Differential binding of human blood group Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins with *Dolichos biflorus* and *Vicia villosa*-B_4 agglutinins

Albert M. Wu[a,*], June H. Wu[b], Winifred M. Watkins[c], Chie-Pein Chen[a], Shuh-Chyung Song[a], Yuen-Yuen Chen[a]

[a] *Glycoimmunochemistry Research Laboratory, Institute of Molecular and Cellular Biology, Chang-Gung Medical College, Kwei-san, Tao-yuan 333, Taiwan*
[b] *Department of Microbiology and Immunology, Chang-Gung Medical College, Kwei-san, Tao-yuan, Taiwan*
[c] *Department of Haematology, Royal Postgraduate Medical School, Imperial College of Science, Technology, and Medicine, Du Cane Road, London W12 ONN, UK*

Received 21 April 1998

Abstract The binding patterns of human blood group Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins (THGPs) with respect to four GalNAc specific agglutinins were studied by quantitative precipitin assay (QPA) and enzyme linked lectinosorbent assay (ELLSA). Of the native and asialo Sd(a+) and Sd(a−) THGP tested by QPA and ELLSA, only native and asialo Sd(a+) bound well with *Dolichos biflorus* (DBA) and *Vicia villosa*-B_4 (VVA-B_4), while Sd(a−) THGP reacted poorly with these two lectins. Neither Sd(a+) nor Sd(a−) THGPs reacted with two other GalNAc α-anomer specific lectins: *Codium fragile* subspecies *tomentosoides* and *Artocarpus integrifolia*. Furthermore, the binding of asialo Sd(a+)THGP-VVA-B_4 and native Sd(a+)THGP-DBA through GalNAcβ→ was confirmed by inhibition assay. These results demonstrate that DBA and VVA-B_4 are useful reagents to differentiate between Sd(a+) and Sd(a−) THGP.

Key words: Binding properties of GalNAc specific lectins; Native and asialo-Tamm-Horsfall glycoproteins

1. Introduction

Human Tamm-Horsfall glycoprotein (THGP) is the most abundant urinary protein in normal individuals [1–5]. Most Tamm-Horsfall glycoproteins carry the Sd(a+) blood group active determinant, GalNAcβ1→4(NeuAcα2→3)Galβ1→4GlcNAcβ1→3Gal, characterized by the presence of GalNAcβ1→4 at the non-reducing end and a repeating *N*-acetyllactosamine unit [5,6]. The Sd(a−) phenotype lacks the GalNAcβ1→ residue at the terminal non-reducing end of the carbohydrate chains [7]. There is considerable variation in the strength of Sd^a antigen in different individuals [8,9]. From genetic aspects and for understanding the biological roles of GalNAcβ1→ in THGP, it is useful to have reagents that are capable of differentiating between Sd(a+) and Sd(a−) THGP. Furthermore, it is of vital importance to establish the carbohydrate binding specificity of a lectin in order for it to be a useful tool for biochemical and immunochemical studies. In previous studies, we reported that a Tamm-Horsfall urinary glycoprotein with human blood group Sd(a+) activity reacts well with many lectins that recognize GalNAc and/or *N*-acetyllactosamine (Galβ1→4GlcNAc) residues [10] and with three toxic lectins [11]. The phenotype Sd(a−) THGP contains ligands mainly for *N*-acetyllactosamine (Galβ1→4GlcNAc) active agglutinins [12]. However, no detailed studies have been carried out on lectins that discriminate between Sd(a+) and Sd(a−) THGP preparations. Therefore, four GalNAc specific agglutinins, which recognize only GalNAc containing ligands but do not react with non-GalNAc containing structural units (Galβ1→4GlcNAc, Galα1→4Gal, Man and L-Fuc etc.), were chosen from our collection of lectins to resolve this problem. They were *Dolichos biflorus* agglutinin (DBA), *Vicia villosa*-B_4 agglutinin (VVA-B_4), *Codium fragile* subspecies *tomentosoides* (CFT) and Jacalin, in which CFT and Jacalin recognize only GalNAcα1→ residue(s). The binding profiles of Sd(a+) and Sd(a−) THGP to these four lectins were analyzed by quantitative precipitin assay (QPA), enzyme linked lectinosorbent assay (ELLSA), and by the inhibition of agglutinin-glycan interaction with sugar ligands. The results indicate that both native and asialo Sd(a+) THGP reacted readily with *Dolichos biflorus* (DBA) and *Vicia villosa*-B_4 (VVA-B_4), but not with *Codium fragile* subspecies *tomentosoides* (CFT) and Jacalin. Neither native nor asialo Sd(a−) THGP reacted with these four GalNAc specific lectins.

