Carbohydrate Research, 239 (1993) 329–335
Elsevier Science Publishers B.V., Amsterdam

Note

An enzyme-linked immunosorbent assay for blood-group A and B enzymes *

Shin Yazawa [a], Tamiko Nakajima [a], Noriaki Kameyama [b], Koh-Ichi Saga [c] and Tetsuya Tachikawa [d]

[a] *Department of Legal Medicine, School of Medicine, Gunma University, Maebashi 371 (Japan)*
[b] *Gunma Red Cross Blood Center, Maebashi 371 (Japan)*
[c] *Sumitomo Seika Chemicals, Hyogo 675-01 (Japan)*
[d] *Otsuka Assay Institute, Tokushima 771-01 (Japan)*

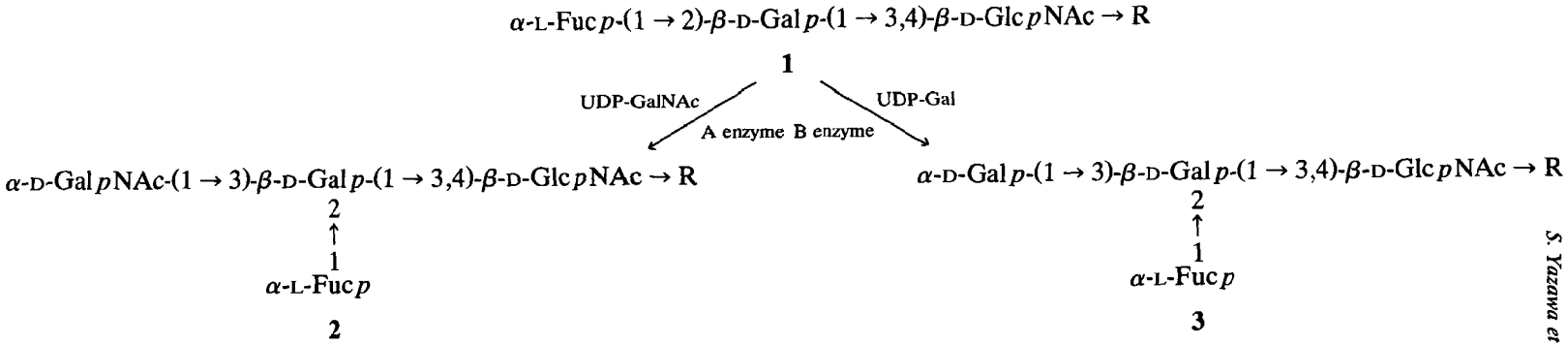
(Received March 28th, 1992; accepted in revised form October 27th, 1992)

The A and B blood group substances are one of the best characterized glycoconjugates and the antigenic determinant of A and B blood groups has been shown to be present predominantly in the terminal sugar residues[2]. All the sugar chains of glycoconjugates are synthesized through the action of a series of glycosyltransferases that catalyze the transfer of each sugar unit from the respective nucleotide sugar to a predetermined position and anomeric linkage[2,3]. The determinants of A (**2**) and B (**3**) blood groups are synthesized from the precursor H substance (**1**) by (1 → 3)-*N*-acetyl-α-D-galactosaminyltransferase (A enzyme) and (1 → 3)-α-D-galactosyltransferase (B enzyme) from A and B blood group subjects, respectively (see Scheme 1). It is also well known that A and B enzymes are primary products of *A* and *B* genes and that A and B antigens are then synthesized by A and B enzymes as shown in Scheme 1. Thus, the detection of A and B enzymes is a useful tool for the determination of A and B blood groups, which are commonly detected by the investigation of A and B antigens on red blood cells, or of anti-A and anti-B antibodies in sera. Furthermore, it is possible to classify A and B blood subgroups and variants by the level of A and B enzymes, even though the A and B antigens on the red blood cells are reduced and hardly detectable[3].

Serum A and B enzyme levels have been determined for a large number of normal groups, subgroups, and variants[4–6]. The enzyme activities are commonly assayed by two different methods. In the first, the conversion of O red blood cells into A or B red blood cells by the enzymes in the presence of UDP-GalNAc or

Correspondence to: Dr. S. Yazawa, Department of Legal Medicine, School of Medicine, Gunma University, Maebashi 271, Japan.

* Part of this work has been reported[1].


α-L-Fuc p-(1 → 2)-β-D-Gal p-(1 → 3,4)-β-D-Glc pNAc → R
1
UDP-GalNAc
UDP-Gal
A enzyme
B enzyme
α-D-Gal pNAc-(1 → 3)-β-D-Gal p-(1 → 3,4)-β-D-Glc pNAc → R
2
↑
1
α-L-Fuc p
2
α-D-Gal p-(1 → 3)-β-D-Gal p-(1 → 3,4)-β-D-Glc pNAc → R
2
↑
1
α-L-Fuc p
3

Scheme 1.

