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Isolation and influenza virus receptor activity of glycophorins B, C and D from human erythrocyte membranes

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(1) Glycophorins (GPs) A^M, A^N, B, C and D were each isolated into a high state of purity from human erythrocyte membranes by a combination of lithium diiodosalicylate (LIS)-phenol extraction, gel-filtration with Bio-Gel A1.5m and HPLC with LiChrospher 1000 TMAE. (2) GPs-B, -C and -D reacted with influenza A and B viruses as well as GPs-A^M and -A^N and the order of reactivities against two viruses of the glycophorins was as follows: GP-B > GP-C > GP-A^M = GP-A^N ≫ GP-D for the former virus and GP-C > GP-B > GP-A^M = GP-A^N ≫ GP-D for the latter virus.

Introduction

Glycophorin (GP) A, which is the major sialoglycoprotein and also the blood group M or N antigen of human erythrocyte membrane, has been known to be the only influenza virus receptor of human erythrocyte membrane [1–4]. Recently, we showed that three sialoglycoprotein fractions were obtained from each of the OMs and ONs human erythrocyte membranes by a combination of the LIS-phenol method and gel-filtration with Bio-Gel A1.5m (M-Frs.1–3 and N-Frs.1–3), and that the reactivities against influenza A and B viruses of fractions containing GPs-B and -C (M-Fr.2 and N-Fr.2) were higher than that of GP-A fractions (M-Fr.1 and N-Fr.1). However, the reactivities of fractions containing GPs-B and -D (M-Fr.3 and N-Fr.3) were lower than that of the other four fractions [5]. These results suggest that human erythrocyte membranes include glycophorins different from GP-A as influenza virus receptors. However, the reactivity of each GPs-B, -C and -D against both influenza A and B viruses could not be elucidated in the preceding paper [5].

GPs-B and -C were purified from human erythrocyte membranes by high-performance ion exchanger and gel permeation chromatographies sufficient for determination of these amino-acid sequences by protein sequencing [6]. However, this method is not really suited for simple and efficient purification of glycophorins. On the one hand, GP-D was obtained as a single component from polyacrylamide gel after electrophoresis in the presence of SDS [7]. However, the preparation of GP-D obtained from SDS-gel has been denatured and is not suitable for the study of serological properties.

Accordingly, straightforward and quick isolation of GPs-B, -C and -D as influenza virus-reactive components is necessary for the study of their influenza virus receptor activity. In this work, we describe the isolation of GPs-B, -C and -D from human OM erythrocyte membranes by a combination of the LIS-phenol extraction, gel filtration with Bio-Gel A1.5m and HPLC with LiChrospher 1000 TMAE. Also, the reactivities of GPs-B, -C and -D against influenza A and B viruses are compared with the reactivities of GPs-A^M and -A^N.

Materials and Methods

Materials. Amphitol 20N (lauryl dimethylamine *N*-oxide, non-ionic detergent) was obtained from KAO Co. Ltd. (Tokyo, Japan). Bio-Gel A1.5m was purchased from Bio-Rad Laboratories (Richmond, CA, USA). LiChrospher 1000 TMAE (trimethylaminoethyl silica) column (1.0 × 5.0 cm) for HPLC was obtained from E. Merck (Frankfurter Straße, Darmstadt, Germany). Blood group anti-M, -N -S and -s sera were obtained from Ortho Diagnostic Systems (Norcross, GA, USA). *Vicia unijuga* anti-N lectin was prepared from their leaves by the method of Yanagi et al. [8]. Influenza A and B viruses used were PR-8 and Osaka strains, respectively.

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Chemical composition analysis. Sialic acid was determined colorimetrically by the method of Jourdian et al. [9]. Glucose, mannose, galactose, fucose, *N*-acetylglucosamine and *N*-acetylgalactosamine were determined by gas-liquid chromatography using a Shimadzu GC-9AM instrument according to the method of Metz et al. [10]. Individual amino acids were measured with a Hitachi L-8500 high-speed amino-acid autoanalyzer according to the method described by Yanagi et al. [8].

