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FULL PAPER

Identification of genes for two major sialoglycoproteins, glycophorin A and glycophorin C in canine red cell membranes

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Abstract

Glycophorins are the major sialoglycoproteins in red blood cell membranes, possessing various physiological and pathological roles. We examined membrane glycoproteins in canine red cells and cloned cDNAs for two major glycophorins, glycophorins A (GPA) and C (GPC) from bone marrow cells. Periodic acid-Schiff staining and immunoblotting analyses showed that canine red cell membranes contained several glycoproteins immunoreactive to an anti-bovine GPC antibody, whereas the most abundant sialoglycoproteins, the candidates for GPA, did not react with an anti-human GPA antibody. The amino acid sequences of the extracellular domains of GPA and GPC had no significant homology to those from other mammalian species, including humans, and had *O*-linked and/or *N*-linked glycosylation sites. On the other hand, the C-terminal cytoplasmic domain and/or the transmembrane helices of GPA and GPC were conserved among species, indicating some functional significance of those regions in red cell membranes that include dimerization of GPA in the membrane-spanning region, and association of GPC with membrane skeletal proteins through binding with protein 4.1 and p55 in the cytoplasmic domain. These findings provide insights for clinical studies to evaluate the involvement of GPA and GPC in the pathogenesis of red cell diseases.

Key Words: dog, glycophorin A, glycophorin C, red cell membranes

Introduction

Glycophorins are the most abundant transmembrane glycoproteins in red blood cells[5,33)]. They are remarkably rich in sialic acid and comprise the vast majority of periodic acid-Schiff (PAS)-stained compounds in red cell membranes. In humans, five glycophorin proteins are known: glycophorins A, B, C, D, and E. Glycophorins A, B, and E are the products of three related genes, whereas

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glycophorins C and D arise from a single gene by alternative mRNA splicing. Full-length human glycophorin A (GPA) consists of 131 amino acid residues and is heavily glycosylated with a single *N*-linked glycan and 15 *O*-linked oligosaccharide units in the extracellular domain containing about 70 amino acid residues.

Glycophorins play important physiological roles in human red cells. Sialic acid moieties of glycophorins account for 60% of the negative charges of the red cell surface, and prevent the cells from aggregating. The sialoglycan moieties of glycophorins also carry blood group antigens, and the major human glycophorins A (GPA) and C (GPC) represent MN and Gerbich blood group antigens, respectively[5]. Moreover, GPA associates with anion exchanger 1 (AE1), a major red cell membrane protein also called band 3, and regulates membrane trafficking and anion exchange activity of AE1[4, 13, 34, 35]. In addition, GPC links the spectrin-actin membrane skeletal network to the cytoplasmic surface of the lipid bilayer through binding with protein 4.1 and p55[10, 11, 19]. Furthermore, glycophorins in human and murine red cells appear to act as receptors for protozoan parasites and viruses, including *Plasmodium*[26, 27] and *Babesia*[18, 31] parasites, Sendai virus[32], and bovine parvovirus[3]. In addition, glycophorins are one of the candidate autoantigens in immune-mediated hemolytic anemia in humans[17].

Thus, glycophorins participate not only in the maintenance of red cell function but also in the pathogenesis of various infectious, immune-mediated and inherited diseases. This is very likely the case in other mammalian species. Actually, a previous study[2] suggested putative roles of PAS-stained glycoproteins that were extracted from red cell membranes as autoantigens in immune-mediated hemolytic anemia in dogs. To date, the primary structures of GPA have been determined for several mammalian species, including the mouse, monkey, pig, and horse[12, 20, 22, 25] as well as for humans[30], and partial amino acid sequences were reported for bovine[23] and canine[24] GPAs. The amino acid sequence of GPC has also been determined for some mammals, including humans[6] and cattle[16]. However, very limited information is available about the polypeptide backbones of glycophorins or the genes encoding glycophorins in small animals such as dogs and cats. To examine the physiological and pathological significance of glycophorins in these animals, it is necessary to identify their glycophorin molecules. In the present study, we isolated cDNA clones for GPA and GPC by rapid amplification of cDNA ends (RACE) in order to determine the characteristics of the major glycophorins in dogs.

Materials and Methods

Protein analyses of canine red cell membranes: Preparation of red cell membranes, SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and immunoblotting were performed as described previously[14, 15, 28]. PAS staining of sialoglycoproteins on SDS-gels was carried out by the method of Fairbanks et al.[8]. A rabbit anti-bovine GPC antibody[16] was raised against a recombinant C-terminal cytoplasmic peptide of bovine GPC, Leu[62]-Ile[109]-COOH[1]. The anti-human GPA antibody used was from Biogenesis Ltd. (Poole, UK). These antibodies were used at 1:25,000 dilution in 5% skim milk, 0.5% Tween 20 in TBS buffer consisting of 20 mM Tris-HCl (pH 7.5) and 137 mM NaCl.

