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Isolation and biochemical characterization of the α- and β-subunits of glycoprotein IIb of human platelet plasma membrane

Juan J. CALVETE and José GONZÁLEZ-RODRÍGUEZ*
Instituto de Química Física, Consejo de Investigaciones Científicas, Serrano 119, 28006 Madrid, Spain

The α- and β-subunits of glycoprotein IIb (GPIIb) of human platelet plasma membrane were isolated in fully reduced, partially reduced and alkylated, and fully alkylated forms, by size-exclusion chromatography after reduction of pure GPIIb. The sugar moiety of GPIIbα accounts for 16.4% of its total weight, whereas that of GPIIbβ accounts for only 10.2%. The molar percentages (per 100 mol of total amino acids) of neuraminic acid and galactose in the α-subunit more than double those in the β-subunit, whereas galactosamine is present only in GPIIbα. From the amino acid and sugar compositions the acidic nature of both subunits was confirmed. The M_r values obtained, 114000 for GPIIbα and 22200 for GPIIbβ, are in very good agreement with those obtained by physical methods. We found by stepwise reduction of pure GPIIb with dithioerythritol that GPIIbα and GPIIbβ are joined by a single interchain disulphide bridge, while the remaining half-cystine residues participate in intrachain bonds, six in GPIIbα and one in GPIIbβ, the intersubunit disulphide bond being that reduced first. Neither of the two subunits is liberated from isolated plasma membranes when this GPIIb interchain bond is reduced in isolated membranes.

INTRODUCTION

Two-dimensional electrophoretic analysis of platelet membrane proteins has shown that GPIIb consists of two subunits, α (GPIIbα) and β (GPIIbβ), joined by disulphide bonds (Phillips & Agin, 1977). In the preceding paper we determined that GPIIb has eight disulphide groups, on the basis of the apparent lack of free thiol groups in isolated GPIIb (Eirín *et al.*, 1986).

In the present paper we describe the isolation of GPIIbα and GPIIbβ from pure GPIIb, their amino acid and sugar compositions, and the presence of a single interchain disulphide bridge in GPIIb, the rest of the half-cystine residues being involved in intrachain disulphide bonds. We have also observed that GPIIbα and GPIIbβ remain in the membrane after cleavage of the interchain disulphide bridge of GPIIb by reduction of isolated platelet membranes.

MATERIALS AND METHODS

Materials

Eosin–maleimide was from Molecular Probes (Junction City, OR, U.S.A.). The rest of the chemicals, biochemicals, chromatographic columns, buffers and preparative procedures for isolation of GPIIb were as described in the preceding paper (Eirín *et al.*, 1986).

Analytical methods

Assays. Assays of protein (Lowry *et al.*, 1951; Markwell *et al.*, 1978), sialic acid (Hammond & Papermaster, 1976), neutral sugars (Alpenfels, 1981), hexosamines (Hjerpe *et al.*, 1980), amino acids, tryptophan (Edelhoch, 1967), free thiol groups (Ellman, 1959) and disulphide groups (Zahler & Cleland, 1968) and SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) were done as indicated in the preceding paper (Eirín *et al.*, 1986). Bound eosin was determined spectrophotometrically at 530 nm ($\epsilon = 85000\ M^{-1} \cdot cm^{-1}$).

Immunoelectroblotting. After gel electrophoresis the glycoproteins were transferred to nitrocellulose by a standard procedure (Towbin *et al.*, 1979) at 250 mA for 2.5–4 h. The first antibodies used, all in the form of 50%-satn.-$(NH_4)_2SO_4$ precipitate of hybridoma supernatants or of antisera, were: mouse anti-GPIIIa monoclonals (P6, P37, P40 and P97) described previously (Melero & González-Rodríguez, 1984), one mouse anti-GPIIb monoclonal (M1), and rabbit anti-GPIIb and anti-GPIIIa sera (J. González-Rodríguez, unpublished work). The second antibodies were anti-(mouse IgG)-peroxidase and anti-(rabbit IgG)-peroxidase conjugates (Sigma Chemical Co., St. Louis, MO, U.S.A.). The substrate was 4-chloro-1-naphthol.