UDP-Gal is monitored by hemagglutination tests with anti-A or anti-B antibody[7]. In the second, the incorporation of a radioactively-labeled sugar (GalNAc or Gal) from a respective nucleotide-sugar into the H-active oligosaccharide is quantitated with a liquid scintillation counter[8]. The former method is simple but is qualitative rather than quantitative, and it is often hard to detect weak enzyme activities[3]. The latter method measures the enzyme activity quantitatively, but it is complicated and time-consuming and, thus, it is difficult to assay many samples simultaneously. Recently simple and quantitative assay methods for many glycosyltransferases have been reported[9–16]. Synthetic oligosaccharides have been used for the specific detection of a single glycosyltransferase[16–21]. Elices and Goldstein[22] showed that synthetic oligosaccharides immobilized to Synsorb beads were acceptors for various glycosyltransferases, and the enzyme activity was determined by measuring the amount of radioactivity incorporated from labeled nucleotide sugars into the beads. We describe herein a simple and more specific assay method for blood group A and B enzymes in plasma samples from normal, subgroups, and variants.

Incubation mixtures containing various amounts of A and B, Synsorb beads were used to establish standard curves of A and B haptens, which were then characterized and quantitated by enzyme-linked immunosorbent assay (ELISA) with anti-A and anti-B antibodies (Fig. 1). These standard curves permit the determination of the ELISA responses of A and B haptens on Synsorb beads, and it can be expected that these ELISA responses are the same as those of the equivalent amount of haptens enzymically synthesized on Synsorb H beads by A and B enzymes. The amount of antibody-detected A hapten, which had been enzymically synthesized by A plasma, was linear with time up to ~8 h and constant after 10 h. The formation of A hapten was proportional to the amount of A plasma and Synsorb H beads, up to 100 μL and 20 mg, respectively. The dose responses for the incubation time, and the amount of enzyme and acceptor beads used in the formation of B hapten by B plasma were almost the same as those in the formation of A hapten by A plasma (data not shown). Thus, both A and B enzyme activities can be determined quantitatively by this ELISA test.

The A enzyme activity of plasma samples from different blood groups subjects was measured under the standard assay condition (Table I). It could be detected in all the samples from A and AB subject tested, and only in samples from A and AB subjects; no A enzyme activity was detected in samples from B or O subjects. There was not significant difference in the levels of A enzyme from A_1 and A_1B individuals. The average A enzyme activity of A_2 and A_3 individuals was ~68 and 19%, respectively, of that of A_1 individuals. The B enzyme activity was also measured in plasma samples from different blood group subjects (Table II). The presence of B enzyme was demonstrated only in samples from B and AB subjects. There was no significant difference in the level of B enzyme between normal B and AB subjects. The average enzyme activity of B_{weak} and B_3 individuals was ~50 and 17%, respectively, of that of normal B individuals. The A and B enzyme activities from common A and B subjects could be measured with < 10 mg of

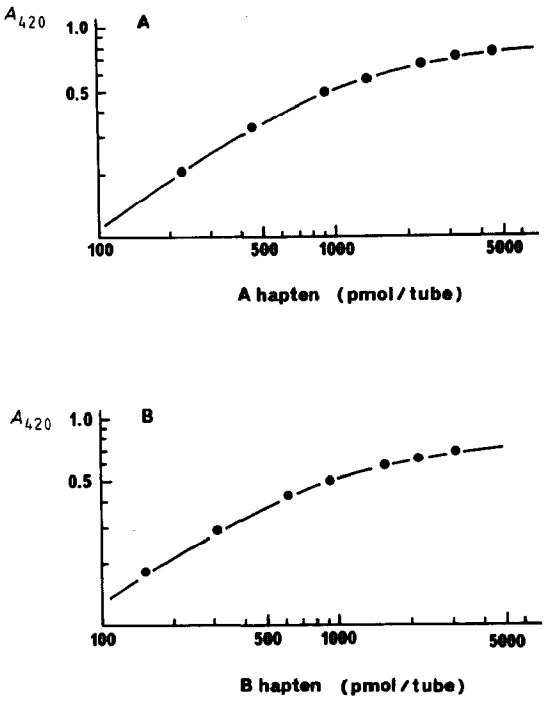


Fig. 1. Standard curves for ELISA response of A (A) and B (B) haptens immobilized on Synsorb beads.

Synsorb H beads, or within a few hours, but those from weak variants were hard to be detected under such conditions (data not shown).