Sodium dodecylsulfate (SDS)-polyacrylamide slab gel electrophoresis (PAGE). SDS-PAGE was carried out according to the method of Ohyama et al. [5]. PAS (periodic acid-Schiff staining)-positive bands in SDS-PAGE of HPLC fractions were measured densitometrically with a Shimadzu CS-9000 (dual-weavelength flying-spot scanner) and identified by reference to the R_F values of glycophorins A, B, C and D found in SDS-PAGE by Furthmayr [4] and Anstee and Tanner [13].

Hemagglutination inhibition test. Hemagglutination inhibition tests [5] with anti-M and -N sera, *Vicia unijuga* anti-N lectin and influenza A and B viruses were carried out by the saline test. Hemagglutination inhibition test [12] with anti-S and -s sera was performed by the indirect anti-globulin test.

Isolation of sialoglycoproteins (glycophorins) from human erythrocyte membranes. Preparation of the sialoglycoprotein mixture (LIS-phenol extract) from human OM erythrocyte membranes by the LIS-phenol method and fractionation of LIS-phenol extract by gel-filtration with Bio-Gel A1.5m was performed according to the method previously described [5]. It has been shown earlier that the LIS-phenol extract is separated into three fractions, M-Frs.1 ~ 3, and that M-Fr.1 was GP-A^M fraction, M-Fr.2 comprised of GP-B and GP-C and M-Fr.3 consisted of GP-B and GP-D [5]. About 5 mg of M-Fr.2 or M-Fr.3 was dissolved in 10 ml of 0.1M Tris-HCl buffer (pH 8.0) containing 1.0% Amphitol 20N and then applied to a LiChrospher 1000 TMAE column (1.0 × 5.0 cm) using a Ryodine sample injector 7125 and Shimadzu constametric pumps LC-6A, respectively. Three-dimensional chromatograms of the fractions in HPLC were measured using a Shimadzu spectromultichannel photodetector LC-M6A. Chromatography was carried out using the linear gradient of NaCl in a concentration of 0 ~ 0.5 M for 70 min at room temperature and 1 ml fractions were collected in a flow rate of 1.0 ml/min. Absorbances at 210 and 280 nm of the effluent were measured and the concentration of NaCl of the effluent was estimated from the refractive index of the solution. The fractions showing absorbance at 210 nm and 280 nm were pooled and fully dialyzed against water at 4°C and then lyophilized. The dry material from each fraction was rechromatographed on a LiChrospher 1000 TMAE column by the method described above. GP-A^M was obtained by chromatography of M-Fr.1 on Bio-Gel A1.5m column; GP-A^N was obtained by chromatography on the Bio-Gel A1.5m column of N-Fr.1 which was prepared by gel-filtration of the LIS-phenol extract of ON erythrocyte membranes with Bio-Gel A1.5m [5].

Results

Isolation and electrophoretic characterization of GPs-B, -C and -D

M-Frs. 2 and 3 obtained by gel-filtration of LIS-phenol extract prepared from OM erythrocyte membranes were chromatographed on LiChrospher 1000 TMAE. As presented in Fig. 1a, M-Fr.2 was separated into two fractions eluting at 0.17 M and 0.25 M NaCl concentration from the column and desingated M-Fr.2-1 and M-Fr.2-2, respectively. As shown in Fig. 1b, M-Fr.3 was also separated into two fractions eluting at 0.13 M and 0.25 M NaCl from the column, designated M-Fr.3-1 and M-Fr.3-2, respectively. Four fractions, M-Fr.2-1, M-Fr.2-2, M-Fr.3-1 and M-Fr.3-2, were chromatographed again on LiChrospher TMAE column, respectively. In three-dimensional chromatogram analysis, M-Fr.2-1, M-Fr.2-2, M-Fr.3-1 and M-Fr.3-2 gave single peaks as shown in Figs. 2a,b,c and d, respectively. Yields of these fractions from the column are shown in Table I.