Isolation of the α- and β-subunits of GPIIb with different degrees of reduction and carboxymethylation

Fully reduced subunits (reduced-GPIIbα and reduced-GPIIbβ) were prepared by reduction of pure GPIIb (5 mg/ml) in h.p.s.e.c. elution buffer with 1% (v/v) 2-mercaptoethanol at 100 °C for 2 min, and further separated by exclusion chromatography with elution buffer containing 0.1% 2-mercaptoethanol, either on a Sephacryl 200 column (1.6 cm × 178 cm) or on an analytical TSK-SW 4000–3000 column system (7.5 mm × 600 mm each, in series). Partially reduced (an average of 2.3±0.4 half-cystine residues reduced per molecule of GPIIbα) and carboxymethylated subunits (GPIIbα and GPIIbβ) were prepared by reduction of GPIIb (5 mg/ml) with a 5-fold molar excess of dithioerythritol (3 μmol/ml) over the theoretical half-cystine content, in 0.15 M-Tris/HCl/1 mM-EDTA/2% (w/v) SDS, pH 8.0, for 2 h at room temperature,

Abbreviations used: GPIIbα and GPIIβ, the α- and β-subunits respectively of glycoprotein IIb (GPIIb), after reduction of the single disulphide bond joining them, and further carboxymethylation; GPIIIa, glycoprotein IIIa; CM-GPIIbα, CM-GPIIbβ and CM-GPIIIa, totally reduced and carboxymethylated forms of GPIIbα, GPIIbβ and GPIIIa respectively; h.p.s.e.c., high-performance size-exclusion liquid chromatography.

* To whom correspondence should be sent.

followed by carboxymethylation with a 2-fold molar excess of iodoacetate over reducing agent. Fully reduced and carboxymethylated subunits (CM-GPIIbα and CM-GPIIbβ) were prepared from GPIIb as above, but with a 150-fold molar excess of dithioerythritol over theoretical half-cystine content. Partially and fully carboxymethylated subunits were isolated by size-exclusion chromatography as above, with h.p.s.e.c. buffer.

Cleavage of GPIIb by reduction

Samples of pure GPIIb (about 3 mg) in 0.05 M-Tris/HCl, pH 9, at different SDS concentrations, were reduced for 2 h with various molar excesses of dithioerythritol over half-cystine content, and the thiol groups formed were titrated with 5,5'-dithiobis-(2-nitrobenzoic acid) (Zahler & Cleland, 1968). Before SDS solubilization and electrophoretic analysis samples were carboxymethylated with a 2-fold molar excess of iodoacetate over reducing agent, and dialysed against electrophoresis sample buffer. Labelling of cleaved GPIIb with eosin–maleimide was done with a 2-fold molar excess of the dye over the reducing agent used in each sample. The labelled and unlabelled subunits were isolated by h.p.s.e.c., as described above.

Reduction and carboxymethylation of platelet plasma membranes

Samples of platelet membranes (about 2.5–5 mg of protein) were dialysed against 0.038 M-Tris/0.1 M-glycine/5 mM-EDTA, pH 9, centrifuged at 150000 *g* ($r_{av.}$ 65 mm) for 1 h at 4 °C, resuspended in the same buffer, and reduced with dithioerythritol with the use of 5–2000-fold molar excesses of reductant over theoretical half-cystine contents of GPIIb and GPIIIa in the membrane. After 2 h of reduction at room temperature, samples were carboxymethylated with a 2-fold molar excess of iodoacetate over reductant, and centrifuged; supernatants and pellets were subjected to protein and electrophoretic analyses. All samples were heated at 100 °C in 1% 2-mercaptoethanol for 2 min to improve resolution, the pellets being previously dialysed against electrophoretic sample buffer. In some experiments dithioerythritol was replaced by 2-mercaptoethanol at molar excesses of 5–15000-fold, and the reduction was carried out at 100 °C for 2 min, the samples being handled as above. Occasionally the buffer ionic strength was raised with NaCl.

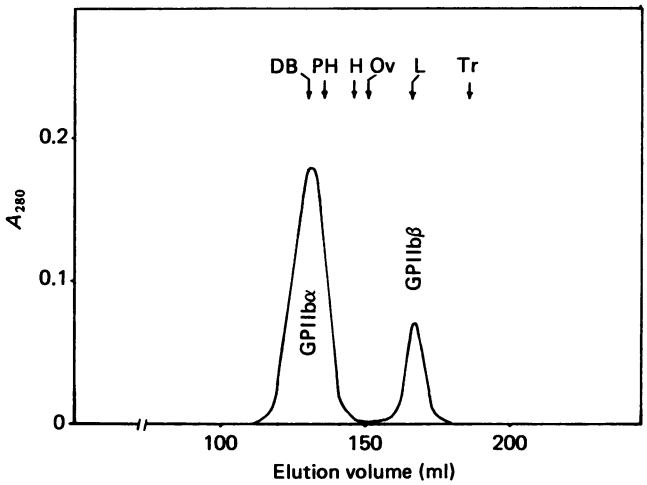

Fig. 1. Isolation of GPIIbα and GPIIbβ from reduced GPIIb by size-exclusion chromatography

Elution profile on a Sephacryl S-200 column (178 cm × 1.6 cm) of 5 mg of pure GPIIb reduced with 1% 2-mercaptoethanol at 100 °C for 2 min and eluted at 7 ml/h with h.p.s.e.c. buffer. Abbreviations: DB, Dextran Blue; PH phosphorylase *b*; H and L, heavy chain and light chain respectively of IgG; Ov, ovalbumin; Tr, trypsin.