2. Materials and methods

2.1. Tamm-Horsfall glycoproteins and Tn containing gp

Tamm-Horsfall glycoprotein Sd(a+) was isolated from the urine of one single donor (W.T.J.M.) with Sd(a+) blood group by the method of Tamm and Horsfall [6,13]. Sd(a−) THGP-A.S. and Sd(a−)-R.D. were prepared from the urine collected from two persons with the Sd(a−) red cell phenotype [6,7,13]. To determine the effect of sialic acid on the sugar residues of the carbohydrate side chains at the non-reducing ends, the native glycoproteins were subjected to mild acid hydrolysis at pH 2.0, 80°C for 80 min [14,15]. The non-dialyzable fraction of the mild acid treated product (molecular mass cut off > 8000) is defined as asialo-THGP [10–12]. Asialo ovine salivary glycoprotein, which contains mainly GalNAcα1→Ser/Thr (Tn) determi-

*Corresponding author. Fax: (886) (3) 328-6456 (lab.) or (886) (3) 328-3031 (college).

Abbreviations: Sugar inhibitors: Gal, D-galactopyranose; Glc, D-glucopyranose; GalNAc, 2-acetamido-2-deoxy-D-galactopyranose; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; Man, D-mannopyranose; NeuAc, sialic acid; Glycoproteins: gp, glycoprotein; THGP, Tamm-Horsfall glycoprotein, and asialo THGP; Asialo OSM, asialo ovine submandibular gp; Lectins: VVA−B_4, *Vicia villosa*-B_4 agglutinin; DBA, *Dolichos biflorus* agglutinin; Jacalin, *Artocarpus integrifolia*; CFT, *Codium fragile* subspecies *tomentosoides*; QPA, quantitative precipitin assay; ELLSA, enzyme linked lectinosorbent assay

PII S0014-5793(98)00617-6

nants, was prepared by the procedure described by Tettamanti and Pigman [14].

2.2. Sugar inhibitors

Galβ1→4GlcNAc, Galβ1→4Glc, GlcNAc, *p*-nitrophenyl α and βGalNAc were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.3. Lectins

Dolichos biflorus agglutinin (DBA) was purified by adsorption to insoluble polyleucyl hog gastric (A+H) mucin [16–18] and eluted with GalNAc [19]. *Vicia villosa*-B_4 (VVA-B_4), *Artocarpus integrifolia* (Jacalin), *Codium fragile* subspecies *tomentosoides* (CFT) agglutinins were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.4. Biotinylation of lectins [20,21]

A solution of 250 μg/ml biotin ester in PBS was obtained by dissolving 500 μg of the ester in 50 μl methanol and mixing this sample with 1.95 ml PBS. The lectin sample (200 μg/250 μl PBS) was mixed with 400 μl of the biotin ester solution (100 μg biotin ester per 200 μg lectin) and left for 30 min at room temperature. The sample was dialyzed for several hours against water and overnight against TBS. After dialysis the sample volume was adjusted to 1 ml with TBS and 20 μl 5% sodium azide was added (final concentrations: 200 μg lectin per ml containing 0.1% NaN_3).

2.5. Lectinochemical assays

The quantitative precipitin assay was performed by a microprecipitation technique [22] using 4.7–6.3 μg of lectin nitrogen mixed with varying amounts of THGP. The mixture was incubated at 37°C for 1 h and kept at 4°C for 1 week. The total N in the washed precipitates was estimated by the ninhydrin method [23].

The enzyme linked lectinosorbent assay was performed according to the procedures described by Duk et al. [20,21]. The volume of each reagent applied was 50 μl/well; all incubations, except for coating, were performed at 20°C. The reagents, if not indicated otherwise, were diluted with TBS containing 0.05% Tween 20 (TBS-T). The TBS buffer or 0.15 M NaCl containing 0.05% Tween 20 was used for washing the plates between incubations. The absorbance was read at 405 nm in a microtiter plate reader, usually after 2 h incubation with THGP and 45 min, with asialo OSM.