Rapid amplification of cDNA ends (RACE) reactions and construction of full-length GPA and GPC cDNA clones: Adapter-ligated cDNAs were prepared from canine bone marrow poly(A)$^+$ RNA using a Marathon cDNA Amplification kit (Clontech, Palo Alto, CA, U.S.A.) according to the manufacturer's instructions as described[28]. Bone marrow aspirates were obtained from a 2-year-old female

[1]Basically, amino acid residues are expressed in 3-letter abbreviations in this study.

beagle according to the Guidelines for the Care and Use of Laboratory Animals of the Graduate School of Veterinary Medicine, Hokkaido University. The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Graduate School of Veterinary Medicine, Hokkaido University. RACE reactions were performed using KOD Plus DNA polymerase (Toyobo, Osaka, Japan) with the adapter primers supplied by the manufacturer and gene-specific primers that were synthesized according to the partial DNA sequences of putative GPA and GPC found in the NCBI canine genome database (Table 1 and Fig. 1). PCR-amplified fragments were cloned into pCR II Blunt TOPO vectors (Invitrogen, San Diego, CA, U.S.A.) and sequenced on both strands. The 5'- and 3'-stretched cDNAs were obtained by PCR amplification using primer pairs of GPAp3 and GPAp4 for GPA and AP1 and GPCp2 for GPC, respectively, and were cloned into pCR II vectors (Fig. 1). DNA sequencing was carried out using a CEQ™ DTCS Quick Start Kit (Beckman Coulter Inc., Fullerton, CA, U.S.A.) on a CEQ™ 8000 DNA sequencer (Beckman Coulter Inc.).

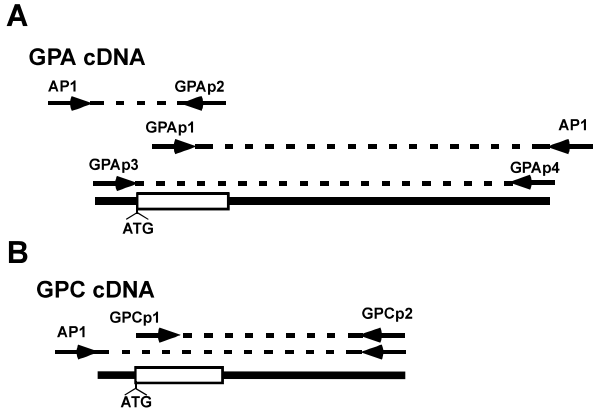


Fig. 1. Cloning of canine GPA and GPC cDNA by rapid amplification of cDNA-ends (RACE). Schematic illustrations of cDNA cloning strategies employing RACE for canine GPA (*A*) and GPC (*B*) are shown. Canine GPA and GPC cDNAs were amplified from an adaptor-ligated canine bone marrow cDNA library by 5'- or 3'-RACE using gene-specific primers (*GPAp* and *GPCp*) and adaptor primer 1 (*AP1*).

Results

Sialoglycoproteins in dog red cell membranes

To define the major sialoglycoproteins in canine red cell membranes, we compared membrane proteins from dog red cells with those from human red cells by SDS-PAGE analysis followed by detection of PAS-positive compounds and glycoproteins reactive to anti-GPA and/or anti-GPC antibodies (Fig. 2A). PAS staining and immunoblotting showed that human red cell membranes contained ~90-kDa, 36-kDa, and 34-kDa PAS-positive sialoglycoproteins that were the dimer (~90 kDa) and monomers (36 kDa) of GPA and GPC (34 kDa) as reported before (for review, see references 5 and 33).

Canine red cell membranes had a pattern of PAS staining similar to that for human red cells, exhibiting ~110-kDa, 66-kDa, and 28~30-kDa

Table 1. Primers used for cDNA cloning of canine glycophorin genes

Genes	Names	Nucleotide sequences	Position*	Purposes
glycophorin A	GPAp1	5'-GTGATAATAGGGATTATTTATGCAG-3'	295→319	3'RACE
	GPAp2	5'-CTTTGTCAGTTGGCCAATACAGAAGGC-3'	355←381	5'RACE
	GPAp3	5'-GGGTTACAGGCACTGAGTTTTGAG-3'	1→25	RT-PCR
	GPAp4	5'-GAGATTTAGATTTTGTACTTTATTTTA-3'	1672←1698	RT-PCR
glycophorin C	GPCp1	5'-GGCCTCAACGGCACGTCGCCGCC-3'	47→69	RT-PCR
	GPCp2	5'-GCTGTTCCCCCCAAATGTCAAGG-3'	738←760	5'RACE
adaptor primer	AP1	5'-CCATCCTAATACGACTCACTATAGGGC-3'		RACE