Table 1. Amino acid and sugar compositions of GPIIbα and GPIIbβ

The experimental results are an average of ten determinations with four different preparations. Abbreviation: N.D., not detected.

	Composition			
	(mol/100 mol of total amino acids)		(residues per molecule)*	
Residue	GPIIbα	GPIIbβ	GPIIbα	GPIIbβ
Asp	10.16	8.19	87	15
Thr	5.05	4.14	43	7
Ser	6.45	4.80	55	9
Glu	13.23	11.07	113	20
Pro	8.38	5.60	72	10
Gly	8.54	8.53	73	15
Ala	5.69	10.60	49	19
Cys	1.43	1.87	12	3
Val	6.72	6.75	57	12
Met	1.39	1.43	12	3
Ile	3.07	2.64	26	5
Leu	11.29	14.86	97	27
Tyr	2.34	2.33	20	4
Phe	3.56	3.57	30	6
His	2.29	1.50	20	3
Lys	3.91	3.10	33	6
NH_3†	11.19	13.70	95	25
Arg	4.47	7.36	38	13
Trp	2.01	1.67	17	3
Gal	2.94	1.31	25	2
Man	1.54	1.68	13	3
GlcNAc	2.80	2.45	24	4
GalNAc	1.11	N.D.	10	—
NeuAc	2.61	1.14	20	2

* Assuming M_r 114000 for GPIIbα and M_r 22200 for GPIIbβ.

† Not included in the total amino acids.

RESULTS

Isolation of the α- and β-subunits of GPIIb

After reduction of pure GPIIb with either 2-mercaptoethanol or dithioerythritol, the subunits, either fully reduced, or partially reduced and carboxymethylated (after cleavage of the single interchain disulphide bond; see below), or fully reduced and carboxymethylated, were separated by exclusion chromatography. In Fig. 1 we show the elution profile of the isolated GPIIbα and GPIIβ fractions. More than 95% of the protein loaded is recovered, of which 82% is in the GPIIbα fraction and 18% in the GPIIbβ fraction.

Table 2. Apparent M_r values of GPIIbα and GPIIbβ determined by gel electrophoresis and by size-exclusion chromatography and from the amino acid and sugar compositions

Procedure	Apparent M_r		
	Reduced GPIIbα and GPIIbα	CM-GPIIbα	Reduced GPIIbβ, GPIIbβ and CM-GPIIbβ
Gel electrophoresis (Laemmli, 1970)	110000	110000	23500
Size-exclusion chromatography on:			
TSK-SW 4000–3000 analytical columns	140000	120000	29500
Sephacryl 200	—	—	23500
Amino acid and sugar analysis	114000	114000	22200

