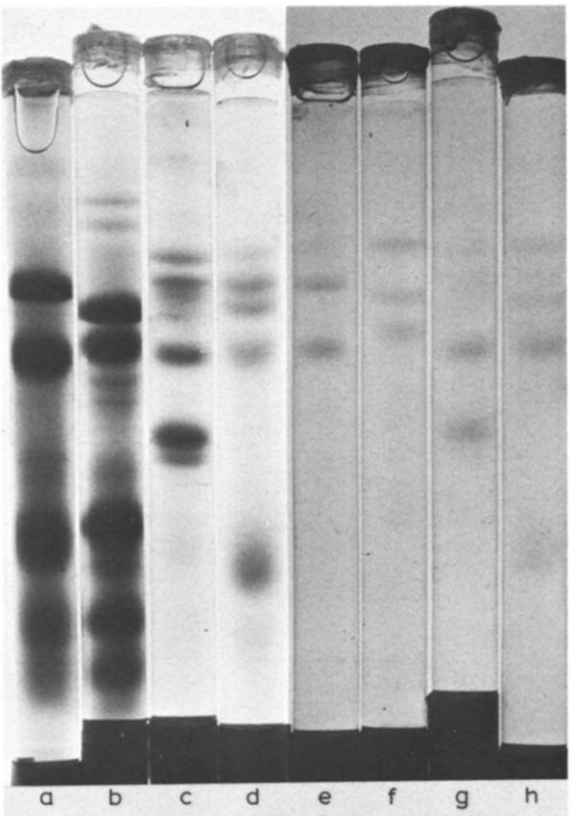

Fig. 5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of fractions of sodium deoxycholate-solubilized platelet membranes from affinity chromatography on *A. precatorius* lectin. a, *A. precatorius* lectin column flow-through, protein distribution, unreduced; b, reduced; c, material bound to *A. precatorius* lectin, protein distribution, unreduced; d, reduced; e—h, as a—d but stained for carbohydrate. The bands in gels, c, d, g and h, at lower molecular weight than the major glycoproteins, which stain for both protein and carbohydrate are *A. precatorius* lectin contaminating this preparation.

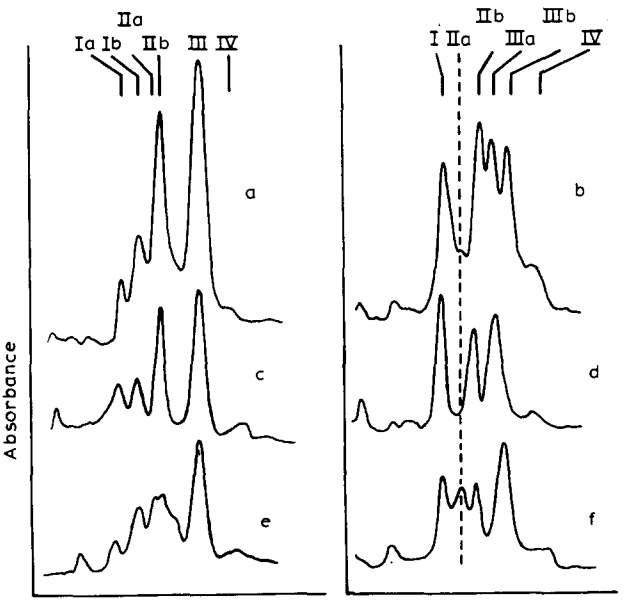

Fig. 6. Microdensitometer scans of sodium dodecyl sulphate-polyacrylamide gels stained for carbohydrate. a, whole membranes, unreduced; b, whole membranes, reduced; c, *A. precatorius* lectin column flow-through, unreduced (Fig. 5, gel e); d, *A. precatorius* lectin column flow-through, reduced (Fig. 5, gel b); e, *A. precatorius* lectin-bound material, unreduced (Fig. 5, gel g); f, *A. precatorius* lectin-bound material