*Position is corresponding to the nucleotide numbers of canine glycophorin A and C cDNA obtained in this study.

bands as predominant sialoglycoprotein constituents (Fig. 2B). Among these bands, the 28～30-kDa bands appeared to be canine GPC molecules since positive signals were observed at the corresponding positions in immunoblotting with anti-GPC antibodies. Canine red cell membranes also possessed an 18-kDa polypeptide recognized by anti-GPC with high signal intensity. However, no

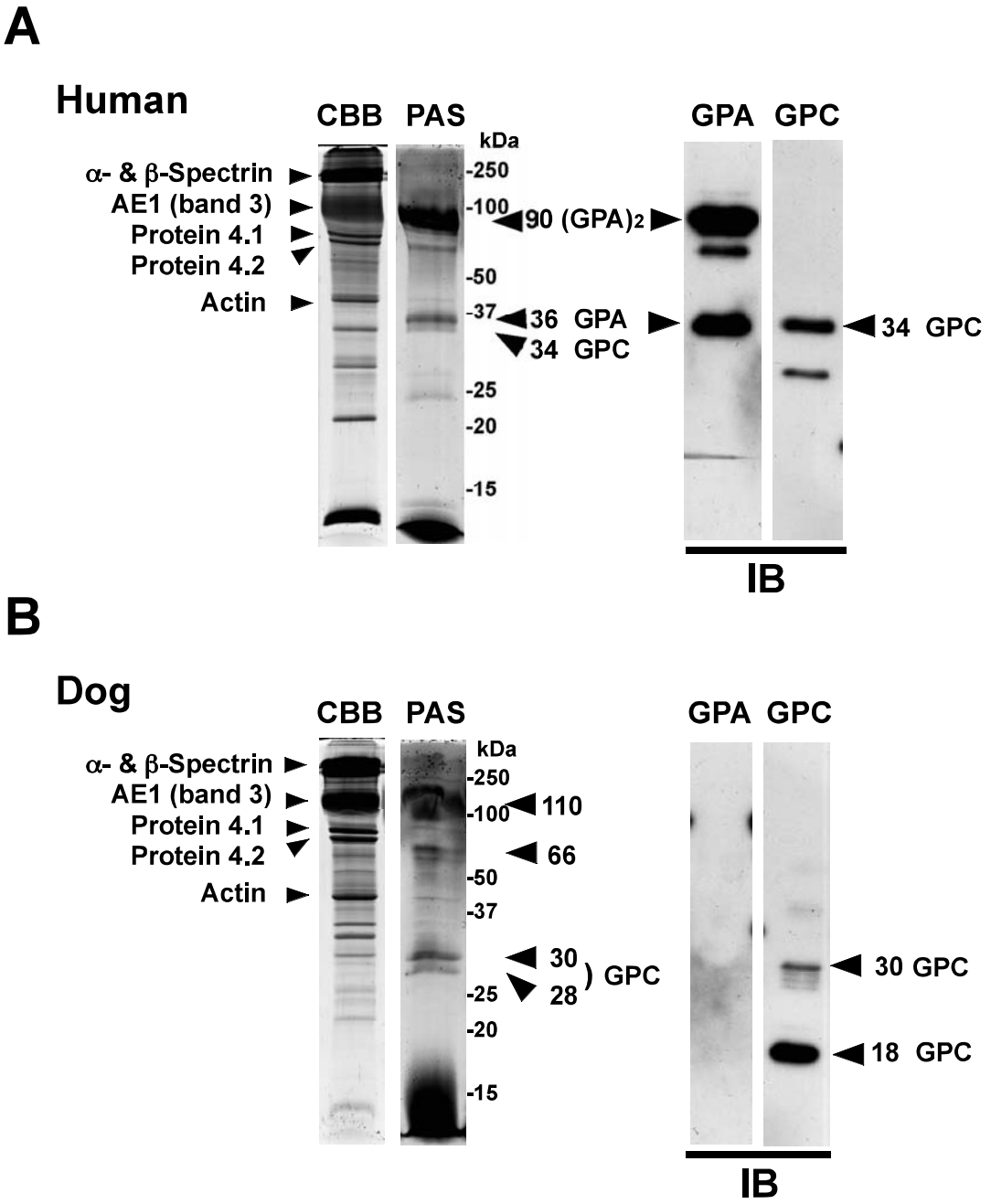


Fig. 2. Sialoglycoproteins in canine and human red cells. Membrane proteins from human (*A*) and dog (*B*) red cells (20 μg/lane) were separated on 12% SDS-PAGE and stained with Coomassie Brilliant Blue (*CBB*) or periodic acid-Schiff (*PAS*). Migrating positions of major red cell membrane proteins are indicated. GPA and GPC in red cell membranes (1 μg/lane) were detected by immunoblotting (*IB*) using anti-human glycophorin A (*GPA*) or anti-bovine glycophorin C (*GPC*). Arrowheads show apparent molecular weights and presumed glycophorin species in the red cells.