For inhibition studies, the serially diluted inhibitor samples were mixed with an equal volume of lectin solution of constant amount. The control lectin sample was diluted two-fold with TBS-T. After 30 min at 20°C, the samples were tested by the binding assay, as described above. The inhibitory activity was expressed as the amount of inhibitor (nmol/well) giving maximum inhibition of the control lectin binding.

All experiments were done in duplicate or triplicate and results are mean values. The standard deviation did not exceed 10% and in most experiments was less than 5% of the mean value. The control wells, where coating or addition of biotinylated lectin was omitted, gave low absorbance values (below 0.1, read against the well filled with buffer) and were used as blank. It showed that blocking the wells before lectin addition was not necessary, when Tween 20 was used in TBS.

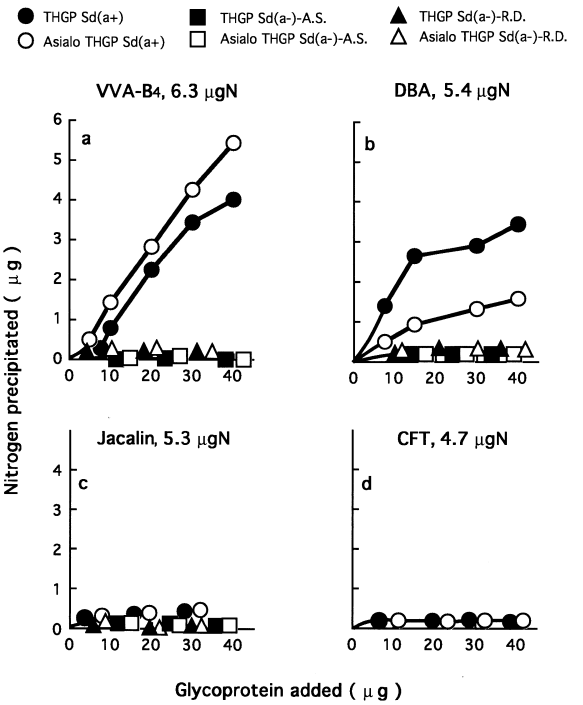

Fig. 1. Quantitative precipitin curves of native and asialo Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins with four GalNAc specific lectins. The amount of lectin nitrogen added ranged from 4.7 to 6.3 μg. Total volume: 300 μl. In contrast to the high affinities of Sd(a+) Tamm-Horsfall glycoproteins (THGP) for VVA-B_4 and DBA, Sd(a−) THGP and its asialo product were inactive or showed insignificant reactivity. Neither Sd(a+), Sd(a−) THGP, nor their asialo products bound to CFT or Jacalin.

3. Results and discussion

During the past two decades, the QPA has been successfully used to characterize the saccharide affinity of lectins [24–32], as such studies can provide insight into the specificities, steric effects, and size parameters of the combining sites of lectins and binding properties of glycoproteins. In this report, four

Table 1
Comparison of affinity of human blood group native and asialo Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins for *V. villosa*-B_4 (VVA-B_4) and *D. biflorus* agglutinin (DBA)

Lectin	Glycoprotein (gp)	A_{405}	Affinity[b]
VVA-B_4	THGP Sd(a+)	1.8	+++
(in Fig. 2a)	Asialo THGP Sd(a+)	2.5	++++
	THGP Sd(a−)-A.S.	0.03	−
	Asialo THGP Sd(a−)-A.S.	0.01	−
	THGP Sd(a−)-R.D.	0.06	−
	Asialo THGP Sd(a−)-R.D.	0.05	−
DBA	THGP Sd(a+)	2.4	++++
(in Fig. 2b)	Asialo THGP Sd(a+)	0.9	++
	THGP Sd(a−)-A.S.	0.01	−
	Asialo THGP Sd(a−)-A.S.	0.01	−
	THGP Sd(a−)-R.D.	0.02	−
	Asialo THGP Sd(a−)-R.D.	0.01	−

[a]100 ng of biotinylated VVA-B_4 and 100 ng of biotin labelled DBA were respectively added to various concentrations of native and asialo Sd(a+) and Sd(a−) THGP ranging from 2 ng to 2.5 μg.
[b]The results were interpreted according to the spectrophotometric absorbance value at 405 nm (i.e. A_{405}) after 2 h incubation as follows: ++++ (A_{405}: ≥2.0), +++ (A_{405}:2.0–1.5), ++ (A_{405}:1.5–0.75), + (A_{405}:0.75–0.2) and − (A_{405}: <0.2).

