Pj variant, a new hybrid MNSs glycoprotein of the human red-cell membrane

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An unusual glycoprotein variant (Pj) was found inherited through a caucasian family exhibiting atypical N and Nvg blood-group reactivities. Pj erythrocytes are blood-group-MS homozygous and have a normal sialic acid content. On sodium dodecyl sulphate/polyacrylamide-gel electrophoresis the variant contains a new component Pj of 24 kDa apparent molecular mass in the monomeric state which is sharply stained by periodic acid/Schiff reagent. Both blood-group-MN (α) and -Ss (δ) glycoproteins were present. Homodimers (Pj_2) as well as heterodimers with MN-glycoprotein (αPj) and the Ss-glycoprotein (δPj) were also identified. The new sialoglycoprotein Pj is trypsin- and chymotrypsin-resistant *in situ* and carries N- and Nvg- but not M- and S-reactivities. The Pj component is labelled by lactoperoxidase-catalysed radioiodination. A 3H label is also easily introduced into the sialic acid or the galactose and galactosamine of the Pj glycoprotein. It is proposed that the Pj is a hybrid glycoprotein containing the N-terminal end of δ -glycoprotein and the C-terminal end of the α -glycoprotein. This proposal is supported by the finding that Pj carries a leucine residue at its N-terminus and is not immunoprecipitated by a monoclonal mouse antibody (R18) reacting specifically with the external domain of glycoprotein α . The red cells from the proposita Pj were found positive for a very low frequency MN antigen named St^a.

The polypeptides and glycoproteins of the human red-cell membrane have been extensively investigated in recent years and one of the most informative tools for such studies was electrophoresis of denaturated membrane components on polyacrylamide gels.

A clear nomenclature of the glycoprotein bands separated by SDS/polyacrylamide-gel electrophoresis in the discontinuous buffer system described by Laemmli (1970) was recently proposed by Anstee *et al.* (1979), who basically identified four major monomeric glycoprotein species denoted α (synonym PAS-2, glycophorin A monomer), β (synonym PAS-2'), γ (synonym glycophorin C) and δ (synonym PAS-3, glycophorin B monomer). The glycoproteins may also exist in homo- or hetero-oligomer entities termed α_2 (dimeric form of GP α , synonym PAS-1), $\alpha\delta$ (heterodimer of GP α and δ , synonym PAS-4), δ_2 (homodimer of GP δ).

Abbreviations used: GP α , GP δ and GP Pj, glycoproteins α , δ and Pj; SDS, sodium dodecyl sulphate; DABITC, 4-*NN*-dimethylaminoazobenzene 4'-isothiocyanate; Mi., Miltenberger antigen.

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No definitive structural information is currently available concerning GP β and GP γ . However, GP α and GP δ have been largely characterized on a molecular basis and have been respectively associated with MN- and Ss-blood-group antigens (Dahr *et al.*, 1977; Wasniowska *et al.*, 1977; Furthmayr, 1978; Dahr *et al.*, 1980a,b).

Although structural analyses of red-cell membrane glycoproteins have progressed well, the biological function of these molecules, if any, remains largely unknown. Indeed, some evidence suggests that these glycoproteins do not play a fundamental role, since several variants lacking GP α (Ena⁻ cells) or GP δ (S⁻ cells) or even both (homozygous Mk cells) were shown not to have impaired red-cell function (for a review, see Anstee, 1980).

Numerous other variants of the MNSs locus are serologically defined (Race & Sanger, 1975), but only a few of them have been investigated on a molecular basis. For instance, M^a and M^c variants were recently characterized as mutations affecting the N-terminal portion of GP α (Anstee & Tanner, 1978; Dahr *et al.*, 1981a,b; Furthmayr *et al.*, 1981;

Blumenfeld *et al.*, 1981), whereas some of the Miltenberger antigens (Mi.III, Mi.IV and Mi.VI) represented still-uncharacterized variants of the GP δ molecule (Anstee & Tanner, 1978; Dahr *et al.*, 1978a; Anstee *et al.*, 1979; Blumenfeld *et al.*, 1981).

