Isolation and characterization of the major oligosaccharide of human platelet membrane glycoprotein GPIb

Philip A. JUDSON,* David J. ANSTEE* and John R. CLAMP†

*Department of Immunochimistry, South Western Regional Blood Transfusion Centre, Southmead, Bristol BS10 5ND, U.K., and †Department of Medicine, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

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Treatment of intact human platelets with chymotrypsin released a glycopolypeptide that was shown to be derived from the major membrane glycoprotein, GPIb. The glycopolypeptide contained 59% carbohydrate on a molar basis and was rich in serine, threonine and proline. Almost all the carbohydrate could be released from the glycopolypeptide by treatment with alkali in the presence of NaBH₄. The major component (comprising 80% of the released sugar) was purified and shown to be a hexasaccharide containing sialic acid, galactose, *N*-acetylglucosamine and *N*-acetyl-galactosaminitol in the molar ratios 2:2:1:1. Two possible structures for this hexasaccharide are proposed on the basis of the known biosynthetic pathways of mucus-type glycoproteins. Our data is consistent with the occurrence of an *O*-glycosidically linked oligosaccharide on one amino acid in four of the glycopolypeptide. These results suggest that glycoprotein Ib can best be described as a membrane-bound mucus-type glycoproteins. Our data are consistent with the occurrence of an *O*-in the process by which platelets adhere to the exposed subendothelium of damaged blood-vessel walls. The possible role of the glycopolypeptide portion of GPIb in this process was investigated. Neither the major oligosaccharide nor the glycopolypeptide itself inhibited ristocetin-induced platelet agglutination at the concentrations tested. It is suggested that the carbohydrate moieties of GPIb molecules at the cell surface interact to form a barrier to macromolecules. Such a barrier could play a major role in modulating platelet function.

Platelets play an essential role in haemostasis, their functions being controlled by the interaction of effector molecules with receptors on the platelet membrane. Specific glycoproteins are thought to be required as receptors during adhesion to subendothelium and also platelet aggregation, both important steps in the haemostatic response to vessel injury (Phillips, 1980).

A major platelet membrane glycoprotein, GPIb, has an apparent M_r of 170 000, consists of two disulphide-linked subunits [GPIba (143 000 Da) and GPIb β (22 000 Da) (Phillips & Poh Agin, 1977)] and is thought to be functionally important in platelet adhesion to subendothelium (Nurden & Caen, 1978). Pepper & Jamieson (1970) showed that treatment of intact platelets with proteinases could release a 'macroglycopeptide' thought to be derived from GPIba (in the present nomenclature). Sub-

sequently Okumura *et al.* (1976) showed that this 'macroglycopeptide' is a proteolytic fragment of a larger glycoprotein, glycocalicin, which itself is likely to be a fragment of GPIba (Solum *et al.*, 1980; Clemetson *et al.*, 1981). GPIb appears to be the major sialoglycoprotein of the platelet membrane, since, when glycocalicin is solubilized from platelets by freeze-thawing (in the absence of proteinase inhibitors and EDTA), 64% of the total acid-labile sialic acid of intact platelets is released (Solum *et al.*, 1980).

We report here the isolation and partial characterization of the major oligosaccharide present on glycoprotein Ib and investigate its possible role in one of the (non-physiological) receptor functions of GPIb, ristocetin-induced platelet agglutination.

Materials and general methods

Outdated human platelet concentrates were available from the South Western Regional Blood Transfusion Centre, Bristol, U.K. α -Chymotrypsin

Abbreviations used: GPIb, glycoprotein Ib; SDS, sodium dodecyl sulphate; SA (in sequences), sialic acid (probably, but not certainly, *N*-acetylneuraminate).

