Studies on human red-cell membrane glyophorin A and glyophorin B genes in glyophorin-deficient individuals

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1. Genomic DNA derived from individuals who lack glyophorin A (GPA), glyophorin B (GPB) or both of these proteins was subjected to Southern-blot analysis using GPA and GPB cDNA probes. 2. Bands on the Southern blots were assigned to the GPA gene, GPB gene or to a putative pseudogene. 3. Genomic DNA derived from an individual of the M^k phenotype was shown to have deletions in the GPA and GPB genes. The simplest model for the results obtained is that a single deletion spans the GPA and GPB genes in the individual studied.

INTRODUCTION

Glyophorin A (GPA) and glyophorin B (GPB) (synonyms sialoglycoproteins α and δ respectively) are integral membrane proteins found in the red-cell plasma membrane (reviewed by Anstee, 1981; Anstee & Tanner, 1986). Both these glyophorins carry a number of blood-group antigens; GPA carries the blood-group-MN antigens, whereas GPB carries the blood-group-N antigen and the blood-group-Ss antigens, and possibly the blood-group-U antigen (reviewed by Issitt, 1985). The cDNA sequences of GPA and GPB share extensive regions of sequence similarity separated by well-defined insertions and deletions (Siebert & Fukuda, 1987; Tate & Tanner, 1988). The 5' non-coding region and the region encoding the leader sequence and the first 26 amino acids of the blood-group-N form of GPA is identical with the corresponding region in the GPB cDNA. Less sequence similarity exists between the regions encoding the membrane-spanning domains, but the 3' non-coding regions of the two mRNAs are totally dissimilar.

Rare individuals are known whose red blood cells lack either GPA [described as En(a[−]) phenotype], GPB (S[−]s[−]U[−] phenotype) or both of these proteins (M^k phenotype). Southern-blot analysis of genomic DNA derived from individuals with the S[−]s[−]U[−] phenotype suggests that the gene encoding GPB has been deleted (Huang *et al.*, 1987), whereas one type of En(a[−]) phenotype [En(Fin)] is characterized by a deletion in the gene encoding GPA (Rahuel *et al.*, 1988a).

The present paper describes Southern-blot analysis of genomic DNA derived from an individual of M^k phenotype and shows that parts of the genes for both GPA and GPB are deleted in this individual.

MATERIALS AND METHODS

Materials

Restriction enzymes were from Amersham International (Amersham, Bucks., U.K.) and Pharmacia Bio-

chemicals (Milton Keynes, Bucks., U.K.). [³²P]dATP was from Amersham International, and Genescreen Plus membrane was purchased from NEN [du Pont (U.K.) Ltd., Stevenage, Herts., U.K.].

Methods

Epstein-Barr-virus-transformed lymphoblastoid cell lines (EBV-LCL) were made from lymphocytes obtained from individuals homozygous for the following genotypes: M^k/M^k (donor R.S.; Okubo *et al.*, 1988), En(Fin)/En(Fin) (donor E.P.; Walker *et al.*, 1987) and S[−]s[−]U[−]/S[−]s[−]U[−] (donor J.B.). Genomic DNA was prepared as described by Tanner *et al.* (1988). The DNA was digested with restriction endonucleases using the manufacturer's recommended buffers. A 15 µg portion of restriction-digested DNA was separated on a 0.5% agarose gel (Maniatis *et al.*, 1982) and transferred on to Genescreen Plus membrane by capillary blotting. Southern blots were probed, using the manufacturer's recommended protocol, with restriction fragments of GPA and GPB cDNA clones previously isolated (Tate & Tanner, 1988). Probe DNA was labelled by using a random priming kit (BCL Ltd., Lewes, East Sussex, U.K.).