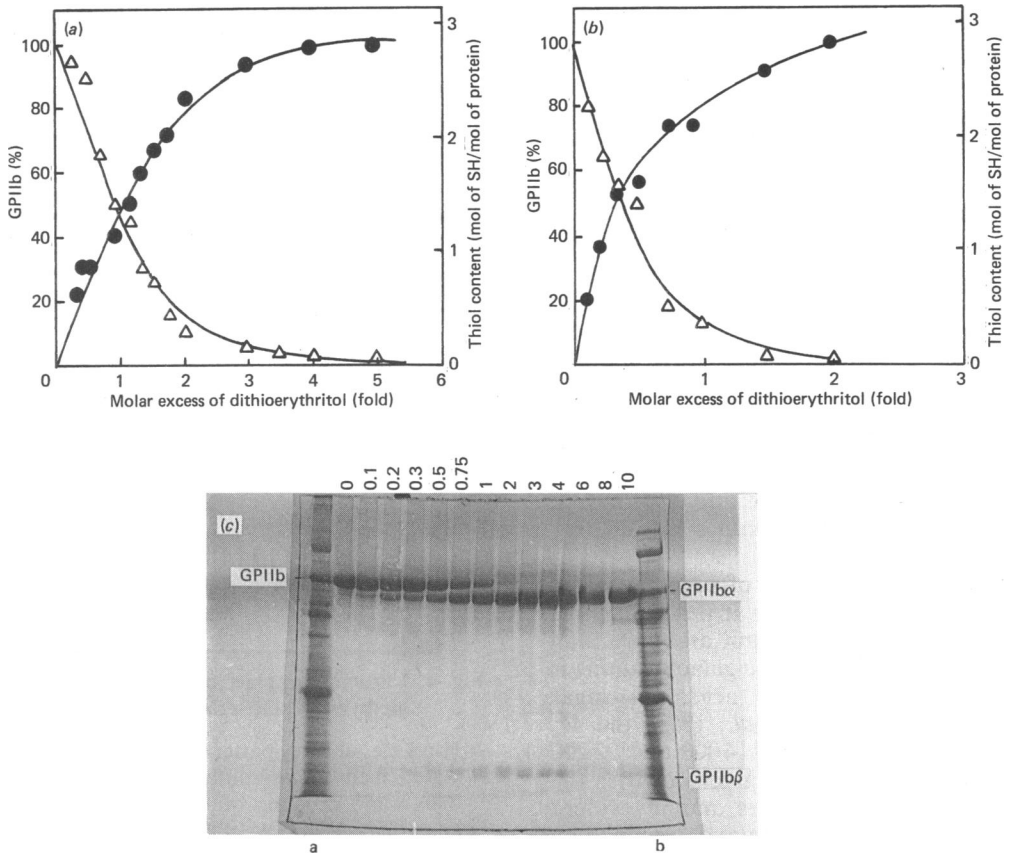

Fig. 2. Cleavage of the interchain disulphide in isolated GPIIb

Stepwise reduction of pure GPIIb with molar excess of dithioerythritol with respect to the half-cystine content of GPIIb as determined by amino acid analysis (Eirín *et al.*, 1986). ●, Number of thiol groups formed in GPIIb determined as described previously (Zahler & Cleland, 1968; Eirín *et al.*, 1986) (for reduction conditions see the Materials and methods section); △, percentage of intact GPIIb obtained by SDS/polyacrylamide-gel electrophoresis (Laemmli, 1970) in 7–12% polyacrylamide gradients and Coomassie Blue staining. (*a*) and (*b*) Reduction of GPIIb in the presence of 0.1% and 2% SDS respectively. (*c*) Electrophoretic analysis of the reduction of GPIIb in 2% SDS as a function of the molar excess of dithioerythritol. Lane a, non-reduced platelet membrane; lane b, reduced platelet membrane. Lanes in between, and from left to right, are pure GPIIb reduced with 0-, 0.1-, 0.2-, 0.3-, 0.5-, 0.75-, 1-, 2-, 3-, 4-, 6-, 8- and 10-fold molar excesses of dithioerythritol.

Amino acid and sugar compositions of GPIIbα and GPIIbβ

In Table 1 we give the amino acid and sugar compositions of GPIIbα and GpIIbβ. The molar percentage of dicarboxylic amino acids in GPIIbβ is lower than that in GPIIbα, whereas the percentage of basic amino acids is higher. Both serine and proline contents are lower in the β-subunit. There are no significant differences in the polarity of the amino acids between the two subunits, which, as also happens with GPIIb, cannot be considered as low-polarity membrane proteins. Ammonia, although also given, is not included in the total amino acids. The amino acid composition of

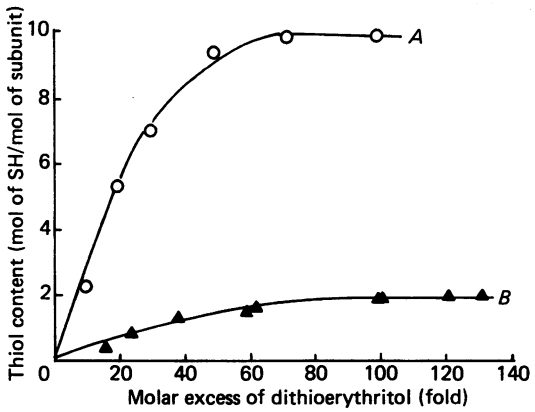

Fig. 3. Quantification of the disulphide bridges in GPIIbα and GPIIbβ

The Figure shows the dependence of the titratable thiol groups in GPIIbα (○) and in GPIIbβ (▲) on the molar excess of reducing agent. The thiol-group titrations were carried out in 0.1% SDS as described previously (Zahler & Cleland, 1968; Eirín *et al.*, 1986). The concentration of dithioerythritol is expressed in molar excess with respect to the expected half-cystine content of GPIIb (for reduction conditions see the Materials and methods section).