In SDS-PAGE, M-Fr.2-1 and M-Fr.3-1 migrated as single bands, reacted with periodate-Schiff (PAS) staining reagent, having relative mobilities of 0.61 and 0.74, respectively (shown in Fig. 3b and d). M-Fr.2-2 gave two bands stained by PAS-reagent and relative mobilities of these bands were 0.53 and 0.83, respectively

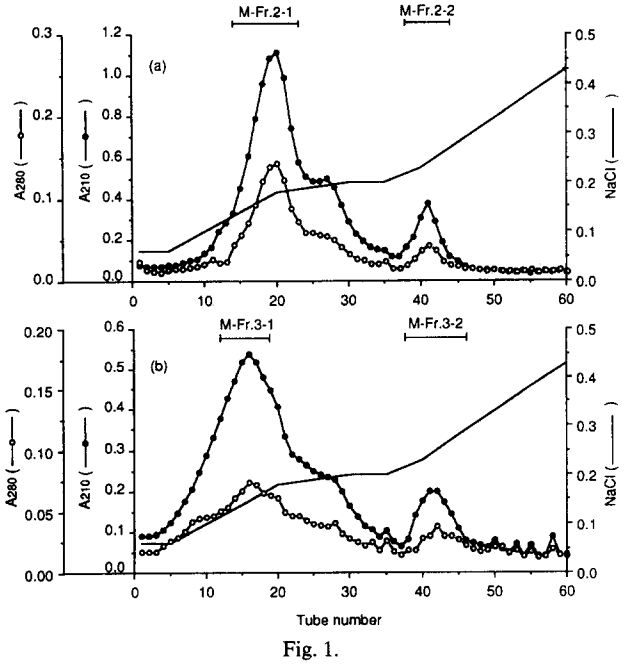

Fig. 1.

(Fig. 3c). The migration profile and a relative mobility of a single band given by SDS-PAGE of M-Fr.3-2 were the same as those of M-Fr.2-2 (data not shown). SDS-PAGE of stroma (blood group M) is shown in Fig. 3a and the relative mobilities of bands given with stroma were good agreement with those reported by Furthmayr [4], Anstee and Tanner [11] and Fairbanks et al. [13]. From the relative mobilities of stroma, M-Frs.2-1, 2-2 and 3-1, and comparing these results with reports of Furthmayr [4], Anstee and Tanner [11], Fairbanks et al. [13] and Anstee [14], it is reasonable to conclude as follows: M-Frs.2-1 and 3-1 correspond to GP-C and GP-D, respectively, and M-Fr.2-2 was a mixture of dimmer and monomer of GP-B. Additionally, GPs-B, -C and -D were isolated into a high state of purity by this method. The results of SDS-PAGE showed that M-Fr.3-2 as well as M-Fr.2-2 comprised dimer and monomer of GP-B; however, the yield of M-Fr.3-2 form M-LIS-phenol extract was significantly lower than that of M-Fr.2-2 (data not shown). Therefore, M-Fr.2-2 was used as GP-B in the following experiments.

Chemical compositions and MNSs activities of GPs-A^M, -A^N, -B, -C and -D

The carbohydrate and amino-acid compositions of GPs-A^M, -A^N, -B, -C and -D are shown in Table I. These chemical compositions indicate that the five glycophorins, GPs-A^M, -A^N, -B, -C and -D, contained 38.63, 44.01, 26.15, 40.39 and 19.40% (w/w) carbohydrate. The contents of mannose, fucose and *N*-acetylglucosamine of GP-B were significantly smaller than those of GPs-A^M, -A^N and -C, and the carbohydrate composition of GP-C was similar to those of both GPs-A^M and -A^N. The carbohydrate composition of GP-D was characterized by smaller galactose and *N*-acetylgalactosamine contents than in GPs-A^M, -A^N, -B and -C. The amino-acid compositions of these glycophorins show that these glycophorins all contain abundant amounts of serine, threonine and glutamic acid.