(type II) was from Sigma (London) Chemical Co. Ltd., Poole, Dorset, U.K. Neuraminidase (from *Vibrio cholerae*) was from Behringwerke AG, Marburg, Germany, and purified wheat-germ agglutinin (from *Triticum vulgaris*), *Phaseolus vulgaris* (red kidney bean) agglutinin and concanavalin A [from jack bean (*Canavalia ensiformis*)] were purchased from Calbiochem (London, U.K.), Difco (Detroit, MI, U.S.A.) and Pharmacia (London, U.K.) respectively and were used without further purification. The lectins from castor bean (*Ricinus communis*) (Thompson and Morgan Ltd., Ipswich, Suffolk, U.K.), osage orange [*Maclura aurantiaca (pomifera)*] (Schumacher Ltd., Sandwich, MA, U.S.A.) and *Arachis hypogaea* (peanut) were purified from crude seed extracts by absorption to, and elution from, formaldehyde-treated erythrocytes (Tanner & Anstee, 1976).

Haemagglutination-inhibition tests using lectins were performed in 7 mm × 50 mm tubes, using standard serological techniques with human group-O erythrocytes. Neuraminidase-treated erythrocytes were used as indicator cells for experiments involving *Arachis hypogaea* lectin and trypsin-treated erythrocytes for those involving wheat-germ agglutinin, concanavalin A and *Phaseolus vulgaris* lectins. The methods of neuraminidase treatment and trypsin treatment of erythrocytes are described elsewhere (Anstee *et al.*, 1979; Tanner *et al.*, 1980).

SDS/polyacrylamide-gel electrophoresis was in slab gels containing 7% (w/v) acrylamide and with a 3% overlay, the discontinuous buffer system of Laemmli (1970) being used. Samples for SDS/polyacrylamide-gel electrophoresis were solubilized by the addition of an equal volume of 10% SDS and boiled for 2 min. A sample was removed for protein determination (Lowry *et al.*, 1951). To the remainder, $\frac{1}{2}$ vol. of sample buffer was added and the sample was boiled again for 2 min. Reduced samples were made 5% with respect to 2-mercaptoethanol, before boiling with sample buffer. Gels were stained with periodic acid/Schiff's-base stain (Zacharias *et al.*, 1969). Chymotrypsin was assayed by the method of Hummel (1959), with benzoyl-L-tyrosine ethyl ester (Sigma) as substrate. Samples for amino acid analysis were hydrolysed in constant-boiling HCl under N₂ at 110°C for 24, 72 and 96 h before determination on a Rank-Hilger Chromaspek amino acid analyser. Values for serine and threonine were extrapolated to zero time to overcome losses occurring during hydrolysis, and values for isoleucine, leucine and valine taken after complete hydrolysis for 96 h. O-Linked oligosaccharides were released from the chymotryptic glycopolyptide (1–2 mg/ml) by treatment with 1 M-NaBH₄/0.05 M-NaOH for 20 h at 50°C in the dark (Farrar & Harrison, 1978). The solution was then cooled in ice, neutralized by the addition of Dowex 50WX8

resin (acid-washed, H⁺ form; BDH) until effervescence ceased, and freeze-dried. The residue was freed of boric acid by repeated addition of methanol and removal by evaporation under reduced pressure and dissolved in 50 mM-acetic acid. Sialic acid was measured by the resorcinol (Svennerholm, 1957) or the thiobarbituric acid method (Aminoff, 1961). Total hexose was assayed by the method of Dische & Danilchenko (1967). G.l.c. of monosaccharides was carried out by the procedure described by Clamp (1977).

T.l.c. on plates of silica-gel G (BDH) was performed with two solvent systems: butanol/propanol/water (3:5:2, by vol.) and butanol/acetic acid/water (75:25:6, by vol.). Samples and standards (1 mg/ml) in water were applied in a volume of 5–15 μ l, and, after chromatography, the spots were made visible with the orcinol spray reagent (Humbel & Collart, 1975). Some samples were desialylated by adding 0.1 M-H₂SO₄ (50 μ l) to the sample (0.3 mg/ml; 50 μ l) and heating for 1 h in a sealed tube. The solutions were cooled and were neutralized with 0.1 M-NaOH (50 μ l).