GPIIb, calculated from the composition of the individual subunits and their contribution to the total mass of GPIIb (82% and 18% for the α- and β-subunits respectively), is in good agreement with the composition of GPIIb determined by amino acid analysis (Eirín *et al.*, 1986). Exceptions are serine and methionine, for which the calculated values are 25–30% lower, and tyrosine and tryptophan, for which the calculated values are 22–30% higher.

GPIIbα contains 16.4% by weight of sugar, whereas GPIIbβ contains only 10.2%. The molar percentages of sialic acid and galactose in the α-subunit are more than twice those in the β-subunit, and all the galactosamine is in the α-subunit. From the amino acid and sugar compositions we calculated (Hoy *et al.*, 1974) the M_r values of GPIIbα and GPIIbβ to be 114000 and 22200 respectively, which are in very good agreement with the values obtained by gel electrophoresis and in evident disagreement with the apparent M_r values determined by h.p.s.e.c. on TSK-SW columns (Table 2).

Cleavage of the interchain disulphide bonds in isolated GPIIb

Stepwise reduction with dithioerythritol of pure GPIIb in SDS solutions selectively cleaves the interchain linkages joining the two subunits of GPIIb, as monitored by SDS/polyacrylamide-gel electrophoresis. When the reduction is carried out in 0.1% SDS, a 5-fold molar excess of dithiothreitol is required for the cleavage of GPIIb in 25 min, exposing 2.6±0.3 thiol groups per cleaved molecule to titration with 5,5′-dithiobis-(2-nitrobenzoic acid) (Fig. 2*a*). When the reduction is done in 2% SDS, a 2-fold molar excess of dithioerythritol is required for complete cleavage, and an average of 3 thiol groups per molecule are now titratable (Figs. 2*b* and 2*c*). In contrast, GPIIb in 0.05% SDS is not appreciably cleaved with a 2-fold molar excess of reductant, even after 2 h of reduction time, requiring a 10-fold molar excess of dithioerythritol to cleave 25% of the molecules of GPIIb in the same time.

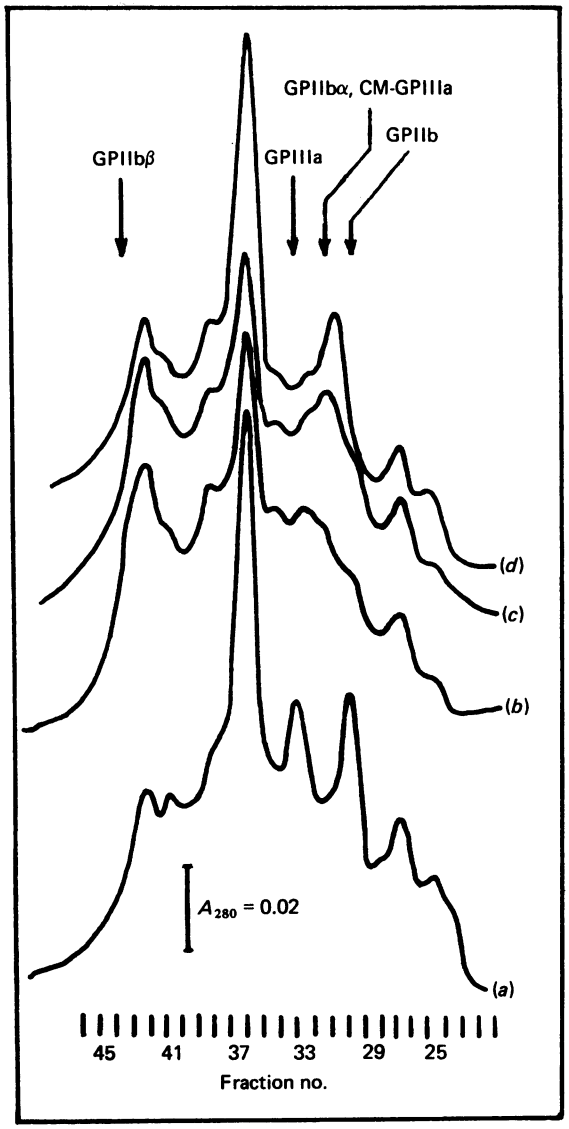

Fig. 4. Chromatographic analysis of reduced and carboxymethylated platelet membranes

H.p.s.e.c. elution patterns of reduced and carboxymethylated membranes solubilized in SDS. About 600 μg of membrane proteins of each sample was loaded on an analytical TSK-SW 4000 column in series with two TSK-SW 3000 columns. Elution and monitoring were done in an LKB system at 0.2 ml/min (Eirín *et al.*, 1986). Trace (*a*) Unmodified membranes; traces (*b*), (*c*) and (*d*) membranes reduced with 5-, 20- and 1000-fold molar excess of dithioerythritol respectively with respect to the half-cystine content of GPIIb and GPIIIa of the membranes.