Southern blots were probed with ALP1a [nts 1–779 of the cDNA encoding GPA (ALP1; Tate & Tanner, 1988)], ALP1b (nts 804–983 of ALP1), ALP2 (nts 1–233 of ALP1) and the 3' non-coding region from the cDNA encoding GPB (DEL3, nts 429–616; Tate & Tanner, 1988). ALP2 was produced by digesting ALP1a with restriction endonuclease *Sac*I, whereas DEL3 was produced by digesting the intact GPB cDNA with *Sph*I; fragments were subsequently purified from low-melting-point agarose gels (Maniatis *et al.*, 1982). Oligonucleotides were labelled by using polynucleotide kinase (Maniatis *et al.*, 1982) and used to probe genomic DNA in dried agarose gels as described by Thein & Wallace (1986).

Abbreviations used: GPA, glyophorin A; GPB, glyophorin B; nts, nucleotides; EBV-LCL, Epstein-Barr-virus-transformed lymphoblastoid cell lines; 1 × SSC, 0.15 M-NaCl/0.015 M-sodium citrate.

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RESULTS AND DISCUSSION

Southern blots of *Eco*RI- and *Hind*III-digested genomic DNA from EBV-LCL were prepared and probed with the GPA probe (ALP1a) (Fig. 1). This probe detected not only the GPA gene, but also hybridized with the coding region and the 5' non-coding region of the GPB gene. The bands missing from the genomic DNA derived from individuals of the M^k phenotype (M^k DNA), the En(Fin) phenotype [En(Fin) DNA] and the S-s-U- phenotype (U- DNA) were compared with DNA derived from a normal control individual (control DNA), and this suggested that these unusual phenotypes arose from deletions. The hybridization pattern of *Eco*RI-digested U- DNA was identical with the control DNA, whereas *Hind*III-digested U- DNA lacked two fragments compared with the control DNA. Siebert & Fukuda (1986) have shown that *Hind*III digestion of normal genomic DNA produces fragments of the GPA gene which are different in size to those fragments generated from the GPB gene, whereas *Eco*RI digestion produces fragments from the GPA and GPB genes which are identical in size.

Bands in the Southern blot of *Hind*III-digested DNA

were assigned to the GPA or GPB genes by comparing the bands in U- DNA and En(Fin) DNA. A band present in En(Fin) DNA but absent from U- DNA is likely to be derived from the GPB gene. Similarly, a band present in U- DNA, but absent from En(Fin) DNA, is likely to be derived from the GPA gene. It was possible to assign most of the bands in the Southern blot of *Hind*III-digested DNA, except for the 7.0, 4.4 and 8.2 kb bands, which were present in both En(Fin) DNA and U- DNA. The latter bands were assigned by probing a Southern blot with ALP2 (nts 1-233 of ALP1) and an oligonucleotide (NTA). Oligonucleotide NTA contained nts 165-191 of the GPA cDNA (Tate & Tanner, 1988), which is identical with the corresponding region of GPB. Table 1 shows that ALP2 hybridized to five fragments, whereas oligonucleotide NTA hybridized to three fragments (7.0, 6.6 and 1.6 kb). The 1.6 and 6.6 kb bands (Table 1) had already been assigned to the GPA gene and GPB gene respectively. Thus the 7.0 kb band, which is present in all the DNA samples, cannot be part of either the GPA or GPB genes. It is possible that the 7.0 kb fragment may be part of a pseudogene related to the GPA and GPB genes (see below).

ALP2 hybridized to two additional bands (8.2 and

Fig. 1. Southern blots of variant DNA

Genomic DNA samples are: lane a, control DNA; lane b, En(Fin) DNA; lane c, M^k DNA; lane d, U- DNA. Genomic DNA was digested with the following restriction enzymes: blot 1, *Eco*RI; blot 2, *Hind*III; blot 3, *Pst*I. Blots 1 and 2 were probed with ALP1a (nts 1-779 of ALP1), whereas blot 3 was probed with DEL3, the 3' non-coding region of a GPB cDNA clone (nts 424-616). Blots 1 and 3 were washed in 0.1 × SSC at 65 °C, whereas blot 2 was washed in 1 × SSC at 65 °C. Bands have been assigned to the GPA gene (A), GPB gene (B) and to the putative pseudogene (P). Assignments (As.) in parentheses are tentative, since the assignment depends on the absence of a GPA-related 5' non-coding region in the pseudogene.