More intriguing variants (Mi.V., En M^k, Ph, J.R.) were found which were suggested to arise from a non-homologous crossing-over between genes coding for membrane glycoproteins (Dahr *et al.*, 1978b; Tanner *et al.*, 1980; Vengelen-Tyler *et al.*, 1981; Langley *et al.*, 1981). As a result, new gene fusion products coding for hybrid molecules are thought to be obtained.

In the present study we report another variant, Pj, which was identified in a Caucasian family where α - and δ -gene products are present, and we provide biochemical evidence supporting the view that Pj erythrocytes contain a hybrid glycoprotein molecule composed of the N-terminal portion of GP δ and the C-terminal portion of GP α . The rare blood-group antigen St^a was found associated with this condition.

Materials and methods

Reagent-grade chemicals were used throughout. Lactoperoxidase from bovine milk (60 units/mg) was obtained from Sigma Chemicals (St. Louis, MO, U.S.A.). Galactose oxidase (2200 units/mg) was obtained freeze-dried from KabiVitrum AB (Stockholm, Sweden). Neuraminidase from *Vibrio cholerae* (1 unit/ml) was purchased from Behringwerke AG (Marburg-Lahn, Germany). Trypsin [1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated] (541 units/mg) and α -chymotrypsin (53.6 units/mg) were purchased from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). The chemicals used for polyacrylamide-gel electrophoresis were acrylamide, NNN'N'-tetramethylethylenediamine from Eastman Kodak Corp. (Rochester, NY, U.S.A.) and sodium dodecyl sulphate from Prolabo (Paris, France).

Na¹²⁵I (16.9 mCi/ μ g of iodine) was bought from The Radiochemical Centre (Amersham, Bucks., U.K.) and NaB³H₄ (7 Ci/mmol) from New England Nuclear Corp. (Dreieichenhain, Germany).

Reagents for N-terminal amino-acid analysis were dansyl (5-dimethylaminonaphthalene-1-sulphonyl) chloride from Eastman Kodak and DABITC from Fluka AG (Buchs, Switzerland).

The propositus, Mrs. Pj, was a caucasian blood donor from the Centre National de Transfusion Sanguine (Paris) found in October 1979.

Intact red cells were radioiodinated by the lactoperoxidase procedure described by Shin & Carraway (1974). The treatment of erythrocytes with a low concentration of metaperiodate followed by reduction with NaB³H₄ was also used to label the

surface sialic acids (Gahmberg & Andersson, 1977). These labelling procedures are given in the legends to the Figures.

The membrane ghosts from labelled erythrocytes were prepared by hypo-osmotic lysis (Steck & Kant, 1974). Membrane pellet obtained by centrifugation for 15 min at 22000 g (4°C) (1 vol.) was dissolved in 0.5 vol. of 30 mM-Tris/HCl buffer, pH 6.8, containing 3 mM-EDTA and 6% (w/v) SDS and boiled for 3 min at 100°C (stock lysate).

Before electrophoresis, 150–300 μ g of protein from the stock lysate was adjusted to 100 μ l in 10 mM-Tris/HCl buffer, pH 6.8, containing 1 mM-EDTA, 2% (w/v) SDS (Laemmli buffer) and 5 μ l of 2-mercaptoethanol. The mixture was heated for 30 min at 37°C, followed by 3 min at 100°C. Before each sample was loaded on the gel, 20 μ l of a solution containing 0.025% Bromophenol Blue in 50% (v/v) glycerol was added (final vol. 0.125 ml).

Electrophoresis of the erythrocyte membranes solubilized in SDS was performed on 10% (w/v) acrylamide gels as described by Laemmli (1970), in a Bio-Rad 220 D electrophoresis system.