bands were detected at the corresponding position in the PAS-stained gel, suggesting that the 18-kDa band consisted of canine GPC lacking or deficient in sialic acids. These data demonstrated that the 28～30-kDa and 18-kDa polypeptides were canine GPC that shared antigenic epitopes in the cytoplasmic domain with bovine and human GPC.

On the other hand, the PAS-positive bands of ～110 kDa and 66 kDa are candidates for the canine homologue of GPA, because they were the predominant component in PAS-positive compounds in red cell membranes as observed for human GPA. The ～110-kDa and 66-kDa bands likely represent dimers and monomers, respectively, of the GPA as in human red cells. In contrast to immunoblotting for GPC, however, no signals were detected for GPA in canine red cell membranes in immunoblotting. The antibody used in this study recognizes the N-terminal extracellular domain of human GPA, and previous studies have demonstrated that amino acid sequences of GPAs from several mammalian species, including humans, have no significant homology with each other except that they share similar sequences in the transmembrane span to some extent[12, 20, 22, 23, 25, 30]. PAS-positive signals in canine and human red cell membranes at the bottom regions of the gel (< 12 kDa) had no immunoreactivity to anti-glycophorins and appeared to be derived from glycolipids.

Based on these observations indicating the presence of glycophorin homologues in dog red blood cells, we attempted to isolate cDNAs for canine GPA and GPC to identify and characterize these major glycophorins in dogs.

Isolation and identification of GPA cDNA

We performed a BLAST search of the canine genome database (NCBI, http://www.ncbi.nlm.nih.gov/) and found no information on the canine GPA gene. We searched for nucleotide sequences similar to the human GPA mRNA sequence (GenBank accession number NM002099) and found a 30-kb candidate region for the GPA gene (*GYPA*) in dog chromosome 15 (nt 43,030,000-43,060,000; GenBank accession number NW876259). However, sequence similarity was found only in the 5'-and 3'-untranslated regions (UTR) and we could not find any DNA sequences relevant to the coding region of human GPA mRNA. Therefore, we performed another BLAST search to find the gene locus in the human genome database possessing sequences similar to the 30-kb candidate for canine *GYPA*. This search resulted in several hits in the human GPA, GPB and GPE genes located in chromosome 4 (4q28-4q31). Most of these hits were found in the UTRs or introns in these genes, whereas one with a short sequence was obtained in the small segment of human *GYPA* encoding the transmembrane region of GPA. Since, as described above, the amino acid sequence of the transmembrane span of GPA has significant sequence similarity among several different species[12, 20, 22, 25, 30], we hypothesized that this short sequence was the one encoding the polypeptide segment for the single transmembrane span of canine GPA. We therefore prepared several gene-specific PCR primers for RACE reactions (Table 1 and Fig. 1A). The RACE reactions using bone marrow cDNAs as the template successfully amplified ～1.5-kb and ～0.5-kb single bands in 3'-and 5'-RACE, respectively (data not shown). After determination of the nucleotide sequences of these products, full-length canine GPA cDNA was amplified by PCR.

Canine GPA cDNA (GenBank accession number AB299408) consisted of 1,727-bp nucleotides containing an open reading frame for a 129 amino acid residue polypeptide (Fig. 3). The 17 N-terminal residues of the polypeptide showed a characteristic of signal peptide that was lost in the mature protein. It was deduced that canine GPA had an extracellular domain containing 13 possible *O*-glycosylation sites and one transmembrane span followed by a short C-terminal cytoplasmic tail. As expected, the amino acid sequence of the membrane-spanning region of canine GPA showed sequence similarity, being 48%－56% identical to those of GPAs from other mammals and the human, whereas the extracellular and cytoplasmic parts of canine GPA had no significant homology