The reactivities of GPs-A^M, -A^N, -B and -C and -D against anti-M, -N, -S and -s sera and *Vicia unijuga* anti-N lectin are presented in Table II. GP-A^M reacted

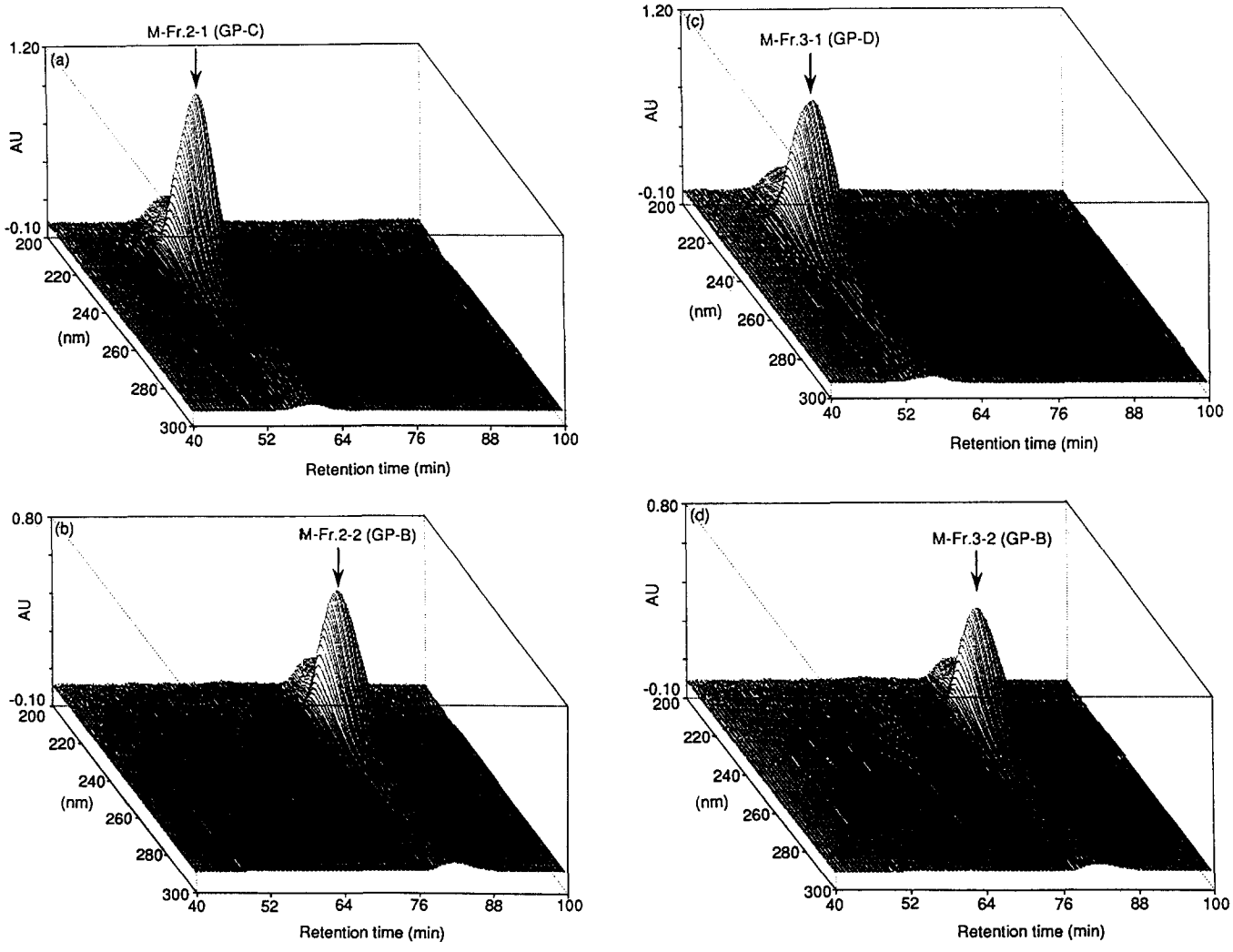

Fig. 2.