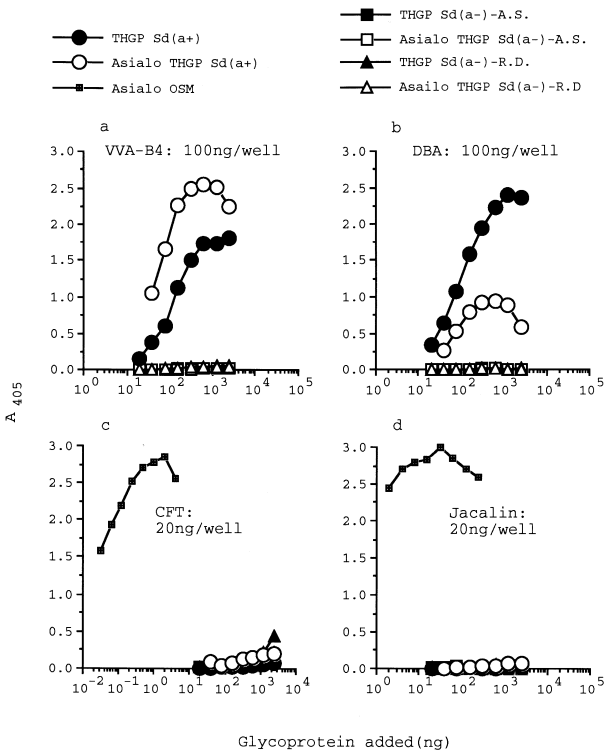

Fig. 2. Binding of various lectins to microtiter wells coated with serially diluted native and asialo Sd(a+) and Sd(a−) Tamm-Horsfall glycoproteins. All lectins were used at a constant quantity of 100 ng/well, except Jacalin which was used at 20 ng/well; total volume, 50 μl; with THGP, A_{405} read at 2 h; with asialo-OSM, A_{405} read at 45 min. Asialo OSM, which contains GalNAcα1→Ser/Thr as carbohydrate chains, was used as a positive control for the reactivities of CFT and Jacalin.

GalNAc specific lectins were selected to study differential binding of the afore-mentioned glycoproteins. These four lectins recognize only GalNAc related ligands, i.e. they recognize one or several of the following GalNAc containing ligands: GalNAcα1→3Gal, GalNAcα1→3GalNAc, GalNAcβ→, GalNAcα1→Ser/Thr and Galβ1→3GalNAcα1→Ser/Thr, but do not react with non-GalNAc containing structural units such as Galβ1→4GlcNAc, Galβ1→3GlcNAc, Galα1→3Gal, Galα1→4Gal, Man and LFuc etc. [24–26]. When these lectins were tested for THGP binding by QPA, both native and asialo Sd(a+) THGP reacted well with DBA and VVA-B_4 (Fig. 1a,b), but not with Jacalin and CFT which recognize mainly GalNAcα1→ residues [24,25]. Native and asialo Sd(a+) THGP precipitated over 50% of the VVA-B_4 nitrogen added (Fig. 1a). They also precipitated 54% and 25% (read at 30 μg gp added) of the DBA nitrogen added, respectively (Fig. 1b). Since Sd(a−) THGP lacks GalNAc residues, neither native Sd(a−) THGP nor its asialo product reacted with VVA-B_4, DBA, CFT, and Jacalin.

The affinity of the four GalNAc specific lectins for the native and asialo Sd(a+) and Sd(a−) THGPs were also examined by ELLSA. ELLSA is a simple and sensitive method where biotinylated lectin is used to detect coated glycans [20,21,25]. As shown in Fig. 2, similar binding profiles (Fig. 1 vs. Fig. 2) were obtained, in which asialo Sd(a+) THGP reacted better with VVA-B_4 than the native one (Fig. 2a). As for Sd(a+) THGP and its asialo product interacting with DBA, the native compound reacted much stronger than the asialo gp (Fig. 2b). DBA and VVA-B_4 showed different binding strengths with Sd(a+) THGP. However, both lectins still are useful to detect the presence of Sd(a+) determinant (GalNAcβ→ as key sugar) (Table 1).

Removal of sialic acid is the most efficient method to study the role of sialic acid in lectin binding. From the results, it is shown that a shielding effect of sialic acids on the binding efficiency was found in the VVA-B_4-THGP interaction (Figs. 1a and 2a); sialic acid also played an important steric role (positive) on the binding of DBA (Figs. 1b and 2b); other factors may also be involved. These have to be further investigated.