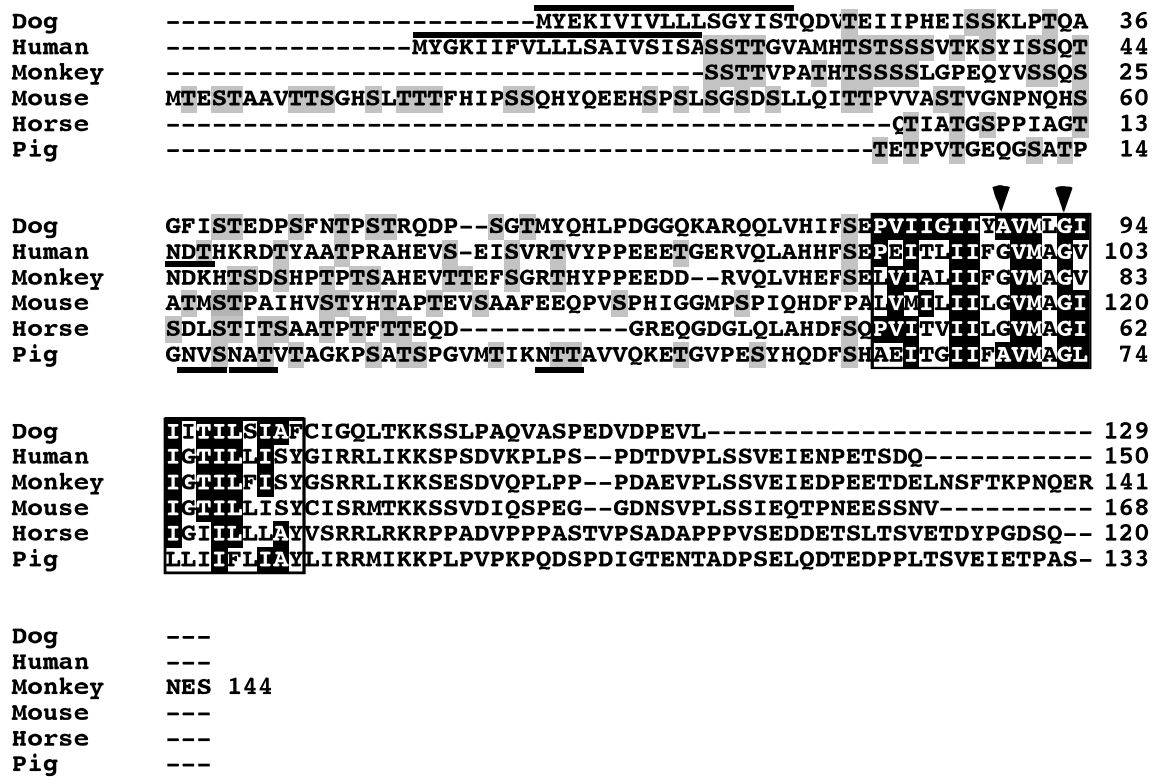


Fig. 3. Alignment of amino acid sequences of glycophorin A from various animals. The amino acid sequences of GPA from dogs (GenBank accession number AB299408) are aligned with those for human (NP002090), monkey (P14221), mouse (NP034499), horse (P027026) and pig (P027025) GPAs in the order of the similarity to the sequence of dog GPA. The amino acid sequences for dogs, humans and mice were deduced from nucleotide sequences of each GPA cDNA. The others were reported as amino acid sequences of purified glycophorin proteins from red cell membranes and the N-terminal leader sequences are unidentified. Putative N-terminal signal peptide regions are overlined. Potential *O*-linked glycosylation sites are shaded and consensus sequences of *N*-linked glycosylation sites are underlined. Transmembrane regions are boxed and the amino acid residues identical to the transmembrane sequence of dog GPA are highlighted. Arrowheads indicates dimerization motif (G/AXXXG).

with any others (Fig. 3).

When cDNAs from canine bone marrow and reticulocytes were amplified by PCR for the coding region of GPA, several variants that were different in size were obtained in addition to the predominant full-length cDNA (Fig. 4A). Two cDNA clones obtained (GPAv1 and GPAv2) had independent in-frame deletions of the nucleotide sequences encoding the extracellular domain, resulting in truncated GPA polypeptides with deletion of Lys^{31}-Gly^{37} and Gly^{37}-Gln^{68} in GPAv1 and GPAv2, respectively. The GPAv1 and GPAv2 variants, therefore, were hypothesized to possess 12 and 5 *O*-linked oligosaccharide units, respectively, whereas they had leader peptide sequences, membrane-spanning and cytoplasmic sequences identical to those in the full-length GPA. Comparison with the nucleotide sequence of the genomic DNA showed that the sequence of full-length cDNA for canine GPA was again found in chromosome 15 (GenBank accession number NW876259), being separated into 7 exons with 6 intervening introns (Figs. 4A and 4B). The nucleotide sequence of the 3' part of exon 2 and the sequence of exon 3 were the same as those deleted in GPAv1 and GPAv2, respectively, suggesting that these variants were derived from distinct mRNA splicing.