TABLE 1

Yields and chemical compositions of glycophorins A^M, A^N, B, C and D

n.d., not detected.

Glycophorin (Fraction)	A^{M} [a] (M-Fr.1)	A^{N} [b] (N-Fr.1)	B [c] (M-Fr.2-2)	C (M-Fr.2-1)	D (M-Fr.3-1)
Yield					
mg/100 mg of LIS-phenol extract	45.24	46.07	4.42	4.56	3.36
mg/100 mg of M-Fr.2	–	–	31.70	39.28	–
mg/100 mg of M-Fr.3	–	–	12.42	–	56.38
Carbohydrate composition (w/w, %)					
Sialic acid	20.06	22.97	14.30	18.35	11.38
Mannose	1.17	2.18	0.77	2.26	0.88
Galactose	6.63	7.22	4.01	6.71	0.79
Fucose	0.64	0.97	0.28	1.11	n.d.
n-Acetylglucosamine	2.89	3.51	0.85	3.98	3.60
n-Acetylgalactosamine	7.24	7.16	5.94	7.98	2.75
Amino acid composition (mole/100 mol)					
Aspartic acid	6.45	6.42	3.20	8.49	8.18
Threonine	11.49	11.15	12.06	11.07	10.76
Serine	13.88	12.63	14.37	10.82	10.06
Glutamic acid	11.46	12.25	9.09	8.87	10.01
Glycine	5.06	5.01	6.85	7.28	8.88
Alanine	4.96	5.09	7.49	10.10	10.82
Cysteine	n.d.	n.d.	n.d.	n.d.	n.d.
Valine	8.03	7.98	8.50	5.23	4.91
Methionine	1.06	1.09	1.99	5.37	4.89
Isoleucine	7.29	7.47	7.82	4.37	3.65
Leucine	5.43	6.31	7.58	5.55	6.34
Tyrosine	3.01	3.00	2.30	3.30	3.47
Phenylalanine	1.59	1.63	2.10	2.10	2.51
Lysine	4.11	3.52	3.48	3.20	3.10
Histidine	4.06	3.19	3.45	3.07	2.80
Arginine	4.14	4.44	4.23	3.85	3.68
Proline	7.96	7.82	4.34	7.32	5.87

[a] Rechromatographed preparation of M-Fr.1.
[b] Rechromatographed preparation of N-Fr.1.
[c] Yield of GP-B was shown as a totaly yield of M-Fr.2-2 and M-Fr.3-2, and GP-B obtained from M-Fr.2 was used for the analysis of chemical composition.

with anti-M serum but did not react with anti-N, -S and -s sera and *Vicia unijuga* anti-N lectin. Also, GP-A^N reacted against anti-N serum and *Vicia unijuga* anti-N lectin but not against anti-M, -S or -s serum. GP-B reacted against anti-N and -s sera and *Vicia unijuga* anti-N lectin. GPs-C and -D did not react with anti-M, -N, -S and -s sera and *Vicia unijuga* anti-N lectin. These serological studies indicate that GPs-A^M and -A^N have M activity and N activity, respectively, that GP-B exhibited both N and s activities, and that GPs-C and -D did not have MNSs activity.

Influenza virus receptor activities of GPs-A^M, -A^N, -B, -C and -D

The reactivities of GPs-A^M, -A^N, -B, -C and -D against influenza A and B viruses are shown in Table II. The reactivity of GP-A^M against influenza A virus was the same as its reactivity against influenza B virus and the reactivity was also the same as the reactivities

TABLE II

Hemagglutination inhibition activities [a] of purified glycophorins A^M, A^N, B, C and D

n.i., no inhibition of hemagglutination at 5000 μg/ml.