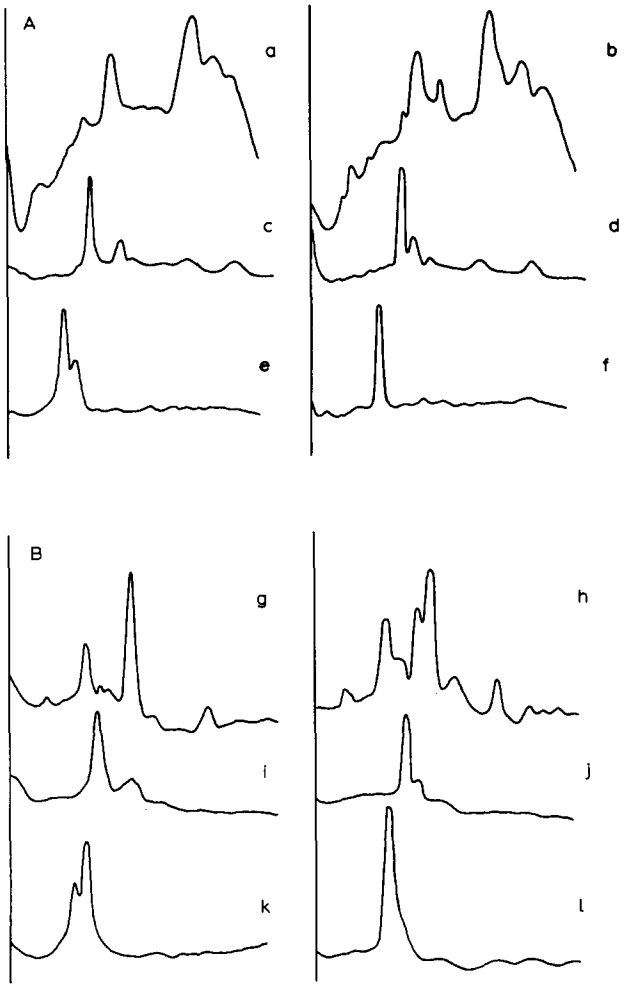

Fig. 7. Microdensitometer scans of sodium dodecyl sulphate-polyacrylamide gel electrophoresis of fractions from affinity chromatography of sodium deoxycholate-solubilized platelet membranes on *L. culinaris* lectin and wheat germ agglutinin. (A) a, *L. culinaris* lectin column flow-through, protein distribution, unreduced; b, reduced; c, material which bound to *L. culinaris* lectin but not to wheat germ agglutinin, protein distribution, unreduced; d, reduced; e, material which bound to both *L. culinaris* lectin and to wheat germ agglutinin, protein distribution, unreduced; f, reduced. (B) g—l, as a—f but stained for carbohydrate.

lectin and not to wheat germ agglutinin. It contains a glycoprotein which runs in the position of IIa unreduced and IIb reduced and a trace of glycoprotein III. Gels e and f show the material which bound to both columns. It contains only glycoprotein Ia which moves to the position of I on reduction.

Wheat germ agglutinin-Sepharose 4B and L. culinaris-Sepharose 4B

The columns were also used in this reverse order and the material eluted from the wheat germ agglutinin was directly applied to the *L. culinaris* lectin column. However, it was found that very little glycoprotein bound to the *L.*

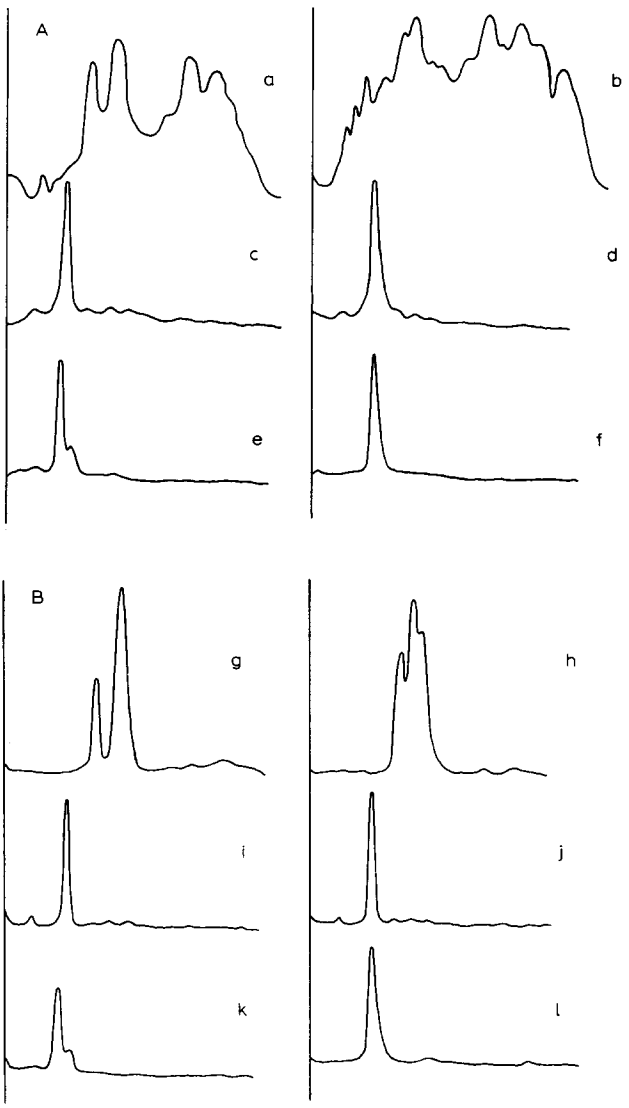

Fig. 8. Microdensitometer scans of sodium dodecyl sulphate-polyacrylamide gel electrophoresis of fractions from affinity chromatography of sodium deoxycholate-solubilized platelet membranes on wheat germ agglutinin and *L. culinaris* lectin. (A) a, wheat germ agglutinin columns flow-through, protein distribution, unreduced; b, reduced; c, material which bound to wheat germ agglutinin but not to *L. culinaris* lectin, protein distribution unreduced; d, reduced; e, material which bound to both wheat germ agglutinin and to *L. culinaris* lectin, protein distribution, unreduced; f, reduced. (B) g—l, as a—f but stained for carbohydrate.