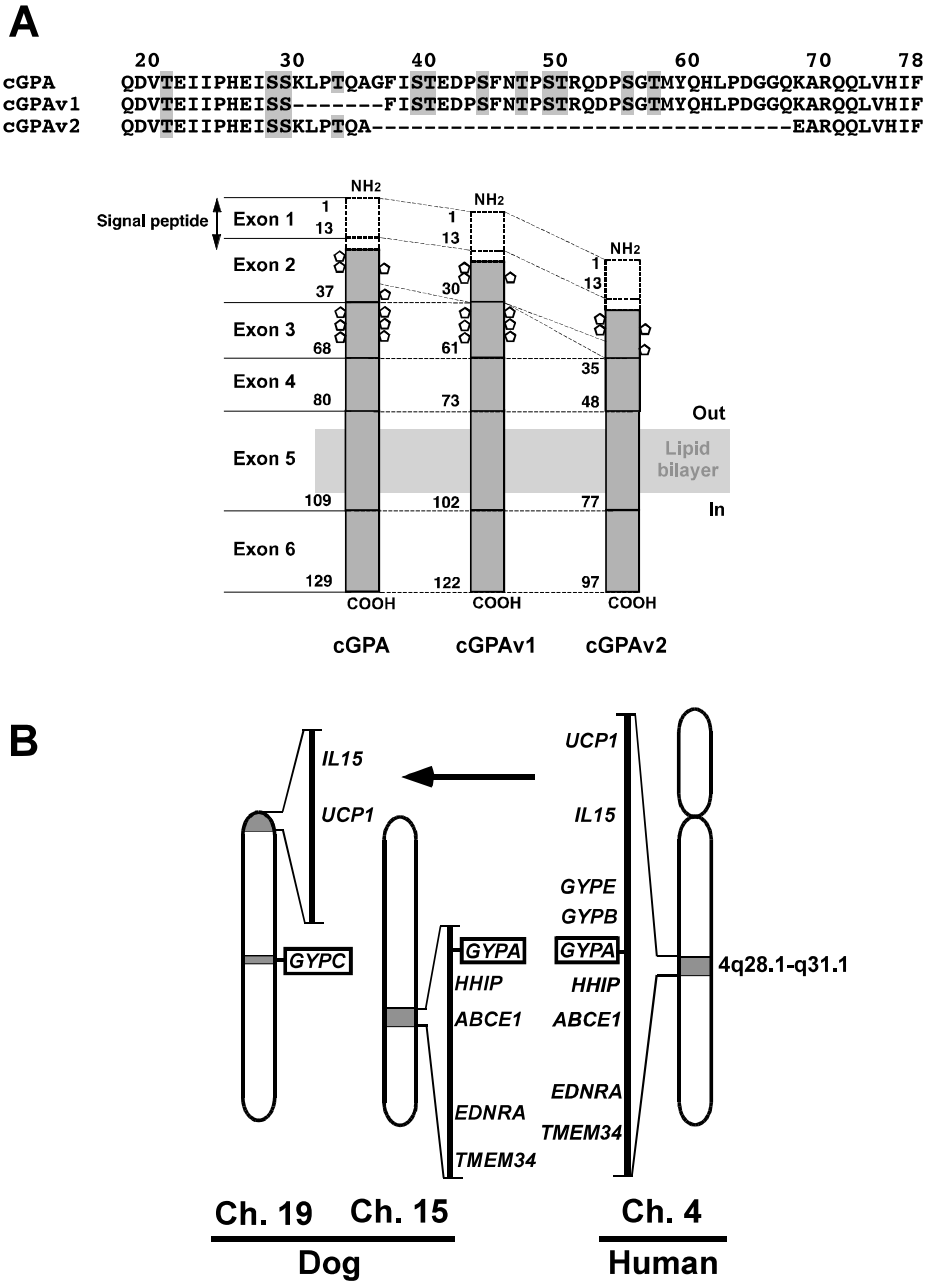


Fig. 4. Splice variants of canine GPA and their deduced amino acid sequences. *A*. The amino acid sequences of fully stretched (*cGPA*) and two splice variants of canine GPA (*cGPAv1 and cGPAv2*) are aligned. Putative *O*-linked glycosylation sites are shaded. The illustrations show the exon organizations of these molecules and the amino acid residue numbers of the exon boundaries. The signal peptide regions are drawn using broken lines. Pentagons represent *O*-linked oligosaccharide units attached to the polypeptides. *B.* Schematic illustrations show genomic organization around the dog and human GPA genes (*GYPA*). Both canine and human *GYPA* genes were flanked by hedgehog interacting protein (*HHIP*), ATP-binding cassette transporter E1 (*ABCE1*), endothelin receptor type A (*EDNRA*) and transmembrane protein 34 (*TMEM34*) at the 5' side in dog chromosome (*Ch.*) 15 and human chromosome 4, respectively. Human genes for glycophorins B (*GYPB*) and E (*GYPE*) are flanked at the 3' side of the *GYPA* gene. Several genes including interleukin 15 (*IL15*) and uncoupling protein 1 (*UCP1*) are found in dog chromosome 19 instead of the 3' side of *GYPA* in chromosome 15, indicating that chromosome rearrangement occurred during evolution at the 3' region close to the *GYPA* locus. Dog chromosome 19 also contains the *GYPC* gene.