Glycophorin: Hemagglutinin [b]	A^{M} [c]	A^{N} [d]	B [e]	C	D
Anti-M serum	156	n.i.	n.i.	n.i.	n.i.
Anti-N serum	n.i.	313	313	n.i.	n.i.
Anti-S serum	n.i.	n.i.	n.i.	n.i.	n.i.
Anti-s serum	n.i.	n.i.	78	n.i.	n.i.
Vicia unijuga anti-N lectin	n.i.	313	313	n.i.	n.i.
Influenza A virus	156	156	39	78	5000
Influenza B virus	156	156	39	20	5000

[a] Hemagglutination inhibition activity is indicated as minimum concentration (μg/ml) of glycophorin giving complete inhibition.
[b] Titer = 8.
[c] Rechromatographed preparations of M-Fr.1.
[d] Rechromatographed preparations of N-Fr.1.
[e] GP-B obtained from M-Fr.2 was used for the analysis of chemical composition.

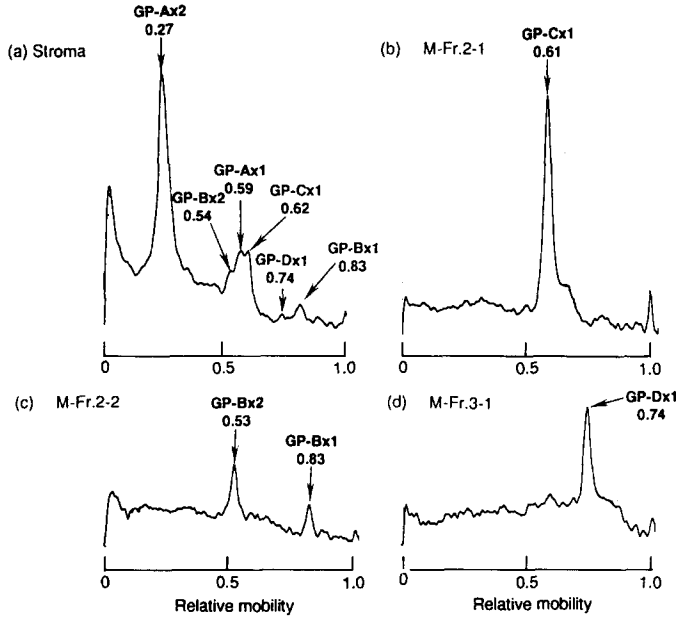

Fig. 3.

of GP-A^N against influenza A and B viruses. Furthermore, GPs-B, -C and -D reacted with influenza A and B viruses as well as GPs-A^M and -A^N and the reactivities of GPs-B and -C against both viruses were significantly higher than those of GPs-A^M and -A^N. The reactivity of GP-C against influenza A virus was lower than its reactivity against influenza B virus. On the other hand, GP-D exhibited the lowest reactivity of all these glycophorins against two viruses. These results indicate that the order of reactivities of glycophorins against influenza A and B viruses was as follows: GP-B > GP-C > GP-A^M = GP-A^N $\gg$ GP-D for the former virus and GP-C > GP-B > GP-A^M = GP-A^N $\gg$ GP-D for the latter virus.

Discussion

From sialoglycoprotein mixture (LIS-phenol extract) prepared from human OMs erythrocyte membranes, sialoglycoprotein (glycophorin) fractions, M-Frs.2-1, 2-2, 3-1 and 3-2, were obtained by gel-filtration with Bio-Gel A1.5m and then HPLC with LiChrospher 1000 TMAE. The results of the SDS-PAGE of the four sialoglycoprotein fractions showed that M-Frs.2-1 and 2-2 were GPs-C and -B and M-Frs.3-1 and 3-2 were GPs-D and -B, respectively, and that these glycophorins were each isolated into a high state of purity (Figs. 1–3). Blanchard et al. [6] purified GPs-B and -C by HPLC with an ion-exchanger and then gel permeation chromatography and revealed the amino-acid sequences of GPs-B and -C. HPLC with LiChrospher 1000 TMAE described here is much less time-consuming than the previous methods described by Blanchard et al. [6]. On the other hand, GP-D was also purified by extraction from polyacrylamide gel after electrophoresis of preparative SDS-PAGE, but only its partial amino-acid sequence was determined [7]. The reactivities of glycophorins against some anti-sera and lectins were reduced and/or faded out by exposure to SDS (data not shown). Consequently, glycophorins denatured by treatment with SDS are not adequate for serological study. A combination of gel-filtration with Bio-Gel A1.5m and HPLC with LiChrospher 1000 TMAE was a good method for purification of GPs-B, -C and -D (also GP-A) in order to study the chemical and serological properties of glycophorins.