Eosin–maleimide labelling and isolation of the individual subunits: quantification of the thiol groups and intrachain disulphide bonds in each subunit

The newly formed thiol groups in GPIIb cleaved by reduction in 0.1% SDS were blocked either with eosin–maleimide or by carboxymethylation (see the Materials and methods section), and the subunits were

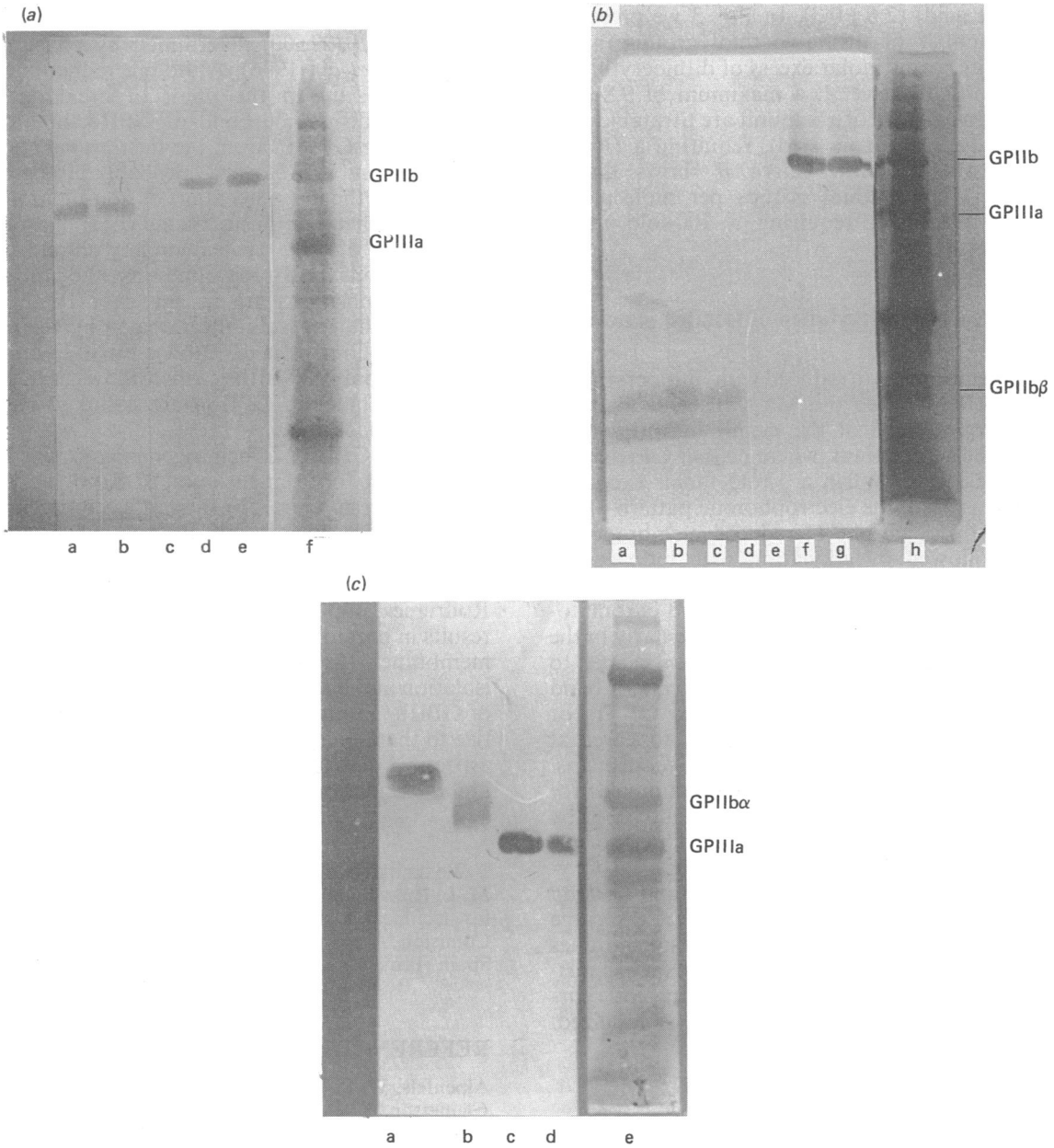

Fig. 5. Identification by immunoblotting of modified GPIIbα, GPIIbβ and GPIIIa in the electrophoretic pattern of platelet membranes reduced with a 1000-fold excess of dithioerythritol and carboxymethylated