Isolation and identification of GPC cDNA

A partially cloned cDNA sequence (GenBank accession number DR105030) and an mRNA sequence predicted from canine genomic DNA (accession number XM847247) were registered for canine GPC, but there was no record for the full-length canine GPC cDNA sequence. Therefore we prepared a 5'-RACE primer in the 3'-UTR, amplified 5'-stretched full-length canine GPC cDNA and determined the nucleotide sequences (Table 1 and Fig. 1B).

The GPC cDNA was 760 bp in length with a 291-bp open reading frame encoding a polypeptide of 96 amino acid residues (GenBank accession number AB299409). The cDNA sequence was identical to the one that was predicted from the canine GPC gene (*GYPC*) on chromosome 19 (XM847247). The canine GPC polypeptide had an N-terminal extracellular domain of 25 amino acid residues with a single *N*-glycosylation site at the 5th Asn residue and 6 potential *O*-glycosylation sites, a membrane-spanning region, and a C-terminal cytoplasmic domain. Since the theoretical molecular weight of the canine GPC polypeptide was about 10 kDa, addition of *O*-linked oligosaccharide units was expected for the 18-kDa GPC with predominant signals as well as the 28−30-kDa polypeptides observed in immunoblotting (Fig. 2).

Comparison of the amino acid sequences demonstrated that the extracellular polypeptide of canine GPC had no significant homology with those from other mammalian species (Fig. 5), as was observed for the GPA molecule (Fig. 3). However, the transmembrane segment and the cytoplasmic domain of canine GPC exhibited high amino acid sequence similarity (78-80%) to those of other mammalian GPCs. The amino acid sequence of the cytoplasmic domain, Leu[49]-Ile[96], had 83% identity with the corresponding sequence in bovine GPC (Leu[62]-Ile[109]), for which the anti-GPC antibody[16] was raised (Fig. 2). The cytoplasmic domain of human GPC possesses a protein 4.1-binding motif adjacent to the lipid bilayer (Leu[62]-Gly[79])[11] and a C-terminal PDZ domain-binding sequence to associate with p55 (Tyr[107]-Phe-Ile[109])[19], and these sequences, Leu[49]-Gly[66] and Tyr[94]-Phe-Ile[96], were well conserved in canine GPC (Fig. 5).

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Dog      ---MSG---LNGTSP----------PRFELDTGTFFMS---------------------- 22
Mouse    ---MSS--PVSKPPP----------ELLEPNPGKSYG---------------------- 22
Cattle   ---MSTQLPLTTTQQGTETAAVMEIPLWSPEPNPGMAS---------------------- 35
Human    MWSTRS--PNSTAWP----------LSLEPDPGMASASTTMHTTTIAEPDPGMSGWPDGR 48

Dog      ------LGANIAIIAGVIAAIAFVLICLLIFMLRYMYRHKGTYYTNEAKGTEFAESADTA 76
Mouse    -------IMEIAIIAAVITAVALVLVCLLFLMLRYLYRHKGTYHTNEAKGTEFAESADAA 75
Cattle   ------SEINIAVIAGVIAAMAFVLVGLLLLMLHYMYRHKGTYHTNEAKGTEFAENADVA 89
Human    METSTPTIMDIVVIAGVIAAVAIVLVSLLFVMLRYMYRHKGTYHTNEAKGTEFAESADAA 108

Dog      LQDDPSLQDTGDNSRKEYFI 96
Mouse    LQSDPALQDAGDTSKKEYFI 95
Cattle   LQDDPSFQDNGGNSKKEYFI 109
Human    LQGDPALQDAGDSSRKEYFI 128
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Fig. 5. Alignment of amino acid sequences of GPCs from various animals. The amino acid sequences (GenBank accession number AB299409) of canine GPC are aligned with those of human (NP002092), mouse (NP 001041672), and bovine (NP001002886) GPC amino acid sequences. Putative *O*-glycosylation sites are shaded and *N*-glycosylation sites are underlined. Transmembrane regions are boxed and the amino acid residues identical to the dog sequence are highlighted. The putative protein 4.1-binding regions are overlined and the C-terminal PDZ domain binding sites are double-overlined.