In order to prove that Sd(a+) THGP-lectin interactions occur through GalNAcβ→ rather than being non-specific, five sugar inhibitors were used to inhibit the THGP-lectin complex. As shown in Table 2, these interactions were inhibited by *p*-NO_2phenyl βGalNAc; Jacalin, which reacts mainly with GalNAcα1→, was used as a negative reference. Jacalin-asialo OSM interaction was inhibited strongly by GalNAcα1→ derivative and weakly by the GalNAcβ1→ compound.

The blood group substances with Sd(a+) activity occur in most human secretions, with the highest concentration in urine and meconium [8,9,33,34]. The binding properties of Sd(a+), Sd(a−) THGP and their asialo products with a panel of lectins exhibiting a broad range of carbohydrate specificities have been characterized by QPA and quantitative precipitin inhibition assay (QPIA) [10–12]. However, no detailed

Table 2
Inhibition of Sd(a+) Tamm-Horsfall glycoprotein interaction with VVA-B_4, DBA and Jacalin lectins by sugar inhibitors[a]

Lectin (amount)	Glycoprotein (amount)	Inhibition (%)[b]				
		120 nmol *p*-NO_2-phenyl α-GalNAc added	95 nmol *p*-NO_2-phenyl β-GalNAc added	339 nmol Man added	251.3 nmol GlcNAc added	259.3 nmol Glc added
VVA-B_4 (100 ng)	Asialo Sd(a+) THGP (100 ng/well)	99.2	99.4	−2.5	−3.0	3.8
DBA (100 ng)	Native Sd(a+) THGP (200 ng/well)	77.1	62.9	2.3	−5.1	2.1
Jacalin[c] (20 ng)	Asialo OSM (4 ng/well)	90.7	30.5	4.2	4.5	4.1

[a]A range from 20 ng to 100 ng of lectins was mixed with the amount of the sugar inhibitors as shown above. After incubation at room temperature for 30 min, the mixture of lectins and inhibitors was added to the Sd(a+) Tamm-Horsfall glycoprotein coated well, and incubated for 30 min.
[b]% inhibition = (difference between A_{405} of the binding affinity without and with inhibitor added/A_{405} of the binding avidity without inhibitor added) × 100.
[c]As reference for the α-anomer of GalNAc.

studies have been carried out on lectins that discriminate between Sd(a+) and Sd(a−) THGP preparations [35]. In this communication, we have tested the binding interaction of both Sd(a+) and Sd(a−) THGP with four selected GalNAc specific lectins by QPA and ELLSA. Asialo OSM was included as a positive control in the tests with the lectins (CFT and Jacalin) that are specific mainly for α-linked GalNAc [24,25]. The results indicate that DBA and VVA-B_4 reacted well only with Sd(a+) THGP and its asialo product, but not with Sd(a−) ones. Moreover, their interactions were inhibited by *p*-NO_2phenyl βGalNAc (Table 2). Therefore, DBA and VVA-B_4 can be used as reagents to differentiate Sd(a+) and Sd(a−) THGP.

Acknowledgements: This work was supported by Grants from the Chang-Gung Medical Research Project (CMRP 676), Kwei-san, Tao-yuan, Taiwan, the National Science Council (NSC 87-2314-B-182-053 and 87-2316-B-182-005), the National Health Institutes (DOH 85-HR-316 and DOH 84-HR-209), Department of Health, Taipei, Taiwan.