Ristocetin-induced platelet agglutination was carried out with platelet-rich plasma prepared from fresh blood by differential centrifugation (Taylor, 1981). The platelet-rich plasma was adjusted to contain 2×10^8 platelets/ml with autologous plasma, and 1 ml was placed in the cuvette of a Bryston aggregometer (Albert Browne Ltd., Leicester, U.K.) and maintained at 37°C with constant stirring. Inhibition of ristocetin-induced agglutination was studied by adding inhibitor diluted in 0.15 M-NaCl (100 μ l), followed 1 min later by 80 or 100 μ l of ristocetin solution (16 mg/ml in 0.15 M-NaCl). Change in light transmission was recorded over a 5-min period. Ristocetin was from Lundbeck, Copenhagen, Denmark, and glycocalicin (Okumura & Jamieson, 1976a) was prepared by the method of Solum *et al.* (1980), the affinity-chromatography step being omitted.

Results

Platelets were obtained from outdated human platelet concentrates by differential centrifugation at room temperature in 150 mM-NaCl/50 mM-sodium phosphate buffer, pH 6.5, containing 1 mM-EDTA (Barber & Jamieson, 1970). The washed platelets (purity >95%) (2×10^9 /ml) were suspended in 150 mM-NaCl/50 mM-sodium phosphate, pH 8.0, containing 1 mg of α -chymotrypsin/ml, for 1.5 h at 37°C. The treated platelets were removed by centrifugation (6000 g/15 min) and analysed by SDS/polyacrylamide-gel electrophoresis (Fig. 1). The results clearly showed a gross reduction of periodate-Schiff staining in the region of GPIIb. A concomitant reduction in the staining intensity of

GPIIb α was also apparent. The other major periodate-Schiff-staining bands appeared unaffected by the treatment.

The clear supernatant obtained after chymotrypsin treatment of the platelet suspension (crude

Fig. 1. SDS/polyacrylamide-gel electrophoresis of normal and chymotrypsin-treated human platelets

Electrophoresis was in a slab containing 7% (w/v) acrylamide with an overlay of 3% acrylamide as described in the Materials and general methods section. (a) Normal platelets under non-reducing conditions; (b) chymotrypsin-treated platelets under non-reducing conditions; (c) chymotrypsin-treated platelets under reducing conditions; (d) normal platelets under reducing conditions. The gel was stained with periodate/Schiff reagent.

glycopolypeptide) was concentrated 10-fold by ultrafiltration (Amicon PM10 membrane) and subjected to gel-permeation chromatography on a column of Sephadex G-200. The elution profile obtained (Fig. 2) was essentially the same as that reported by Barber & Jamieson (1971). The major sialic acid-rich peak (Peak I) was eluted just after the material at the void volume of the column. The fractions comprising this sialic acid-rich peak (glycopolypeptide) were pooled and concentrated by ultrafiltration (Amicon PM10 membrane). About 9 mg of Peak I material (purified glycopolypeptide) was obtained from 2×10^{11} platelets.

Analysis of the glycopolypeptide by haemagglutination inhibition using selected lectins (Table 1) established that this glycopolypeptide is derived from GPIb α , since *Arachis hypogaea* (peanut) lectin, which binds exclusively to GPIb α in desialylated platelet membranes (Judson & Anstee, 1979; Clemetson *et al.*, 1981), is inhibited by desialylated glycopolypeptide. The amino acid and carbohydrate composition of the glycopolypeptide is given in Table 2. The glycopolypeptide contains 59 mol % of carbohydrate, the predominant sugars being galactose, *N*-acetylglucosamine, *N*-acetylgalactosamine and sialic acid. It is rich in serine, threonine and proline, but lacks cysteine. *O*-Glycosidically linked oligosaccharides were isolated by treatment with an alkaline solution of NaBH_4 , followed by gel-permeation chromatography (Fig. 3). Assay of the eluted material for hexose and sialic acid revealed three peaks, and the relevant fractions were pooled as indicated (Fig. 3 and Table 3). The major carbohydrate-containing peak (I-3) was further purified by

Fig. 2. Gel-permeation chromatography of peptides released by chymotrypsin digestion of intact platelets

Concentrated supernatant (10 ml) obtained after α -chymotrypsin treatment of intact platelets was applied to a column of Sephadex G-200 (86 cm $\times$ 2.5 cm) and eluted at a flow rate of 9 ml/h. Fractions (5 ml) were collected. Percentage transmission at 280 nm ($\%T_{280}$) of the effluent was measured continuously (----) and fractions were assayed for sialic acid (—) and chymotrypsin activity (▨) as described in the Materials and general methods section. Fractions 30–35 were pooled and denoted 'Peak I'. V_0 indicates the void volume of the column.