Erythrocyte ghosts from Pj were extracted by the chloroform/methanol procedure (Hamaguchi & Cleve, 1972). The glycoproteins solubilized in the upper aqueous phase were separated by SDS/polyacrylamide-gel electrophoresis and stained with Coomassie Blue (Cleveland *et al.*, 1977). The bands α , α Pj and Pj were cut off from the gel with a razor blade and eluted by 18 h incubation of the homogenized gel in 10 vol. of 50 mM-NH₄HCO₃/SDS (0.05%)/NaN₃ (0.02%) (Weber & Osborn, 1975). The gel was removed by centrifugation at 500 g and was further extracted by 2 vol. of the elution buffer. The combined supernatants were freed of residual gel particles by filtration on a Millipore 0.45 μ m-pore-size filter and freeze-dried. The dried material was redissolved and extensively dialysed against distilled water. The Coomassie Blue stain was eliminated by three extractions with 2 vol. of water-saturated butanol and the pooled colourless aqueous phases were dialysed against distilled water and freeze-dried. Portions of the glycoproteins extracted by this procedure were redissolved in 10 mM-Tris/HCl buffer, pH 6.8, containing 1 mM-EDTA, 2% (w/v) SDS and adjusted to a final volume of 100 μ l with the standard Laemmli buffer. The purity of glycoproteins was checked by SDS/polyacrylamide-gel electrophoresis as described above.

Standard agglutination tests were performed with either proteinase-treated or untreated erythrocytes by using a routine procedure (Mollison, 1974) with various reagent including human anti-M and anti-N sera, *Vicia graminea* seed extracts and human anti-S and anti-s sera.

The protein content of the samples was measured

as described by Lowry (1951), with bovine serum albumin as standard. Sialic acids were determined by the Warren (1959) assay. The glycoproteins eluted from acrylamide gels were first desialylated (0.1 M-H₂SO₄, 80°C, 1h) before N-terminal sequence analysis by the Dansyl-Edman degradation procedure. Dansylation and identification of the dansyl derivatives was done by the method described by Hartley (1970), whereas Edman degradation was conducted by using DABITC as described by Chang *et al.* (1978).

Results

Serological observations

Mrs. Pj was a caucasian blood-group-O donor, first typed as M⁺ N⁺w S⁺s⁻. However, by using human anti-M and anti-S antibodies, which exhibit dosage effects, we were able to demonstrate by agglutination kinetics on a continuous-flow system (Rouger *et al.*, 1980) that Pj red cells were indeed homozygous for M- and S-antigens. Careful examination of Mrs. Pj's red cells showed an unusually high reactivity with human anti-N reagents compared with other MM-typed erythrocytes. As shown in Table 1, the N and Nvg (Nvg stands for N-reactivity detected by *Vicia graminea* lectin) reactivities of Pj red cells were trypsin- and α-chymotrypsin-resistant and stronger than expected for homozygous MM individuals. After α-chymotrypsin treatment, which cleaves mainly band-3 protein and GPδ *in situ* (Dahr *et al.*, 1975), the 'N' receptor of normal MM erythrocytes is severely decreased as compared with trypsin-treated cells. This is not the case for Pj erythrocytes, which therefore behaved more like N than M red cells. As also indicated in Table 1, the M- and S-antigens of Pj erythrocytes were normally expressed, but were respectively destroyed by trypsin or α-chymotrypsin treatment. These results suggest that Pj red cells are MS-homozygous and carry N and Nvg receptors possibly located, in addition to

the normal GPδ, on some new component of the red-cell membrane.

Investigations carried out on blood from Mrs. Pj's family showed that the same atypical N and Nvg reactivities were present on her mother's and first sister's red cells (I-2 and II-2 respectively in Fig. 1), but not on her father's (I-1) or second sister's (II-1) erythrocytes. Enough blood was available from II-2 to demonstrate that her red cells were also MS-homozygous. In addition, none of the following MNSs-related antigens were present on red cells from II-2 or II-3: M^s, Mi^a, Vw, Mur, Hut, Hil and Far.

SDS/polyacrylamide-gel electrophoresis

After SDS/polyacrylamide-gel electrophoresis and staining by the periodate/Schiff procedure (Fig. 2), a typical pattern showing the four monomeric bands α, β, γ and δ (corresponding to apparent molecular masses 36, 32, 23 and 20kDa respec-

Fig. 1. Pedigree of the Pj family
Determination of MNSs blood group and genotypes. ● indicates atypical N-reactivity in individuals carrying the abnormal Pj sialoglycoprotein. The proband (II-3) is indicated by an arrow.