References

[1] Muchmore, A. (1990) Kidney Int. 37, 1395–1401.
[2] Lambert, C., Steele, B.J. and Rook, G.A.W. (1993) Immunology 79, 203–210.
[3] Rindler, M.J., Naik, S.S., Li, N. and Hoops, T.C. (1990) J. Biol. Chem. 265, 20784–20789.
[4] Hard, K., Van Zadelhoff, G., Moonen, P., Kamerling, J.P. and Vliegenthart, J.F.G. (1992) Eur. J. Biochem. 209, 895–915.
[5] Donald, A.S.R., Yates, A.D., Soh, C.P.C., Morgan, W.T.J. and Watkins, W.M. (1983) Biochem. Biophys. Res. Commun. 115, 625–631.
[6] Donald, A.S.R., Soh, C.P.C., Watkins, W.M. and Morgan, W.T.J. (1982) Biochem. Biophys. Res. Commun. 104, 58–65.
[7] Soh, C.P.C., Morgan, W.T.J., Watkins, W.M. and Donald, A.S.R. (1980) Biochem. Biophys. Res. Commun. 93, 1132–1139.
[8] Macvie, S.I., Morton, J.A. and Pickles, M.M. (1967) Vox Sang. 13, 485–492.
[9] Renton, P.H., Howell, P., Ikin, E.W., Giles, C.M. and Goldsmith, K.L.G. (1967) Vox Sang. 13, 493–501.
[10] Wu, A.M., Watkins, W.M., Song, S.C., Herp, A. and Wu, J.H. (1995) Biochem. Biophys. Res. Commun. 209, 103–110.
[11] Wu, A.M., Watkins, W.M., Chen, C.P., Song, S.C., Chow, L.P. and Lin, J.Y. (1995) FEBS Lett. 371, 32–34.
[12] Wu, J.H., Wu, A.M., Watkins, W.M., Chen, C.-P. and Song, S.-C. (1996) FEBS Lett. 384, 231–234.
[13] Tamm, I. and Horsfall, F.L. (1950) Proc. Soc. Exp. Biol. Med. 74, 108–114.
[14] Tettamanti, G. and Pigman, W. (1968) Arch. Biochem. Biophys. 124, 41–50.
[15] Wu, A.M. and Pigman, W. (1977) Biochem. J. 161, 37–47.
[16] Kabat, E.A. (1956) Blood Group Substances: Their Chemistry and Immunochemistry, 2nd edn., Academic Press, New York.
[17] Tsuyuki, H., von Kley, H. and Stahmann, M.A. (1956) J. Am. Chem. Soc. 78, 764–767.
[18] Kaplan, M.E. and Kabat, E.A. (1966) J. Exp. Med. 123, 1061–1081.
[19] Etzler, M.E. and Kabat, E.A. (1970) Biochemistry 9, 869–877.
[20] Duk, M., Lisowska, E., Wu, J.H. and Wu, A.M. (1994) Anal. Biochem. 221, 266–272.
[21] Lisowska, E., Duk, M. and Wu, A.M. (1996) in: Laboratory Guide to Biotin-labeling in Biomolecule Analysis (Meier, T. and Fahrenholz, F., Eds.), pp. 115–129, Birkhäuser Verlag, Basel.
[22] Kabat, E.A. (1961) Kabat and Mayer's Experimental Immunochemistry, 2nd edn., C.C. Thomas, Springfield, IL.
[23] Schiffman, G., Kabat, E.A. and Thompson, W. (1964) Biochemistry 3, 113–120.
[24] Wu, A.M., Song, S.C., Sugii, S. and Herp, A. (1997) Ind. J. Biochem. Biophys. 34, 61–71.
[25] Wu, A.M., Song, S.C., Wu, H.J., Kennth, S.S. and Kabat, E.A. (1997) Glycobiology 7, 1061–1066.
[26] Wu, A.M. and Sugii, S. (1988) Adv. Exp. Med. Biol. 288, 205–263.
[27] Wu, A.M., Wu, J.H. and Shen, F.S. (1994) Biochem. Biophys. Res. Commun. 178, 251–256.
[28] Wu, A.M., Shen, F.S., Herp, A. and Wu, J.H. (1994) Mol. Immunol. 31, 485–490.
[29] Wu, A.M., Lin, S.R., Chin, L.K., Chow, L.P. and Lin, J.Y. (1992) J. Biol. Chem. 267, 19130–19139.
[30] Wu, J.H., Herp, A. and Wu, A.M. (1993) Mol. Immunol. 30, 333–339.
[31] Wu, A.M., Kabat, E.A., Gruezo, F.G. and Allen, H.J. (1980) Arch. Biochem. Biophys. 204, 622–639.
[32] Wu, A.M., Song, S.C., Wu, J.H., Pfüller, U., Chow, L.P. and Lin, J.Y. (1995) Biochim. Biophys. Acta 1243, 124–128.
[33] Morton, J.A. and Terry, A.M. (1970) Vox Sang. 19, 151–161.
[34] Morton, J.A., Pickles, M.M. and Terry, A.M. (1970) Vox Sang. 19, 472–482.
[35] Morgan, W.T.J., Soh, C.P.C., Donald, A.S.R. and Watkins, W.M. (1981) Blood Transfus. Immunohaematol. 24, 37–51.