Blanchard et al. [6] reported from the analysis of carbohydrate compositions of purified GPs-B and -C that the carbohydrate content of GP-C is higher than that of GP-B. On the basis of the results of SDS-PAGE and PAS- and Coomassie blue-staining, EL.-Maliki et al. [7] suggested that GP-D is much less glycosilated than GP-C. The results of the carbohydrate composition analysis of GPs-B, -C and -D (Table I) showed that the order of the carbohydrate content is GP-C > GP-B > GP-D. The carbohydrate contents of GPs-B, -C and -D are in fair agreement with the findings of Blanchard et al. [6] and EL.-Maliki et al. [7], and the amino-acid compositions of GPs-B and C were nearly the same as those shown by Blanchard et al. [6]. These findings suggest that GPs-B, -C and -D are not affected chemically during purification procedures.

GP-A expresses M or N activity and this expression is governed by the nature of the five amino-acid residues from N-terminal of the peptide chain [14] and the N-terminal 26 residues of the amino-acid sequence of GP-B was found to be identical with that of GP-A^N [3,6,15,16]; accordingly, GP-B has N antigen activity [15]. GP-B also expresses the Ss antigen and this expression is governed by amino-acid substitutions at residue 29 [15,16]. The complete amino-acid sequence of GP-C has been determined both by protein sequencing [6] and by nucleotide sequencing of cDNA clones [17]. While GPs-A and -B derive from separate highly homologous genes [18], GPs-C and -D are suggested to be the products of a single gene [19,20] and GP-D would have the same sequence as GP-C from amino-acid residue 22 to the carbohydrate terminus [20,21]. GPs-C and -D do not have an N-terminal amino-acid sequence similar to that of GPs-A and -B, which express M or N activity [6,7,22]. Furthermore, GP-C has an amino-acid sequence quite different from that of GP-B in the vicinity of residue 29, which expresses the Ss activity [6,16]. The results shown in Table II suggest that GPs-A^M and -A^N exhibit M and N activities, respectively, GP-B expresses N activity and GPs-C and -D did not show any MNS activity. These results are compatible with the facts mentioned above that GPs-C and -D have not any MNS activity. From these results of the serological properties, it is reasonable to infer

that no artificial change of the serological properties of GPs-B, -C and -D results from the procedure.

The influenza virus receptor on the surface of human erythrocytes is known to be GP-A [23]. Purification of GPs-B, -C and -D and their chemical and serological properties have been defined [6,7,14,15,16]; however, GPs-B, -C and -D have not been described as influenza virus receptors. Our investigation of influenza virus receptor activities of GP-A^M, -A^N, B, -C and -D show clearly that GPs-B, -C and -D had influenza virus receptor activities against both influenza A and B viruses as well as GP-A^M and -A^N. Additionally, the activities of GPs-B and -C were much higher than those of GP-A^M and -A^N (shown in Table II). From the result in this paper that GP-D also had reactivity against influenza A and B viruses but the reactivity was lower than that of other GPs, and the fact reported previously that the GP-D has the same amino-acid sequence as GP-C from amino-acid residue 22 to the carboxy terminus [20,21], we conclude that the glycopeptide moiety from the N-terminus to amino-acid residue 22 of GP-C is related to the high reactivity of GP-C against the two influenza viruses. The results in this paper justify our previous suggestion derived from the results of SDS-PAGE and serological study of glycophorin fractions separated by gel-filtration that human erythrocyte membranes comprise GP-A and at least two kinds of glycophorin different from GP-A as influenza virus-reactive components [5]. Further, one of these is a glycophorin possessing influenza virus receptor activity which is remarkably higher, and the other one considerably lower, than the influenza virus receptor activity of GP-A [5].