Table 1. *Lectin-binding properties of platelet glycopolypeptide*

The ability of glycopolypeptide (Peak I material prepared as in Fig. 2) to inhibit lectin-mediated agglutination was determined as described in the Materials and general methods section. The numbers indicate the minimum quantity of glycopolypeptide (μg ; based on hexose assay) necessary to inhibit three haemagglutinating doses of lectin.

Lectin ...	Wheat-germ agglutinin	<i>Phaseolus</i> <i>vulgaris</i>	Concanavalin A	<i>Ricinus</i> <i>communis</i>	<i>Maclura</i> <i>aurantiaca</i>	<i>Arachis</i> <i>hypogaea</i>
	24	6	>24	12	6	>24 8*

* Desialylated glycopolypeptide.

Table 2. *Composition of glycopolypeptide*

Amino acid and carbohydrate analysis was carried out as described in the Materials and general methods section. The material contained glucose but since Sephadex was used in the purification procedure (Fig. 2), the value obtained was considered unreliable and was not used for the calculation of the molar ratios.

	Content (mol %)
Aspartic acid	1.29
Threonine	7.15
Serine	4.55
Glutamic acid	3.17
Proline	6.08
Glycine	0.99
Alanine	1.4
Valine	1.3
Cysteine	—
Methionine	0.13
Isoleucine	1.38
Leucine	2.7
Tyrosine	1.81
Phenylalanine	2.9
Lysine	1.4
Histidine	2.9
Arginine	1.12
Mannose	4.2
Galactose	17.8
Glucose	—
<i>N</i> -Acetylglucosamine	10.8
<i>N</i> -Acetylgalactosamine	10.9
Sialic acid	14.9
Fucose	0.3

Table 3. *Carbohydrate composition of products of alkaline-borohydride digest of the glycopolypeptide*

Carbohydrate compositions were determined as described in the Materials and general methods section and expressed as nmol/mg. Fractions I-1, I-2, and I-3 were prepared as described in Fig. 3. Fraction I-3A was prepared as described in Fig. 4. The value obtained for glucose was not used in the calculation of molar ratios (See Table 2).

Fraction no. ...	Composition (nmol/mg)			
	I-1	I-2	I-3	I-3A
Fucose	—	17	—	—
Mannose	—	37	—	—
Galactose	15	84	526	157
Glucose	515	15	30	—
Glucuronic acid	79	—	—	—
<i>N</i> -Acetylglucosamine	6	50	221	67
<i>N</i> -Acetylgalactosamine	44	26	34	—
Sialic acid	—	100	652	168
<i>N</i> -Acetylgalactosaminitol	—	—	254	75

acetylglucosamine, galactose and sialic acid in the molar ratios 1.0:0.9:2.1:2.2 respectively.

The material in Peak I-3A gave a single symmetrical peak when subjected to gel-permeation chromatography on a column of Sephadex G-25 (145 cm $\times$ 13 cm) and appeared homogeneous both before and after desialylation when examined by t.l.c. with the butanol/acetic acid/water solvent mixture. With butanol/propanol/water (Fig. 5) Peak I-3A material gave a major spot with R_F 0.09 and a faint spot with R_F 0.20. When desialylated, Peak I-3A material migrated as a major spot with R_F 0.20, and a very faint spot with R_F 0.45. Estimation of the apparent molecular weights of Peak I-3A and desialylated Peak I-3A material on a calibrated column of Sephadex G-25 (Fig. 6) gave values of 1400 and 900 respectively.