Chemically synthesized oligosaccharides have been used in the past as acceptors for a number of glycosyltransferases, and the assay procedure was further simplified by having hydrophobic aglycons as acceptors[21,23]. The A and B enzyme activity of subgroups and weak variants could only be determined previously by the conventional radiochemical assay method[4–6]. The method described in this study enabled us to determine both A and B enzyme activities quantitatively in plasma samples from subgroups and variants, as well as normal groups. It has been

TABLE I

Blood-group A enzyme activity in sera of various blood types [a]

Blood type	Number of sera	Enzyme activity (units)
A_1	20	2278 ± 669
A_1B	20	2315 ± 682
A_2 (A_2B)	8	1559 ± 624
A_3	4	444 ± 394
O	20	0
B	20	0

[a] Enzyme activity is expressed as mean ± SD.

TABLE II

Blood-group B enzyme activity in sera of various blood types [a]

Blood type	Number of sera	Enzyme activity (units)
B	20	1465 ± 578
A_1B	20	1516 ± 426
B_{weak}	5	732 ± 607
B_3	3	242 ± 197
O	20	0
A	20	0

[a] Enzyme activity is expressed as mean ± SD.

proposed that defective A and B enzymes are present in the subgroups and variants, and that these enzymes might be involved in the expression of such unusual blood group types. Although there might be some qualitative difference between the A and B enzymes investigated in this study, a strong correlation could be demonstrated between the level of A and B enzyme in plasma samples and the strength of A and B antigens on red blood cells. Since the concentration of hapten bound to Synsorb beads can be easily modified, the present method, thus, has several advantages over previous methods and it could serve as a general method for the assay of glycosyltransferases in addition to the blood-group typing. In conclusion, A and B enzyme activities were measured quantitatively with the present ELISA, test and clear-cut differences in enzyme activity could be found between common, subgroups, and variants.

EXPERIMENTAL

Materials.—The AB-transferase assay kit, Galserve AB, which contains UDP-GalNAc, UDP-Gal, and buffers for A and B enzymes, as described below, was obtained from Sumitomo Seika Chemical, Japan. Synsorb A, B, and H beads were obtained from Chembiomed, Canada, and these beads contained covalently bound A {α-D-Gal*p*-NAc-(1 → 3)-[α-L-Fuc*p*-(1 → 2)]-β-D-Gal*p* → R}, B {α-D-Galp-(1 → 3)-[α-L-Fuc*p*-(1 → 2)]-β-D-Gal*p* → R}, and H [α-L-Fuc*p*-(1 → 2)-β-D-Gal*p*-(1 → 4)-β-D-Glc*p*NAc → R] haptens whose degrees of substitution were 0.225, 0.155, and 0.414 μmol/mg of beads, respectively. Purified anti-A (SYNAFF A) and anti-B (SYNAFF B) mouse monoclonal antibodies were from Chembiomed, Canada. Purified goat anti-mouse IgM conjugated to peroxidase was purchased from Tago, CA. ABTS, ovain albumin, and gelatin were from Sigma. Plasma samples were collected from blood group types A_1, A_2, A_3, B, B_{weak}, B_3, A_1B, A_2B, and O and stored at −80°C until used.

Methods.—A and B enzyme assays were carried out in plastic tubes containing 20 mg of the H Synsorb beads (8.28 nmol H hapten) and the following components (standard assay condition): For the A enzyme assay, Galserve A buffer (100 μL, 0.1 M cacodylate, pH 6.5, and 0.1 M $MnCl_2$), UDP-GalNAc (30 nmol), and plasma

(100 μL) to be tested were incubated at 37°C for 12 h with rotation. The beads were then washed three times with water (2 mL), and then anti-A (200 μL, 39 μg/mL in 50 mM PBS containing 0.5% ovain albumin and 0.05% thimerosal) was added and the mixture was incubated at room temperature for 1 h. The beads were washed three times with water and of peroxidase-conjugated anti-mouse IgM (200 μL, 91.1 unit/mL in PBS containing 0.5% gelatin) was added and the mixture was incubated at room temperature for 2h. The beads were washed three times with water and the substrate (200 μL, 0.78 mg ABTS in 0.01% H_2O_2) was added. The increase in absorbance at 420 nm was monitored after addition of 1% oxalic acid (1 mL). Control assays were carried out without UDP-GalNAc and the increase in absorbance was subtracted from each assay. In order to determine the amount of A hapten formed, a standard curve was prepared by conducting the ELISA test on various amounts of Synsorb A beads (0–4.5 nmol of hapten/0–20 mg beads) in the same incubation mixtures as described above. For the B enzyme assay, the incubation mixture was the same as for the A enzyme assay, except that Galserve B buffer (100 μL, 0.1 M PIPES, pH 7.0, and 0.1 M $MnCl_2$) and UDP-Gal (30 nmol) were added. Anti-B monoclonal antibody (20 μg/mL) and Synsorb B beads (0–3.1 nmol of hapten/0–20 mg beads) were also used for the ELISA test of the B enzyme. A unit of enzyme activity is defined arbitrarily as the quantity of enzyme producing an ELISA response equivalent to one pmol of A or B hapten immobilized to Synsorb beads under the standard assay conditions.