Table 1. Probing Southern blots of *Hind*III-digested normal DNA with fragments of GPA cDNA

'+' indicates that the probe hybridized to the band in question. Abbreviations: DNA, DNA used to probe Southern blots; NTA, oligonucleotide NTA.

Band size (kb)	Hybridization			
	DNA ...	ALP1a	ALP1b	ALP2 NTA
8.2		+		+
7.0		+		+
6.6		+		+
4.4		+		+
3.9		+		
3.7		+		
2.1		+		
1.6		+		+
1.1		+	+	

4.4 kb) compared with oligonucleotide NTA (Table 1). These two bands were assigned to the 5' non-coding regions of the GPA and GPB genes (but see below) by comparing the results obtained in the present work with those previously published. Neither Huang *et al.* (1987) nor Rahuel *et al.* (1988a) observed bands corresponding to the 8.2 and 4.4 kb fragments, because the GPA cDNA probes used in their studies lacked most of the 5' non-coding region. In comparison, ALP1a and ALP2 contain 59 nts of the 5' non-coding region (Tate & Tanner, 1988).

ALP1b (nts 804–983 of ALP1), part of the 3' non-coding region of the GPA cDNA, was used to probe a Southern blot of *Hind*III-digested genomic DNA. This probe hybridized only to the 1.1 kb band (Table 1), thus assigning this fragment to the 3' non-coding region of the GPA gene.

Bands present in the Southern blot of *Eco*RI-digested genomic DNA have been assigned to the GPA or GPB genes by Siebert & Fukuda (1987). The 1.9 kb band represents fragments from both the GPA and GPB genes encoding the *N*-termini of GPA and GPB. The 2.7 kb band represents a fragment of the GPA gene encoding the *C*-terminus of the protein. The 3.4 kb band contains fragments from both the GPA and GPB genes, encoding the membrane-spanning domains of the two proteins. Rahuel *et al.* (1988a) assigned the 1.1 kb band to the 3' non-coding region of the GPA gene. Neither the 11.5 nor the 8.1 kb band has been previously observed (Huang *et al.*, 1987; Rahuel *et al.*, 1988a) and they have thus been assigned to the 5' non-coding regions of the GPA and GPB genes.

The deletion in M^k DNA

Fig. 1 shows that M^k DNA lacks all the fragments of the GPA and GPB genes which encode the mature proteins expressed in the red blood cell. The 7.0 kb *Hind*III fragment found in M^k DNA is clearly derived from the putative pseudogene discussed above. Oligonucleotide NTA still hybridizes to a 1.9 kb *Eco*RI fragment in M^k DNA, which is identical in size with the corresponding fragments derived from the GPA and GPB genes (Fig. 1). Thus oligonucleotide NTA is hybridizing to the same part of the putative pseudogene which is found in the 7.0 kb *Hind*III fragment. The identity in size of the 1.9 kb *Eco*RI fragment from the putative

pseudogene and the 1.9 kb *Eco*RI fragments from the GPA and GPB genes adds further weight to the suggestion that it is a pseudogene. The 8.1 kb *Eco*RI fragment and 4.4 kb *Hind*III fragment were assigned (see above) to part of the 5' non-coding region of either the GPA or GPB gene. It seems more likely that these bands represent part of the GPA gene. The order of the GPA and GPB genes on chromosome 4 is GPA–GPB (Murray *et al.*, 1988). If these fragments represent part of the GPB gene, then two separate deletions would be required to remove the coding regions of the GPA and GPB genes to give rise to the alterations observed in M^k DNA. It seems more likely that a single deletion spans both the GPA and GPB genes and that M^k DNA retains part of the GPA 5' non-coding region. If this is the case, then (a) the 8.1 kb *Eco*RI fragment and 4.4 kb *Hind*III fragment are derived from the GPA gene, whereas the 11.5 kb *Eco*RI fragment and 8.2 kb *Hind*III fragment are part of the GPB gene, and (b) the putative pseudogene cannot be situated between the GPA and GPB genes. This result suggests that the 5' break-point of the deletion in M^k DNA is within the 5' non-coding region of the GPA gene. However, it should be borne in mind that the extent, if any, of sequence similarity between the putative pseudogene and the 5' non-coding region of the GPA gene is unknown. If the 8.1 kb *Eco*RI fragment and 4.4 kb *Hind*III fragment were also present in the pseudogene, then the deletion in M^k DNA may include the 5' non-coding region of the GPA gene.