Table 1. Effect of proteinase treatment on the MNSs blood typing of Pj variant and control erythrocytes
Portions (1 vol.) of red cells were treated with 4 vol. of trypsin [1-chloro-4-phenyl-3-L-tosylamidobutan-2-one ('TPCK')-treated] (T) or α-chymotrypsin (αCT) [2.5 mg/ml in phosphate-buffered saline (10 mM-phosphate/150 mM-NaCl, pH 7.2) pH 8.0] for 30 min at 37°C, followed by four washes in phosphate-buffered saline. The results of agglutination experiments are given as reciprocals of the agglutination titres.

Erythrocytes	Antibody Source Enzyme treatment	Anti-N Human			Anti-N <i>Vicia graminea</i>			Anti-M Human			Anti-S Human		Anti-s Human	
		None	T	αCT	None	T	αCT	None	T	αCT	T	αCT	T	αCT
		2 ¹	2 ³	2 ¹	2 ¹	2 ⁵	2 ⁵	2 ³	0	2 ⁴	2 ⁴	0	0	0
Pj variant (II-3)														
Control														
MMSS		0	0	0	2 ¹	2 ⁴	2 ¹	2 ³	0	2 ³	2 ³	2 ¹	0	0
NNss		2 ⁶	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁵	0	0	0	0	0	2 ⁴	2 ¹

Fig. 2. SDS/polyacrylamide-gel electrophoresis of red-cell membranes from Pj and normal erythrocytes

Electrophoresis of ghosts was performed on a 1.5 mm slab gel containing 10% (w/v) acrylamide, 0.27% (w/v) bisacrylamide with a stacking gel of 3% (w/v) acrylamide in the discontinuous buffer system of Laemmli (1970). Protein (150 μ g) was loaded on the gel. The slab gel was stained with periodic acid/Schiff reagent and scanned on a Vernon spectrophotometer equipped with green filter (no. 57A). (a) Normal erythrocytes; (b) donor Pj (II-3 in Fig. 1). The arrows indicate the four additional bands identified in Pj erythrocytes. The nomenclature of these new bands is given in the text and in Fig. 4. The nomenclature of the glycoproteins is that given by Anstee *et al.* (1979); L indicates lipid.

tively) was obtained with the control red cells, as well as higher-molecular-mass band components named α_2 , $\alpha\delta$, or δ_2 , which represent either homo or hetero association of the monomeric band components (Anstee *et al.*, 1979). In striking contrast, the glycoprotein profile from Pj membranes showed four additional periodate/Schiff-positive bands with respective apparent molecular masses of 68, 58, 50 and 24 kDa. The 68 kDa component was predominant and had almost the same periodate/Schiff-staining intensity as that of the α_2 -band. It was noticed that the new 24 kDa band component migrated with apparently the same mobility as the

γ -band, which usually stained very poorly with periodate/Schiff reagent.

A similar periodate/Schiff staining profile was obtained with erythrocytes from Mrs. Pj (II-3), her mother (I-2) and her sister (II-2), demonstrating that this new character is inherited according to Mendelian laws. As deduced from Warren (1959) assays, the neuraminidase-sensitive sialic acid content of Pj-type erythrocyte was found to be similar to control erythrocytes.

On a quantitative basis the components α_2 , $\alpha(+\beta+\delta_2)$, $\alpha\delta$ and δ of normal erythrocytes represented 48, 31, 5.5 and 8.5% respectively of the total periodate/Schiff stain, a set of values in good agreement with those obtained by Dahr *et al.* (1976). In Pj erythrocytes the extra bands corresponding to 68, 58, 50 and 24 kDa represented (18.7, 3, 1.8 and 6.9% respectively of the total periodate/Schiff staining intensity, whereas α_2 , $\alpha(+\beta+\delta_2)$, $\alpha\delta$ and δ represented 27, 28, 6.8 and 7.8. However, the relative intensity of the 24 kDa band should be decreased by about 0.7% to account for the co-migration of band γ .