Immunoelectroblotting was carried out as described previously (Towbin *et al.*, 1979; Eirín *et al.*, 1986). (*a*) Localization of modified GPIIbα by using rabbit anti-GPIIb sera (at 1/500 dilution). Lane a, reduced and carboxymethylated membranes; lane b, CM-GPIIbα; lane c, GPIIIa; lane d, GPIIb; lane e, unmodified membranes; lane f, electroblotting control of unmodified membranes stained with Amido Black. Electrophoresis was carried out in a 7–12% polyacrylamide gradient gel. (*b*) Localization of modified GPIIbβ by using a mouse anti-GPIIbβ monoclonal antibody (M1, at 1/500 dilution). Lane a, CM-GPIIbβ; lane b, reduced and carboxymethylated membranes; lane c, h.p.s.e.c. fractions 43–45 of reduced and carboxymethylated membranes (Fig. 4, trace *d*); lane d, GPIIIa; lane e, CM-GPIIbα; lane f, unmodified platelet membranes; lane g, GPIIb; lane h, electroblotting control of unmodified membranes stained with Amido Black. Electrophoresis was performed in a 7–15% polyacrylamide gradient gel. (*c*) Localization of modified GPIIIa by using a mouse anti-GPIIIa monoclonal antibody (P37, at 1/100 dilution). Lane a, reduced and carboxymethylated membranes; lane b, CM-GPIIIa; lane c, unmodified platelet membranes; lane d, GPIIIa; lane e, electroblotting control of unmodified platelet membranes stained with Amido Black. All samples were reduced in 1% 2-mercaptoethanol at 100 °C for 2 min, before electrophoresis in a 7% polyacrylamide gel.

separated by h.p.s.e.c. for a new quantification of the thiol groups exposed after cleavage of the interchain disulphide bond and determination of the intrachain disulphide bonds. From the eosin content we found that 2.3 ± 0.4 and 0.9 ± 0.04 thiol groups per molecule of GPIIbα and GPIIbβ respectively were formed after cleavage. This is in very good agreement with the value for the number of thiol groups per molecule of cleaved GPIIb determined before h.p.s.e.c. either from the eosin content (2.7 ± 0.5) or by titration with 5,5′-dithiobis-

(2-nitrobenzoic acid) (2.6±0.3). In Fig. 3 we present a plot of the number of titratable thiol groups in the isolated subunits versus molar excess of dithioerythritol. In the conditions of curve *A*, a maximum of 9.8±0.2 thiol groups per molecule of α-subunit are titratable with 5,5′-dithiobis-(2-nitrobenzoic acid), requiring a 60-fold molar excess of reductant. Curve *B* shows that a maximum of 1.8±0.08 thiol groups per molecule of β-subunit are titratable, requiring a 100-fold molar excess of dithioerythritol.

Reduction and carboxymethylation of isolated platelet membranes

If platelet membranes are reduced with dithioerythritol or 2-mercaptoethanol (see the Materials and methods section), up to one-third of the membrane proteins is solubilized in the supernatant, where neither GPIIbα nor GPIIbβ can be found. When a 5-fold molar excess of reducing agent is used, the electrophoretic pattern of the pellet is the well-known one of reduced membranes. If reduction is followed by alkylation with iodoacetate, again neither the α- nor the β-subunits can be found in the supernatant, but the electrophoretic and chromatographic patterns of the pellets change, particularly in the region of GPIIb and GPIIIa (Fig. 4). GPIIbα seemed to be in the expected position, but there was no major band in the expected positions of either reduced GPIIIa or CM-GPIIIa. However, a new band, which appeared at lower mobility than GPIIb and stained for glycoproteins, was identified as modified GPIIIa by immunoelectroblotting (Fig. 5*c*). GPIIbα and GPIIbβ were identified by electrophoretic analysis, glycoprotein staining and immunoelectroblotting of the reduced and carboxymethylated membranes and of the GPIIbα and GPIIbβ fractions obtained by analytical h.p.s.e.c. of the same membranes (Figs. 5*a* and 5*b*). When these membranes were extracted in 10 mM-Tris/0.15 M-NaCl/2 mM-EDTA/0.1% (v/v) Triton X-100 at 0 °C with gentle stirring for 1 h, neither GPIIbα nor GPIIbβ was solubilized.