culinaris lectin column. When the *N*-acetylglucosamine was first removed (by dialysis against 0.5% sodium deoxycholate) the binding pattern expected was observed. Fig. 8 shows the densitometer scannings of the gels run on fractions from these columns. Fig. 8A shows gels stained for protein and Fig. 8B gels stained for carbohydrate. Gels a and b are of the flow-through from the wheat germ agglutinin-Sepharose column and are identical to those in Fig. 4. Gels c

and d show the material which bound to wheat germ agglutinin and not to *L. culinaris* lectin. The main component is glycoprotein Ib, and there are some traces of other components. Glycoprotein Ia is absent. Gels e and f show the material which bound to both wheat germ agglutinin and *L. culinaris* lectin. It contains only glycoprotein Ia.

Discussion

Sodium deoxycholate has been shown to be an efficient solubilizing agent for the major glycoproteins of the platelet membranes. The insoluble residue from the sodium deoxycholate treatment contained virtually no glycoproteins but was rich in other platelet components.

Affinity chromatography of the solubilized platelet membrane on lectin-Sepharose columns either singly or in combination can be used to yield some glycoproteins in a virtually pure state and others extensively purified and is a relatively simple technique. In the course of this work it became apparent that the pattern of glycoproteins present in platelet membranes is much more complex than had previously been described. Electrophoresis of the glycoproteins in the presence and absence of a reducing agent has permitted the distinction of at least three glycoproteins in addition to those previously described (Table I). Glycoproteins of typical membrane preparations demonstrated by gel electrophoresis and subsequent densitometry are shown in Fig. 6a and b. The nomenclature used therein is that proposed in this paper and used below.

The fact that glycoprotein Ia has an apparent higher molecular weight in the absence of a reducing agent may be due to oxidising conditions during isolation and may therefore be artifactual. However, it has distinct lectin binding properties which indicate that it is indeed a separate molecular species.

The molecular weight of glycoprotein Ib does not appear to depend on its reduction state. It appears to be the same glycoprotein as that described by Lombart et al. [32] which could be purified from soluble material obtained during platelet membrane isolation by glycerol-lysis or by sonication of washed platelets, although it is not yet known for certain if this latter glycoprotein is exposed at the membrane surface. However, this had the same lectin binding properties as glycoprotein Ib (Clemetson, K.J., unpublished observations).

The so-called "thrombin-sensitive protein" [11] does not occur in the platelet membrane but can be distinguished from glycoproteins Ia and Ib in whole platelets by its high molecular weight (approx. 300 000) in the unreduced state [12].

The glycoproteins hitherto termed IIa and IIb appear to refer to the same molecule in different states of reduction. This molecular species can be greatly purified by passing the glycoproteins bound to *L. culinaris* lectin through a wheat germ agglutinin-Sepharose column where it constitutes the greater part of the flow-through. Since a further glycoprotein occurs in the same position as IIa, does not move on reduction and does bind to the *A. precatorius* lectin and not to the other two, we propose that the term IIa should be reserved for this and that the reducible *L. culinaris* lectin binding glycoprotein should be termed IIb in both the reduced and unreduced states. After polyacrylamide gel electrophoresis of both membranes and the supernatant from sodium deoxy-

cholate-solubilized membranes, glycoprotein III in the unreduced state stains strongly for both carbohydrate and protein. On reduction, this band splits into two components, one which stains heavily for protein, contains about half of the material staining for carbohydrate and which moves to an apparently higher molecular weight, just below glycoprotein IIb, and the other which remains in about the unreduced position, contains the other half of the material staining for carbohydrate and stains much more weakly for protein.

Both the *L. culinaris* lectin and the *A. precatorius* lectin columns bind part of glycoprotein III. The *L. culinaris* lectin column appears to bind selectively the component which moves to a higher molecular weight on reduction and the flow-through from this column is depleted in this component although it is not entirely removed. The *A. precatorius* lectin column appears to bind rather the other component which does not move appreciably on reduction.