Experiments to determine the involvement of GPIb in ristocetin-induced platelet agglutination showed that neither the major oligosaccharide I-3A nor the glycopolypeptide had a significant inhibitory

ion-exchange chromatography on DEAE-Sephadex A-25 (Farrar & Harrison, 1978). Two sialic acid-containing peaks were resolved (Fig. 4). The major peak (I-3A) accounted for more than 80% of the material released by β -elimination of the glycopolypeptide and had the carbohydrate composition shown in Table 3. Insufficient material corresponding to peak I-3B was obtained for analysis. Peak I-3A contained *N*-acetylgalactosaminitol, *N*-

Fig. 3. Gel-permeation chromatography of alkaline-borohydride digest of platelet glycopolypeptide. The products from alkaline-borohydride treatment of the glycopolypeptide (5 mg) were separated on a column (115 cm $\times$ 1.5 cm) of Sephadex G-50 and eluted at a flow rate of 20 ml/h with 50 mM-acetic acid. Fractions (3 ml) were collected. The A_{280} of the effluent was measured continuously (Δ) and fractions were assayed for sialic acid ($\bullet$) and hexose ($\circ$). Fractions 24–27, 41–47 and 51–61 were pooled and termed 'I-1', 'I-2' and 'I-3' respectively.

Fig. 4. Ion-exchange chromatography of fraction I-3. Fraction I-3 (85 μ g of hexose) was prepared as in Fig. 3 and applied to a column (25 cm $\times$ 1.5 cm) of Sephadex A-25 equilibrated with 50 mM-pyridine/acetate buffer, pH 5.0. The column was washed with a further 50 ml of equilibrating buffer after sample addition, before application of a linear gradient of 50–650 mM-pyridine/acetate buffer, pH 5.0 (200 ml). Fractions (5 ml) were collected at a flow rate of 40 ml/h. A portion of each fraction was freeze-dried, reconstituted in distilled water (0.1 ml) and assayed for sialic acid. —, Sialic acid; ----, buffer gradient. Fractions 21–23 (I-3B) and Fractions 32–36 (I-3A) were pooled and freeze-dried.

Discussion

Treatment of intact platelets with chymotrypsin results in the solubilization of a number of membrane-derived peptides without the concomitant initiation of the platelet-release reaction (Barber & Jamieson, 1971; Davey & Lüscher, 1967). Although a number of membrane proteins may be affected (Podolsak, 1977), the major soluble product originates from GPIb and is a glycopolypeptide of high molecular weight that can be readily purified by gel-permeation chromatography on Sephadex G-200. Similar material was designated 'macro-glycopeptide' by Pepper & Jamieson (1970). This term indicates high-molecular-weight carbohydrate units (polysaccharide) attached to peptide. Our results, however, show that the material consists of large numbers of oligosaccharide units attached to a polypeptide. The preferred term therefore is 'glycopolypeptide' (Reid & Clamp, 1978) and is the term used throughout the present paper.

Features of interest in the glycopolypeptide are a high content of serine, threonine and proline, low levels of asparagine, hydrophobic amino acids and an absence of cysteine. The glycopolypeptide is heavily glycosylated, consisting of 59% carbohydrate on a molar basis. A glycopolypeptide isolated from combined tryptic and Pronase digests of intact platelets (Pepper & Jamieson, 1970) had very similar amino acid and carbohydrate com-

effect (Figs. 7a and 7b) at the concentrations tested. However, glycalicin at 0.2 mg/ml reduced the extent of agglutination to about 25% of that of the control (Fig. 7c).

Fig. 5. *T.l.c. analysis of I-3A*

I-3A and desialylated I-3A was analysed by t.l.c. as described in the Materials and general methods section. Butanol/propanol/water was the solvent. (a) I-3A; (b) desialylated I-3A; (c) standards: glucose (G), melibiose (M), raffinose (R) and stachyose (S).

positions to those reported here (correlation coefficient, $r = 0.94$).

Our data indicate that most of the carbohydrate is *O*-glycosidically linked through *N*-acetylgalactosamine to serine or threonine, which together comprise about 25% of the total amino acids of the glycopolypeptide. Assuming a single *N*-acetylgalactosamine residue per oligosaccharide, this indicates that, on average, every fourth amino acid in the glycopolypeptide is glycosylated (approx. 60 oligosaccharide chains per molecule). The high level of substitution with *O*-linked oligosaccharides and the overall amino acid composition of the glycopolypeptide is similar to the glycosylated, Pronase-resistant regions of blood-group substances and mucus glycoproteins (Donald, 1973; Goodwin & Watkins, 1974). Furthermore, both GPIb and mucus glycoproteins consist of disulphide-linked subunits (Phillips & Poh Agin, 1977; Allen, 1978), which are comprised of glycosylated and non-glycosylated regions (Okumura *et al.*, 1976; Allen, 1978).