ACKNOWLEDGMENTS

The authors thank Drs. R. Madiyalakan and N. Kochibe for valuable discussions. This study was supported, in part, by Grants-in-Aid for Scientific Research No. 6257061 and No. 01010001 from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1 S. Yazawa, T. Nakajima, K. Saga, and T. Tachikawa, *Abstr. Int. Carbohydr. Symp. XVth*, (1990) 208.
2 W.M. Watkins, *Adv. Human Genet.*, 10 (1980) 1–136.
3 C. Salmon, J.-P. Cartron, and P. Rouger, *The Human Blood Groups*, Masson, New York, 1984, pp 94–140.
4 J.-P. Cartron, A. Gerbal, J. Badet, C. Ropars, and C. Salmon, *Vox Sang.*, 28 (1975) 347–365.
5 J.-P. Cartron, J. Badet, C. Mulet, and C. Salmon, *J. Immunogenet.*, 5 (1978) 107–116.
6 J. Badet, C. Ropars, and C. Salmon, *J. Immunogenet.*, 5 (1978) 221–231.
7 H. Schenkel-Brunner and H. Tuppy, *Eur. J. Biochem.*, 17 (1970) 218–222.
8 A. Kobata, E.F. Grollman, and V. Ginsburg, *Biochem. Biophys. Res. Commun.*, 32 (1968) 272–277.
9 J.A. Voynow, T.F. Scanlin, and M.C. Glick, *Anal. Biochem.*, 168 (1988) 367–373.
10 H. Iwase, I. Ishii, T. Saito, S. Ohara, and K. Hotta, *Anal. Biochem.*, 173 (1988) 317–320.
11 C.L.M. Stults, B.J. Wilbur, and B.A. Macher, *Anal. Biochem.*, 174 (1988) 151–156.
12 P.A. Ram, M. Fung, C.F. Millette, and D.R. Armant, *Anal. Biochem.*, 178 (1989) 421–426.
13 S.C. Crawley, O. Hindsgaul, G. Alton, M. Pierce, and M.M. Palcic, *Anal. Biochem.*, 185 (1990) 112–117.

14 X. Gu, T. Gu, and R.K. Yu, *Anal. Biochem.*, 185 (1990) 151–155.
15 T. Taki, S. Nishiwaki, K. Ishii, and S. Handa, *J. Biochem. (Tokyo)*, 107 (1990) 493–498.
16 M.M. Palcic, R.M. Ratcliffe, L.R. Lamontagne, A.H. Good, G. Alton, and O. Hindsgaul, *Carbohydr. Res.*, 196 (1990) 133–140.
17 M.A. Chester, A.D. Yates, and W.M. Watkins, *Eur. J. Biochem.*, 69 (1976) 583–592.
18 R.A. DiCioccio, J.J. Barlow, and K.L. Matta, *Clin. Chim. Acta*, 108 (1980) 41–48
19 R. Madiyalakan, S. Yazawa, S.A. Abbas, J.J. Barlow, and K.L. Matta, *Anal. Biochem.*, 152 (1986) 22–28.
20 S. Yazawa, R. Madiyalakan, H. Izawa, T. Asao, K. Furukawa, and K.L. Matta, *Cancer*, 62 (1988) 516–520.
21 S. Yazawa, R. Madiyalakan, R.K. Jain, N. Shimoda, and K.L. Matta, *Anal. Biochem.*, 187 (1990) 374–376.
22 M.J. Elices and I.J. Goldstein, *Arch. Biochem. Biophys.*, 254 (1987) 329–341.
23 M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconjugate J.*, 5 (1988) 49–63.
24 H. Fujii and A. Yoshida, *Proc. Nat. Acad. Sci. U.S.A.*, 77 (1980) 2951–2954.
25 A. Yoshida, H. Yamaguchi, and Y. Okubo, *Am. J. Hum. Genet.*, 32 (1980) 332–338.
26 A. Yoshida, H. Yamaguchi, and Y. Okubo, *Am. J. Hum. Genet.*, 32 (1980) 645–640.