Lactoperoxidase radioiodination of Pj erythrocytes

Intact red cells from control and Pj family members were 125 I-labelled and analysed by SDS/polyacrylamide-gel electrophoresis. The labelled polypeptides were detected by autoradiography of the dried gels (Fig. 3). With control erythrocytes (Fig. 3, lane 4) the two monomeric species α and δ were 125 I-labelled, but the β - and γ -bands were not (or to a very low extent), as was also shown by others (Mueller *et al.*, 1976). However, the band 3 as well as the oligomeric bands α_2 , $\alpha\delta$ and δ_2 were also strongly labelled by this procedure.

With erythrocytes from Mrs. Pj (II-3) and her sister (II-2), four additional 125 I-labelled bands were seen (Fig. 3, lanes 1 and 3), which had apparent molecular masses of 70, 56, 49 and 24 kDa respectively, a set of values virtually identical with those found with periodate/Schiff-stained gels. These bands have been denoted ' α Pj', ' Pj_2 ', ' δ Pj' and ' Pj ', since their molecular composition was deduced from two-dimensional SDS/polyacrylamide-gel electrophoresis as reported in Fig. 4. Indeed, on diagonal electrophoresis in the second dimension, the 24 and 56 kDa components each gave only two spots of 24 and 56 kDa, indicating that they were derived from each other by interconversion of a monomeric (Pj) $\rightleftharpoons$ dimeric (Pj_2) equilibrium. The same picture demonstrates that the 49 kDa component might be a heterodimer of the Pj and δ -glycoproteins which partly dissociates and gives respectively the δ Pj, and Pj bands. However, the expected δ_2 and Pj_2 bands were not detectable on Fig. 4, but were seen in other experiments (results not shown). Similarly the 70 kDa component partly dissociates in the second

Fig. 3. SDS/polyacrylamide-gel electrophoresis of ^{125}I -labelled red-cell membranes from Pj and normal erythrocytes

Electrophoresis of the labelled ghosts was performed as described in Fig. 2. Packed red cells (0.5 ml) were radioiodinated by using 0.5 mCi Na^{125}I and 100 μg of lactoperoxidase. Iodination was initiated by 25 successive additions of 0.02 ml H_2O_2 (2.5 mM) at 20 s intervals. The cells were washed in phosphate-buffered saline (see Table 1) and 150 μg of each labelled ghosts were added to the gel. The gel stained with periodate/Schiff reagent or Coomassie Blue R250 was dried on a filter paper and exposed to Kodak X-Omat MA film for 1–10 days. (1) Radiolabelled ghosts from Pj variant (donor II-3, Fig. 1); (2) radiolabelled ghosts from normal erythrocytes; (3) radiolabelled ghosts from Pj variant (donor I-2, Fig. 1); (4) radiolabelled ghosts from non-affected member of Pj family (father I-1, Fig. 1). Nomenclature of bands and abbreviations as in Fig. 2. The band denoted * might be either a minor component or a degradation product of band 3.

dimension to give mainly α and Pj components. The α_2 and Pj₂ bands theoretically expected could not be seen on this map, since the former spot is masked by the strongly radioactive polypeptides migrating on the diagonal axis and the latter dimer is quantitatively formed only to a small extent.

After digestion of the ^{125}I -labelled Pj erythrocytes by trypsin (Fig. 5), the bands α_2 , αPj and $\alpha\delta$ were removed, but the Pj and Pj₂ bands were not affected. The δ -band was only slightly decreased.

Proteolytic digestion by α -chymotrypsin mainly cleaved band-3 and band- δ proteins, but did not significantly affect GP α , Pj and Pj₂ (Fig. 5). Therefore the Pj component appears to be resistant *in situ* to hydrolysis by trypsin and chymotrypsin. Since M- and S-antigens were respectively destroyed by such enzymes, the results suggest that Pj has N- and Nvg- but no M- or S-determinants.