Discussion

Although glycophorins in red cell membranes are known to play significant physiological and pathological roles in the function and survival of red cells[5,33], only limited information has been available for canine glycophorins. This includes a partial amino acid sequence of the major PAS-stained sialoglycoprotein[24], a partial nucleotide sequence (GenBank accession number DR105030) and the sequence predicted from genome analysis (GenBank accession number XM847247) for putative GPC, and some roles of PAS-positive sialoglycoprotein in immune-mediated hemolytic anemia in dogs[2]. In the present study, we isolated and identified cDNAs for two major glycophorins, GPA and GPC in canine red cells.

The amino acid sequence of the extracellular domain of canine GPA deduced from the cDNA sequence (Gln^{18}-Glu^{80}) had no significant homology with those in humans and other mammals (Fig. 3). However, it was mostly identical to the partial amino acid sequence of putative canine glycophorin (GenBank accession number P02727) that was described in a study by Murayama *et al.*[24], except that their data lacked 11 amino acid residues on the C-terminal side (Ala^{70}-Glu^{80}). They obtained that sequence by direct amino acid sequencing of tryptic and chymotryptic glycopeptides from the most abundant PAS-positive sialoglycoprotein purified from canine red cell membranes, but they could not define it as the glycophorin relevant to human GPA. Analysis of the human genome indicates that human *GYPA* is flanked by several reference genes including *HHIP* for hedgehog-interacting protein, *ABCE1* for ATP-binding cassette protein, and *EDNRA* for endothelin receptor type A at the 5' side, and *IL15* for interleukin-15 and *UCP1* for uncoupling protein 1 at the 3' side. The nucleotide sequence for canine GPA cDNA is found in chromosome 15 as described in Results. At the 5' side of this region, there are several genes similar to *HHIP, ABCE1,* and *EDNRA,* supporting the idea that the GPA cDNA and the gene for it we identified in this study actually represent the *GYPA* gene in dogs (Fig. 4B). Likewise, the PAS-positive sialoglycoprotein analyzed by Murayama *et al.*[24] is now demonstrated to be the canine homologue of human GPA. However, it is unclear yet if the GPA is the component of the 66-kDa and ~110-kDa PAS-stained glycoproteins in dog red blood cells (Fig. 2). Identification of canine GPA on SDS-gels stained with PAS or in immunoblotting remains to be accomplished to determine the contents in red cell specimen.

The diversity in the amino acid sequence and the involvement of a dozen potential *O*-glycosylation sites of the extracellular domain indicate that canine red cell GPA should have physiological and pathological significance as demonstrated in humans and mice[5,13,17,18,26,27,31,34] and as has been suggested in the pathogenesis of hemolytic anemia in dogs[2]. The presence of splicing variants (Fig. 4A) may generate more diverse characteristics of canine GPA as putative red cell antigens or as receptors for pathogens.

In contrast to the extracellular domain, the membrane-spanning region of canine GPA had the amino acid sequence relatively conserved among different mammalian species (Fig. 3) as reported by previous studies[12,20,22,25,30], suggesting that this region would play some role in red cell membrane organization. Possible roles may involve a function of the amino acid sequence Ala^{89}-Val-Met-Leu-Gly^{93} found in transmembrane helices as the dimerization motif GXXXG or AXXXG (Gly-$(Xaa)_3$-Gly or Ala-$(Xaa)_3$-Gly) sequence[7,29] and have some effect on the expression and activity of AE1 (band 3) in red cells as previously reported[1,21,34].

The present study also identified canine GPC cDNA. The data obtained indicated that the *GYPC* gene was located in dog chromosome 19 as has been supposed based on genome analysis (GenBank accession number XM847247) and this gene produced 28−32-kDa GPC and 18-kDa PAS-negative GPC molecules in canine red cell membranes (Fig. 2). One of the structural characteristics of canine GPC involves the short-stretched extracellular domain with *N*-linked and *O*-linked glycan modification, suggesting its significance as

the cell surface molecule to present antigens or to bind pathogens as supposed for GPA. The most characteristic feature of canine GPC is in the well-conserved cytoplasmic domain containing the binding motifs for one of the principal membrane skeletal protein, protein 4.1[11] and the PDZ domain of p55[19].

In conclusion, we identified canine *GYPA* and *GYPC* genes by cDNA cloning. Comparisons of the amino acid sequences of GPA and GPC from several mammals suggest that these glycophorins are remarkably variable in the extracellular domain enriched in *O*-and/or *N*-sialoglycans, but are conservative in the cytoplasmic domain and/or the transmembrane span, indicating the possible physiological and pathological significance of red cell GPA and GPC in red cell investment in dogs as well as in humans. Thus, our data will provide information for clinical studies of the involvement of GPA and/or GPC in the etiology of red cell diseases in dogs.

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