Although this separation of glycoprotein III into two components on reduction has not been described previously, few authors have published results on gel electrophoresis of platelet membranes under strongly reducing conditions. Nachman et al. [15] used 2% 2-mercaptoethanol at 100° C for 10 min and in the gel scannings which they present glycoprotein III appears as a broad peak with a flattened top when stained for carbohydrate, and when stained for protein, as a sharp peak coincident with the higher molecular weight side of the carbohydrate peak. Hagen et al. [12] using 1.5% 2-mercatoethanol at 37° C for 60 min found only one band for glycoprotein III, after reduction, at a slightly higher molecular weight. George et al. [4] using 40 mM dithiothreitol also show similar results. It is possible, however, that the acrylamide concentration or the degree of cross-linking of the gel is critical as the mobility of glycoproteins on sodium dodecyl sulphate-polyacrylamide gel electrophoresis may vary from that of non-glycosylated proteins.

Both Nachman et al. [15] and Ando and Steiner [33] have shown that, within the glycoprotein region of gels, most of both the free thiol groups and the disulphide groups are associated with the glycoprotein III region. It has been reported that 2% 2-mercaptoethanol does not reduce glycoprotein IIb (our nomenclature) efficiently [5] and it is possible that glycoprotein III requires much stronger conditions than the other components for reduction. It is proposed to term the component which moves to a higher molecular weight on reduction glycoprotein IIIa and that which remains in about the same place IIIb.

In addition to the previously mentioned glycoproteins, a more diffuse band staining for carbohydrate at a lower molecular weight than III can be seen on gels run on membranes and solubilized membranes. This component does not move on reduction but it stains more intensely for carbohydrate. It also does not appear to bind to any of the lectins used in this study. It is proposed to call this glycoprotein IV.

The observation that the *A. precatorius* lectin binds a part of some of the glycoproteins (with the exceptions discussed above) is possibly due to incomplete addition of the terminal sugar to the carbohydrate moieties of the glycoproteins since galactose is often the penultimate sugar.

Several different experimental approaches have implicated membrane glycoproteins in the response of platelets to various stimuli. Greenberg and Jamieson

[18] have found that, whereas lectins with specificities towards galactose such as *R. communis* and towards *N*-acetylglucosamine (wheat germ agglutinin) were strong inducers of platelet aggregation and the release of both ADP and serotonin, lectins with specificities towards glucose and mannose, such as *L. culinaris*, at similar concentrations, had little effect.

Comparison of the glycoproteins which bind to wheat germ agglutinin and *A. precatorius* lectin (specificity similar to *R. communis)* with those which bind to *L. culinaris* lectin shows that the only glycoprotein which the first two bind in common, which is not bound by the *L. culinaris* lectin, is glycoprotein Ib. In addition Greenberg and Jamieson [18] have shown that the action of the lectin from *Phaseolus coccineus* (sugar specificity unknown), which is also a strong inducer of platelet aggregation and release, can be inhibited by their "glycopeptide I" which appears to be derived from glycoprotein Ib [14].

In studies of a bleeding disorder, Bernard-Soulier syndrome, where response to ristocetin-von Willebrand Factor is abnormal due to a platelet defect, platelet glycoprotein expression has been shown to be abnormal [5,7]. Enzyme treatment of normal platelets reduces their response to ristocetin-von Willebrand Factor in parallel with hydrolysis of membrane glycoproteins [5].

In Glanzmann's thrombasthenia, a bleeding disorder caused by a platelet defect, where response to ADP is abnormal, abnormalities in membrane glycoprotein expression differing from those of Bernard-Soulier syndrome have been found [6—8] and Glanzmann's thrombasthenia patients have been shown to produce antibodies to a 120 000 daltons component present on normal platelets [34].

The observation that it is possible to distinguish two components of glycoprotein III has implications for Glanzmann's thrombasthenia. Phillips et al. [6] have shown using the lactoperoxidase-iodination technique, that in this disorder "normal" glycoprotein III (the authors' nomenclature) appears to be absent and is replaced by a lower molecular weight component. A simpler explanation would be to postulate that the two components of III, a and b, are not labelled to the same extent by the lactoperoxidase technique, that under mild reducing conditions there is little separation between IIIa and IIIb and that the heavily labelled component (IIIa) completely masks the presence of the other (IIIb). Thus in Glanzmann's thrombasthenia, if IIIa is completely absent or for some reason not accessible to iodination then, if IIIb was labelled to a normal degree, it would be seen as "III", apparently shifted to a lower molecular weight.

These findings all tend to implicate platelet membrane glycoproteins in the aggregation response. The observation that glycoprotein Ib seems to be essential for platelet aggregation to lectins provides an additional piece of evidence for the role played by this component. The isolation of this and other glycoproteins of the platelet membrane in a pure or purified state by a relatively simple technique should permit direct testing of their function in platelet response.

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