DISCUSSION

The main aims of the present work were to isolate and biochemically characterize the α- and β-subunits of GPIIb, to determine the number of interchain disulphide bonds joining the two subunits, and to establish whether any of the subunits was liberated from the membrane after cleavage of the interchain disulphide bridges. Stepwise reduction, electrophoretic analysis and thiol-group determination of reduced and carboxymethylated GPIIb showed that a single interchain disulphide bridge joins the α- and β-subunits, that this is the disulphide bond first reduced in GPIIb, and that the rest of the half-cystine residues of the molecule form seven intrachain disulphide bonds, six in GPIIbα and one in GPIIbβ, the latter one being apparently the most difficult to reduce. In this way we were able to prepare, besides fully reduced subunits and fully carboxymethylated subunits after full reduction of GPIIb, subunits with all their intrachain disulphide bonds practically intact.

The chemical compositions of the α- and β-subunits and the relative mass distribution after their isolation, GPIIbα (82%), and GPIIbβ (18%), are in very good agreement with the composition of GPIIb and with the M_r values of GPIIb (136500), GPIIbα (114000) and GPIIbβ (22500) determined by physical methods (Usobiaga *et al.*, 1986). Although reduction of platelet membranes, up to the point of breaking the single interchain disulphide bond of GPIIb, liberates neither GPIIbα nor GPIIbβ from the membrane, and therefore both subunits are very probably integral to the membrane, the low percentage of non-polar amino acids present in their compositions indicates that only a small proportion of their protein moiety should actually be facing the bilayer. In the same way, the amino acid and sugar compositions are in agreement with the acidic nature of the two subunits revealed by their isoelectric points (McGregor *et al.*, 1981). Finally, galactosamine was found only in GPIIbα, which allows us to predict that the sugar chains in GPIIbβ are *N*-linked only through *N*-acetylglucosamine.

Isolated GPIIbβ is heterogeneous in size when it is subjected to one-dimensional SDS/polyacrylamide-gel electrophoresis (Fig. 5*b*), and it is apparently heterogeneous both in pI and in size when analysed by two-dimensional (isoelectric focusing/SDS/polyacrylamide) gel electrophoresis (J. J. Calvete & J. González-Rodríguez, unpublished work), which agrees with earlier results in patterns of whole platelets and isolated plasma membranes (Clemetson, 1985; Crawford, 1985). The isolation and biochemical analysis of the different species of GPIIbβ, and the elucidation of the mechanism giving rise to them, may provide relevant information on some aspects of platelet physiology and platelet removal from blood circulation.

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REFERENCES

Alpenfels, W. F. (1981) Anal. Biochem. **114**, 153–157
Clemetson, K. J. (1985) in Platelet Membrane Glycoproteins (George, N., Nurden, A. T. & Phillips, D. R., eds.), pp. 67 and 75, Plenum Press, New York
Crawford, N. (1985) in Platelet Membrane Glycoproteins (George, N., Nurden, A. T. & Phillips, D. R., eds.), p. 39, Plenum Press, New York
Edelhoch, H. (1967) Biochemistry **6**, 1948–1954
Eirín, M. T., Calvete, J. J. & González-Rodríguez, J. (1986) Biochem. J. **240**, 147–153
Ellman, G. L. (1959) Arch. Biochem. Biophys. **82**, 70–77
Hammond, K. S. & Papermaster, D. S. (1976) Anal. Biochem. **74**, 292–297
Hjerpe, A., Antonopoulos, C. A., Classon, B. & Engfeldt, B. (1980) J. Chromatogr. **202**, 453–459
Hoy, T. G., Ferdinand, W. & Harrison, P. M. (1974) Int. J. Pept. Protein Res. **6**, 121–140
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Markwell, M. A. K., Haas, S. M., Bieber, L. L. & Tolbert, N. E. (1978) Anal. Biochem. **87**, 206–210
McGregor, J. L., Clemetson, K. J., James, E., Capitanio, A., Greenland, T., Lüscher, E. F. & Dechavanne, M. (1981) Eur. J. Biochem. **116**, 379–388
Melero, J. A. & González-Rodríguez, J. (1984) Eur. J. Biochem. **141**, 421–427

Phillips, D. R. & Agin, P. P. (1977) J. Biol. Chem. **252**, 2121–2126
Towbin, H., Staehelin, T. & Gordon, J. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 4350–4354
Usobiaga, P., Calvete, J., Saiz, J. L., Eirín, M. T. & González-Rodríguez, J. (1986) Eur. Biophys. J., in the press
Zahler, W. L. & Cleland, W. W. (1968) J. Biol. Chem. **243**, 716–719

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