Fig. 6. *Molecular-weight determinations of I-3A*

I-3A, desialylated I-3A and standard reference sugars were applied separately to a column (145 cm $\times$ 1.3 cm) of Sephadex G-25 equilibrated in 50 mM-acetic acid, at a flow rate of 20 ml/h. Their elution volumes (expressed as fraction numbers) were measured and plotted against molecular weight (expressed on a logarithmic scale).

The major *O*-glycosidically linked oligosaccharide fraction (I-3A) of the glycopolypeptide was isolated, purified, and the composition determined (Table 3). The results would be consistent with an oligosaccharide unit containing two residues of galactose, one of *N*-acetylglucosamine and two of sialic acid linked through *N*-acetylgalactosamine to a hydroxy amino acid. The *N*-acetylglucosaminitol content suggests a structure with an equivalent weight of 1330 and 748 when desialylated, in good agreement with the experimental mol.wts. of 1400 and 900 for I-3A and desialylated I-3A respectively as determined by gel-permeation chromatography.

The presence of the sialotetrasaccharide:

as found on the erythrocyte glycoprotein α (Thomas & Winzler, 1969), cannot be ruled out. The contaminants detected by t.l.c. of Peak I-3A material may correspond to this native sialotetrasaccharide and its desialylated product $\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}$ (R_F 0.20 and 0.45; Figs. 5a and 5b respectively). Glockner *et al.* (1978) detected the disaccharide $\text{Gal}\beta 1 \rightarrow 3\text{GalNAc}$ in the desialylated material released by alkaline NaBH_4 treatment of platelet membranes. The specificity of *Arachis hypogaea* lectin has been studied extensively (Uhlenbruck *et al.*, 1969; Terao *et al.*, 1975; Lotan *et al.*, 1975;

Fig. 7. Inhibition of ristocetin-induced platelet agglutination by glycocalicin, glycopolyptide and platelet oligosaccharide I-3A

Inhibition of ristocetin-induced agglutination of fresh platelets in autologous plasma was measured as described in the Materials and general methods section. Inhibitor (I), followed by Ristocetin (R), was added to a stirred platelet suspension ($2 \times 10^8/\text{ml}$) and the change in light transmission at 610nm with time recorded. A 1.6mg portion of ristocetin (R) was added in all cases, except (c)1 and (c)2, where 1.3mg was added. (a) Effect of glycopolyptide: 1, I = 0.7mg of glycopolyptide; 2, I = 1.4mg of glycopolyptide; 3, I = physiological saline (0.9% NaCl). (b) Effect of oligosaccharide I-3A: 1, no inhibitor added; 2, I = 0.27mg of oligosaccharide I-3A. (c) Effect of glycocalicin: 1, I = 0.2mg of glycocalicin; 2, I = physiological saline.

Pereira *et al.*, 1976), and it is widely accepted that it has a preferential reactivity for the disaccharide

Gal β 1 $\rightarrow$ 3GalNAc. Since *Arachis hypogaea* lectin binds exclusively to desialylated GPIb (Judson &

Anstee, 1979; Clemetson *et al.*, 1981), the presence of the sialotetrasaccharide described above on GPIb would not be unexpected. The major *O*-linked oligosaccharides of GPIb are, however, sialohexasaccharides containing *N*-acetylglucosamine. This sugar in an *O*-glycosidically linked oligosaccharide has not previously been described as a constituent of a human blood-cell membrane, although Emerson & Kornfeld (1976) report such oligosaccharides in bovine erythrocyte membranes. Nevertheless, *N*-acetylglucosamine is usually found as a constituent of *O*-glycosidically linked oligosaccharides in mucus glycoproteins such as the blood-group-active glycoproteins of ovarian-cyst fluid, where it may be linked to either the *N*-acetylgalactosamine or the galactose residue of the Gal β 1 $\rightarrow$ 3GalNAc core structure (Kabat, 1970; Rovis *et al.*, 1973).