Fig. 4. Diagonal electrophoresis of ^{125}I -labelled membrane from Pj erythrocytes

Erythrocytes of donor Pj (II-3 Fig. 1) were ^{125}I -labelled as described in the legend of Fig. 3. The ghosts were separated in the first dimension by SDS/polyacrylamide-gel electrophoresis as described in Fig. 2. A longitudinal piece of the gel (1 cm width) containing the ^{125}I -labelled proteins separated in the first dimension was layered at the top of a second slab with similar characteristics of the first one. The slice was equilibrated in Laemmli buffer containing 40 mM-dithiothreitol and the electrophoresis was run in the second dimension. The gel was finally dried on filter paper and exposed to Kodirex film for 1–10 days. The position of the membrane glycoproteins located on the diagonal axis is indicated at the top of the Figure as deduced from the migration of the bands in the first dimension. The spots located above or below the diagonal axis are glycoproteins species which result from the association–dissociation equilibrium of oligomers separated in the first dimension. The nomenclature of these spots is given on the right-hand side of the autoradiograph as deduced from the migration of the glycoproteins in the second dimension. The nomenclature of the bands is as described by Anstee *et al.* (1979).

^3H -labelling of cell-surface residues

After periodate/ NaB^3H_4 treatment, bands α_2 , $\alpha\delta$, δ_2 , α , β and δ were labelled both in control and Pj erythrocytes (results not shown). As expected, the four additional sialic acid-rich bands, αPj , Pj₂, δPj and Pj, already seen with periodate/Schiff staining and [^{125}I]iodination were also strongly labelled. It was noticed that the Pj monomer appeared as a heavily labelled band migrating on the gel at the same position as band γ . This latter glycoprotein, however, was only slightly labelled in control erythrocytes, suggesting that the sialic content is higher in Pj than γ .

In another experiment, the galactose and N-

Fig. 5. SDS/polyacrylamide-gel electrophoresis of ^{125}I -labelled red-cell membrane from Pj and normal erythrocytes treated with proteinases

The erythrocytes were ^{125}I -labelled and treated with proteinases as described in Fig. 3 and Table 1. After extensive washings the ghosts were solubilized with SDS and electrophoresed as described in Fig. 2. The gel was then dried on a filter paper and exposed to a Kodirex film for 10 days. Radioiodinated ghosts from untreated, trypsin-treated and chymotrypsin-treated control erythrocytes are respectively seen on lanes 1, 3 and 5. Radioiodinated ghosts from untreated, trypsin-treated and chymotrypsin-treated Pj erythrocytes are respectively seen on lanes 2, 4 and 6. Numerous bands corresponding to degradation products are visible. The nomenclature of bands is as in Fig. 2.

acetylgalactosamine residues were selectively labelled with ^3H after sequential treatment of intact red-cells with galactose oxidase and reduction with NaB^3H_4 , with and without prior neuraminidase treatment (Gahmberg, 1976). The Pj component was strongly labelled by this procedure as well as band γ on control erythrocytes (result not shown).

N-terminal amino-acid analysis

N-Terminal analysis of GP α isolated from Mrs. Pj erythrocytes established the presence of serine and glycine in positions 1 and 5 respectively, which is consistent with the findings of others when analysis was performed on GP α derived from MM individuals (Dahr *et al.*, 1977; Wasniowska *et al.*, 1977).

Both the DABITC and the dansyl procedures established that a leucine residue was present at the N-terminus of GP Pj. The amino acids in positions 2, 3 and 4 are also very probably glycosylated, but only the serine in position 2 was identified, owing to

the scarcity of material isolated. From these experiments it was concluded that, in the red cells from Mrs. Pj, the GP α and Pj have different N-terminal amino acids.

Immunoprecipitation of membrane proteins

The mouse monoclonal LICR.LON/R18 antibody reacted with an internal sequence of the terminal extracellular domain of GP α , which is located between residues 40 and 72 (Mawby *et al.*, 1981). By using this antibody (a gift from Dr. David Anstee, Department of Biochemistry, University of Bristol, Bristol, U.K.), we found that only band α_2 and α , both from Pj and control erythrocytes, were immunoprecipitated. Since GP Pj is not precipitated, it is concluded that the determinant recognized by the R18 antibody is absent from this glycoprotein.

Discussion

We have described a new sialic acid-rich glycoprotein, Pj, in the erythrocyte membrane from several homozygous MS members of a Caucasian family. This component of 24 kDa molecular mass in the monomeric state carries N- and Nvg-reactivities and rather surprisingly exhibits some properties usually associated with GP α and GP δ respectively.