The following conclusions are reached. A simple and convenient method for purification of GPs-B, -C and -D using a combination of the LIS-phenol method, gel-filtration with Bio-Gel A1.5m and HPLC with LiChrospher 1000 TMAE has been developed. Then, glycophorins of human erythrocyte membrane have been isolated to a high state of purity by the method without any artifactual effects, chemically or serologically. GPs-B, -C and -D had influenza virus receptor activities against influenza A and B viruses and the order of the activities was GP-A.GP-B > GP-C > GP-A^M = GP-A^N ≫ GP-D for influenza A virus and GP-C > GP-B > GP-A^M = GP-A^N ≫ GP-D for influenza B virus.

References

1 Kathan, R.H., Winzler, R.J. and Johnson, C.A. (1961) J. Exp. Med. 113, 37–45.
2 Springer, G.F., Nagai, Y. and Tegtmayer, H. (1966) Biochemistry 5, 3254–3272.
3 Tomita, M., Furthmayr, H. and Marchesi, V.T. (1978) Biochemistry 17, 4756–4770.
4 Furthmayr, H. (1978) J. Supramol. Struct. 9, 79–95.
5 Ohyama, K., Yamauchi, S., Endo, T. and Ohkuma, S. (1991) Biochem. Biophys. Res. Commun. 179, 79–84.
6 Blanchard, D., Dahr, W., Hummel, M., Latron, F., Beyreuther, K. and Cartron, J.-P. (1987) J. Biol. Chem. 262, 5808–5811.
7 E.L.-Maliki, B., Blanchard, D., Dahr, W., Beyreuther, K. and Cartron, J.-P. (1989) Eur. J. Biochem. 183, 639–643.
8 Yanagi, K., Ohyama, K., Yamakawa, T., Hashimoto, K. and Ohkuma, S. (1989) Int. J. Biochem. 22, 43–52.
9 Joudian, G.W., Dean, L. and Roseman, S. (1971) J. Biol. Chem. 246, 430–435.
10 Metz, J., Ebert, W. and Weicher, H. (1971) Chromtographia 4, 345–350.
11 Anstee, D.J. and Tanner, M.J.A. (1986) Br. J. Haematol. 64, 211–215.
12 Technical Manual (1985) 9th Edn., pp. 155–160, American Association of Blood Banks, Arlington, VA.
13 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606–2617.
14 Anstee, D.J. (1990) Vox Sang. 58, 1–20.
15 Furthmayr, H. (1978) Nature 271, 519–524.
16 Dahr, W., Beyreuther, K., Steinbach, H., Gielen, W. and Krüger, J. (1980) Hoppe-Seyler's Z. Physiol. Chem. 361, 895–906.
17 Colin, Y., Rahuel, C., London, J., Romeo, P.-H-, Auriol, L., Galibart, F. and Carton, J.-P. (1986) J. Biol. Chem. 261, 229–233.
18 Tale, C.G. and Tanner, M,J,A. (1988) Biochem. J. 254, 743–750.
19 Le Van Kim, C., Colin, Y., Blanchard, D., Dhar, W., London, J. and Carton, J.-P. (1987) Eur. J. Biochem. 165, 571–579.
20 Tanner, M.J.A., High, S., Martin, P.G., Anstee, D.J., Judson, P.A. and Jones, T.J. (1988) Biochem. J. 250, 407–414.
21 Anstee, D.J. (1990) Vox Sang. 58, 1–20.
22 Dhar, W. and Beyreuther, K. (1985) Biol. Chem. Hoppe-Seyler 366, 1067–1070.
23 Burness, A.T.H. (1981) in Receptors and Recognition, Series. B, Vol. 8 (Lonberg-Holm, K., ed.), pp. 63–84, Chapman & Hall, London.