Two possible structures for the sialohexasaccharides of GPIb are:

A number of oligosaccharides have been isolated from human ovarian-cyst glycoproteins (Rovis *et al.*, 1973), two of which (Lewis R_L 0.44 and Lewis R_{IMS} 0.78 respectively) are closely related to the two possible sialohexasaccharide structures described above. The two sialohexasaccharides and the sialotetrasaccharide all have the Gal β 1 $\rightarrow$ 3GalNAc core structure, and their relative proportions in GPIb would depend on the relative activities of the appropriate transferase enzymes involved in their biosynthesis.

Our data do not give an unequivocal answer to the question of the presence of *N*-linked oligosaccharides in the glycopolypeptide. Both the untreated and alkaline-borohydride-treated glycopolypeptide contained a small amount of asparagine and also mannose, which is usually present in *N*-linked oligosaccharides together with *N*-acetylglucosamine and galactose (Table 2). However, the molar amount of *N*-acetylglucosamine and galactose does not appear to be sufficient to allow for the presence of a complex-type unit such as that found in the major sialoglycoprotein of the human erythrocyte (Yoshima *et al.*, 1980).

The functional role of GPIb is not clearly defined, but its importance as a receptor in the adhesion of platelets to subendothelial structures and in ristocetin-induced platelet agglutination, both of which require Factor VIII/von Willebrand factor in plasma, has been emphasized (Phillips, 1980). We investigated the ability of I-3A, the major oligosaccharide of glycoprotein Ib, the glycopolypeptide and glyocalicin to inhibit ristocetin-induced agglu-

tionation of normal fresh platelets. Only glyocalicin showed some inhibition at a concentration of 1.3 μM (calculated from mol.wt. 140000). Okumura & Jamieson (1976b) reported similar inhibition with glyocalicin in the concentration range 0.25–2 μM . However, Cooper *et al.* (1979) reported that their glyocalicin preparation (concentration not specified) did not inhibit bovine Factor VIII-induced platelet agglutination, whereas purified GPIb was an effective inhibitor. It is now generally accepted that glyocalicin is a proteolytic cleavage product of GPIb, probably due to the action of a platelet-derived Ca²⁺-activated proteinase (Phillips & Jakábova, 1977; Solum *et al.*, 1980; Clemetson *et al.*, 1981). The probable relationship between the glycopolypeptide, glyocalicin and GPIb is shown in Fig. 8. The evidence from our inhibition experiments and those of others suggests that the numerous *O*-glycosidically linked oligosaccharides

on the glycopolypeptide portion of glycoprotein Ib are not specific receptors for Factor VIII/von Willebrand factor. Nevertheless, the fact that chymotrypsin-treated platelets are not agglutinated by ristocetin (Jenkins *et al.*, 1976) suggests that the presence of the glycopolypeptide is important for maintaining the correct conformation of the Factor VIII/von Willebrand factor receptor at the platelet surface. Other, as yet unidentified, changes resulting from chymotrypsin treatment may, however, be involved.

The function of carbohydrate at cell surfaces is poorly understood. Our results suggest that the carbohydrate of GPIb does not play a specific role in platelet adhesion. However, as described above, GPIb has structural features similar to those of mucus glycoproteins. The main functions of mucus glycoproteins in the gastrointestinal tract, namely lubrication and protection, depend on the formation of a visco-gelatinous covering of the endothelial lining which results from the interaction between the carbohydrate side chains and water molecules (Allen, 1978). The numerous carbohydrate side chains of GPIb might give rise to an analogous covering at the platelet surface. Such covering could present a barrier preventing the contact of macromolecules with the membrane (Edwards, 1978). Since the two primary functions of platelets, adhesion and aggregation, are mediated by macromolecules (Factor VIII/von Willebrand factor and fibrinogen respectively; Phillips, 1980), the existence of such a barrier could have a major role in modulating platelet function.

Fig. 8. Possible orientation of GPIb in the platelet plasma membrane (modified from Phillips, 1980)

The presumed sites of action of trypsin (T), α -chymotrypsin (C) and the Ca^{2+} -activated proteinase (Ca^{2+} -P) are indicated. $\rightarrow$ indicates the presence of O-glycosidically linked oligosaccharides.

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