In situ, Gp Pj is able to form oligomers with other red-cell membrane proteins. For instance, homodimers Pj₂ (58 kDa) as well as heterodimers δ Pj (50 kDa) and α Pj (68 kDa) were identified. Since α Pj is the predominant species, it is suggested that Pj might have some structural similarities with GP α molecules, at least within the hydrophobic domain of the molecule which is responsible for aggregation properties (Furthmayr & Marchesi, 1976; Silverberg *et al.*, 1976). Moreover, *in situ*, both GP α and Pj are chymotrypsin-resistant. However, glycoprotein Pj has no detectable M-determinant and a lower molecular mass than GP α .

Obviously, there are structural similarities between GP δ and Pj. Both molecules are trypsin-resistant *in situ* and exhibit N- and Nvg-reactivities. In addition, we have shown that the N-terminus of GP Pj is a leucine residue, an amino acid also found at the N-terminal end of GP δ (Dahr *et al.*, 1977; Wasniowska *et al.*, 1977; Furthmayr, 1978). However, GP Pj differs from δ , since the former has no S-reactivity and a higher molecular mass.

A red-cell glycoprotein with properties similar to that of Pj has been fairly recently described by Tanner *et al.* (1980), in several individuals of a negro family (Ph) either homozygous or heterozygous for the S⁻ condition. The possibility was put forward that GP Ph might be a hybrid molecule carrying sequences of amino acids derived both from α and δ sialoglycoproteins. This phenomenon would have arisen from a chromosomal misalignment and

unequal crossing-over between α and δ genes in a manner similar to which Lepore and anti-Lepore haemoglobins are gene-fusion products between the δ - and β -globin genes (Bunn *et al.*, 1977). To account for its properties, the Ph component was postulated to be a hybrid molecule denoted $(\delta-\alpha)^{Ph}$, composed of the *N*-terminus from $GP\delta$ and the *C*-terminus from $GP\alpha$. Support for this model was very recently provided by Mawby *et al.* (1981), who demonstrated that the Ph component was immunoprecipitated by a specific rabbit antibody (denoted I16) directed against the *C*-terminal domain of $GP\alpha$, but not by a monoclonal mouse antibody (R18) recognizing the external domain of $GP\alpha$ between amino acids 40 and 72. Our results have clearly established that the Pj component is not immunoprecipitated by the monoclonal R18 antibody and exhibited a leucine residue at its *N*-terminus. These properties added to those stated above strongly suggest that Pj is also a hybrid molecule formed by the *N*-terminal end of $GP\delta$ and the *C*-terminal portion of $GP\alpha$. This new hybrid might be denoted $(\delta-\alpha)^{Pj}$. Since $(\delta-\alpha)^{Pj}$ has no S-reactivity, the contribution of $GP\delta$ to the hybrid molecule should be less than the 29 first amino acids and include no cleavage site *in situ* for chymotrypsin. The contribution of $GP\alpha$ to the Pj component was established from indirect evidence. The absence of M- and R18-determinants on $(\delta-\alpha)^{Pj}$ suggest that the $GP\alpha$ molecule in the hybrid might be cut off by approx. 40–72 amino acid residues from the *N*-terminus and include *in situ* no cleavage site for trypsin.

The final $(\delta-\alpha)^{Pj}$ molecule has a molecular mass intermediate between those of $GP\alpha$ and δ . This may be accounted for if we consider the Pj hybrid as a 'GP α -like' molecule carrying no *N*-glycosidically linked oligosaccharide chains (since the *N*-terminus of the molecule is made of $GP\delta$) and a deletion of an undetermined number of amino acids (some being glycosylated) at the junction between the δ - and α -molecules. Therefore there are obviously several possible $(\delta-\alpha)$ hybrid molecules depending on the localization of the unequal crossing-over between α - and δ -genes.

Two distinct chromosomes are formed after this kind of crossing-over.