In order to define the 3' end of the deletion in M^k DNA, a Southern blot of *Pst*I-digested genomic DNA was probed with the 3' non-coding region of GPB cDNA (DEL3) (Fig. 1). Although the Southern blot was washed at high stringency (0.1 × SSC, 65 °C), a faint ladder of bands was present in addition to the four more intense bands. This could be due to unrecognized repetitive DNA elements in the 3' non-coding region of the GPB cDNA, although no significant sequence similarity was found between the 3' non-coding region and sequences in the EMBL and GenBank databases. The number of *Pst*I fragments which hybridized to DEL3 was also surprising. DEL3 is only 184 nts long [excluding the poly(A) tail], and since no *Pst*I sites are present in the cDNA, the result might imply the presence of at least three introns. However, only one band (6.1 kb) can be positively assigned to the GPB gene, as this band is absent from U–DNA (Fig. 1). Two other bands (7.2 and 6.8 kb) were present in all the DNA samples and could not be assigned. The 10 kb band was absent from En(Fin) DNA and M^k DNA, but present in U–DNA. The simplest explanation for this unexpected result is if the 3' non-coding region of the GPB cDNA arose from an intron within the GPA gene and that DEL3 is hybridizing to this intron within the GPA gene in addition to the GPB 3' non-coding region. As the origin of the 7.2 and 6.8 kb fragments is uncertain, it is not possible to define the 3' end point of the deletion in M^k DNA. However, the deletion does extend into at least part of the 3' non-coding region of the GPB gene, since the 6.1 kb *Pst*I fragment is absent.

En(Fin) DNA and U–DNA

The results in Fig. 1 show that En(Fin) DNA and U–DNA contain deletions of the GPA and GPB genes respectively. Rahuel *et al.* (1988a) described the deletion of the GPA gene in an individual of the En(Fin)

phenotype which seems to be very similar to the deletion studied here. The 3.4 kb *EcoRI* fragment corresponding to the GPB gene in En(Fin) DNA in Fig. 1 is absent because this Southern blot was washed at a stringency sufficient to remove the GPA probe from this less similar part of the GPB gene. The deletion in our example of U- DNA appears very similar to that studied by Huang *et al.* (1987), although this work shows that at least part of the GPB 3' non-coding region is also absent and that the 5' non-coding region of the GPB gene may be present. Recently a detailed assignment of the *HindIII* restriction fragments of the GPA and GPB genes has been published (Rahuel *et al.*, 1988b). Rahuel *et al.* (1988b) studied genomic DNA from an individual of En(UK)/M^k genotype and inferred the deletion of the GPA and GPB genes in this individual. These results are compatible with the results described here.

The presence of a putative pseudogene of sequence highly similar to that of the GPA and GPB genes could provide a mechanism for the origin of two of the deletions studied here. If the pseudogene were adjacent to the GPA/GPB gene cluster and in the order GPA-GPB-pseudogene, then an unequal crossing-over event between the GPA gene and the pseudogene could generate the M^k phenotype, the resultant gene being a GPA × pseudogene hybrid. Similarly, the U- phenotype could be generated by an unequal crossing-over event between the GPB gene and the pseudogene, although this assumes that the GPB × pseudogene product is either not expressed or that it cannot insert into the membrane. The En(Fin) phenotype could also have arisen from a GPA × GPB cross-over event. However, unlike the case for En(UK), the cross-over would have to be between the regions encoding the N-termini of GPA and GPB to produce a cross-over product indistinguishable from GPB. Clearly further work on the structure of the putative pseudogene and sequence analysis of the normal and variant DNAs will be required to define the exact locations of the deletions in the variant individuals and to establish whether the deletions could arise from the mechanisms suggested above.

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