(i) The first type of chromosome should carry an α -gene, the $(\delta-\alpha)$ -gene product and the δ -gene in that order. Our Pj variant has one copy of such a chromosome, together with a copy of a normal chromosome carrying the α and δ genes, since we have clearly shown that neither the father (I-1) nor the second sister (II-1) of Mrs. Pj are affected. Furthermore it is established that Pj erythrocytes have two doses of M- and S-antigens respectively, and $GP\alpha$ and $GP\delta$ are normally present in Pj red cells. However, homozygotes for the recombinant chromosome (not yet found) would also have

normal levels of $GP\alpha$ and $GP\delta$, but twice the amount of $(\delta-\alpha)$ -gene product. In the Ph family described by Tanner *et al.* (1980), one member, M.P., is said to be homozygous for the S⁻ condition and carries the hybrid molecule $(\delta-\alpha)^{Ph}$. Since M.P. has no detectable $GP\delta$ on his red cells, but should carry a recombinant chromosome, it was suggested that the unequal crossing-over in this negro family occurred between a normal chromosome and one carrying a 'silent' or altered δ -gene (S⁻) which is frequent in negroid populations. The recombinant chromosomes carrying the $(\delta-\alpha)$ gene-fusion products are reminiscent of those producing 'anti-Lepore' haemoglobins (Bunn *et al.*, 1977).

(ii) The second kind of chromosome produced by the unequal crossing-over between α and δ genes carries only a fused ($\alpha-\delta$) gene, but no longer any normal α - or δ -genes. Variants with such a chromosome produce a hybrid glycoprotein denoted $(\alpha-\delta)$, which is made of the *N*-terminal end of $GP\alpha$ and the *C*-terminal end of $GP\delta$. When found in the homozygous state, the presence of the recombinant chromosome mimics the En(a⁻) phenotype as is the case for the so-called EnM^k phenotype (M.E.P. family) and the homozygous Mi.V individuals (Dahr *et al.*, 1978b; Vengelen-Tyler *et al.*, 1981). Individuals heterozygous for such recombinant chromosome have been found in the J.R. family (Langley *et al.*, 1981). The recombinant chromosomes carrying the $(\alpha-\delta)$ gene-fusion products are reminiscent of those producing 'Lepore' haemoglobins (Bunn *et al.*, 1977).

Before we can get a clear picture of the various hybrid molecules postulated, a detailed structural analysis is required, but it is well-established that the occurrence of such variants is not associated with any impairment of red-cell function. The observation of hybrid molecules determined by unequal crossing-over between α - and δ -genes is in agreement with our present understanding of the MNSs locus (Dahr *et al.*, 1975), which states that α - and δ -genes are closely linked genes on chromosome no. 4 (Cook *et al.*, 1981), which have evolved from a duplication of a common N ancestor gene during evolution.

Since the original version of this paper was submitted, a new sample from Mrs. Pj became available for the complete red-cell typing within the low frequency MN-antigens. The cells were found positive for the rare St^a antigen (results from Miss P. Tippet, Medical Research Council Blood Group Unit, 4, Stephenson Way, London NW1 2HE, U.K.) but negative for the following: Cl^a, D^s, He, M₁, Mit, Mt^a, M^v, Ny^a, Raddon, Ri^a, Sul, Vr. As expected, the St^a reactivity was found to be both trypsin- and chymotrypsin-resistant. This is in agreement with a recent finding from Anstee *et al.* (1982) showing that eight examples of

St(a⁺) blood were very likely carrying hybrid molecules of the anti-Lepore type denoted (δ -a)St glycoproteins. A sample known to have the St(a⁺) phenotype (E.Z., from Dr. M. N. Metaxas, Blood-Group Laboratory, CRS, Hirschengraben 60, 8001 Zurich, Switzerland), was investigated in our laboratory and found to have a glycoprotein profile very similar to that of Mrs. Pj's blood. It is not known, however, whether all these St(a⁺) variants are characterized by hybrid molecules with a complete identical amino acid sequence, but clearly the St^a determinant might be defined as the junction product of the N-terminal and C-terminal parts of normal GP δ and GP α respectively. Indeed, the first reported example of anti-Lepore variant (Tanner *et al.*, 1980) was found St(a⁻), but resulted from an unequal crossing-over between a normal chromosome and one carrying an altered δ gene.

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