A NEW ASSAY METHOD FOR BLOOD GROUP A- AND B-GLYCOSYLTRANSFERASES

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1. Introduction

The differences between blood groups (ABO) are known to be determined by the terminal carbohydrate residues attached to common carbohydrate chains on the red-cell surface. A specific *N*-acetylgalactosaminyltransferase (A-enzyme) of blood-group A subjects transfers *N*-acetylgalactosamine from UDP-*N*-acetylgalactosamine to the terminal galactose of H-substance on the O red-cell surface, while a specific galactosyltransferase (B-enzyme) of blood-group B subjects transfers galactose from UDPgalactose to the H-substance [1–3]. The transferases were commonly assayed by the following two methods:

(a) O red cells were incubated with enzyme (A or B) and the sugar donor, and the newly produced blood group substance (A or B) was semi-quantitatively assayed using anti-A or anti-B agglutinin [4];

(b) 2-Fucosyllactose was incubated with the enzyme and the sugar donor labeled with radioisotope, and radioactivity transferred into 2-fucosyllactose was determined [3,5].

These methods have some drawbacks. Method (a) is not really quantitative, since serial double-fold dilution of agglutinin was used and judgment of the end point of agglutination was subjective. Method (b) may not indicate real biological activity of the blood-group transferases, since 2-fucosyllactose is not a natural acceptor substance for the enzymes. In the present method, O red-cell membranes were incubated with UDP-*N*-acetylgalactosamine (for A-enzyme assay) or with UDPgalactose (for B-enzyme assay) and the enzyme, and amounts of A (or B) substance synthesized by the enzyme were quantified by utilizing lectins (A or B) coupled with peroxidase.

2. Materials

Human blood-group glycosyltransferases: A-enzyme was partially purified to ~ 100 000-fold from plasma of blood-group A by treatment with Sepharose 4B, and B-enzyme was partially purified to ~ 1000-fold from plasma of blood-group B by column chromatography with carboxymethyl–Sephadex and gel filtration with Sephadex G-200, as in [6,7]. Red-cell membranes: Red-cell ghosts were prepared from human red cells with O, A_1 and B blood types by the method in [8].

Lectin coupled with peroxidase: Lectin from *Bandeiraea simplicifolia*, 5–10 mg, (A- and B-specific, from Sigma Chem. Co.) and *Streptomyces* hemagglutinin, 2.5–5 mg, (B-specific), prepared as in [9], were conjugated with horseradish peroxidase, 10 mg, (type VI, from Sigma Chem. Co.), by treatment with glutaraldehyde, as in [10]. The preparation (1–1.5 ml) was dialyzed against saline–phosphate buffer (0.01 M Na_2HPO_4–KH_2PO_4 (pH 6.8) containing 0.15 M NaCl) and stored at 4°C. Peroxidase was fully active after the conjugation procedures.

UDP-*N*-acetylgalactosamine was synthesized as in [6]. UDPgalactose was purchased from Sigma Chem. Co. UDP-*N*-acetyl[1-^{3}H]galactosamine and UDP[6-^{3}H]galactose were purchased from New England Nuclear and Amersham. Purity of these materials was checked by ion-exchange column chromatography with Dowex-1 and found to be $\geq 97\%$. Concentrations of nucleotide sugars were determined from A_{262} (molar absorbance 10.0×10^3) at pH 7.0.

3. Methods and results

3.1. *Properties of lectins coupled with peroxidase*

Streptomyces hemagglutinin coupled with perox-

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Table 1
Hemagglutinating and membrane binding activities of lectins coupled with peroxidase

		Streptomyces lectin–peroxidase		*Bandeiraea* lectin–peroxidase	
		Prep. 1	Prep. 2	Prep. 1	Prep. 2
Hemagglutination Titer[a]	A	0	0	240	640
	B	640	480	320	640
	O	0	0	0	0
Membrane-binding activity[b] ($\Delta A_{510}.min^{-1}$)	A	0.024	0.051	0.191	0.368
	B	0.398	0.401	0.052	0.121
	O	0.036	0.035	0.021	0.050

[a] Hemagglutination titer is expressed as the highest dilution of the lectin–peroxidase causing detectable agglutination of red-blood cells

[b] Membrane-binding activity is expressed as peroxidase activity bound with red-cell membranes. A_1, B or O red-cell membranes (100 μg) together with 400 μg O red-cell membranes, (500 μg total red-cell membranes) were suspended in 100 μl saline–phosphate buffer, containing 2.5 μl lectin–peroxidase. After incubation for 6 h at 4°C, peroxidase activity bound with the membranes was assayed

idase (SH-P) can agglutinate blood-type B red cells but not A or O red cells, and it binds only to B red-cell membranes, while *Bandeiraea simplicifolia* lectin coupled with peroxidase (BL-P) reacts on both A and B red cells and red-cell membranes, but not on O red cells and O membranes (table 1). Although the hemagglutinating activity of BL-P was similar on A_1 and B red cells, it had higher affinity to A_1 red-cell membranes than to B red-cell membranes (table 1). Therefore, BL-P was used only for the assay of blood-group A-enzyme.

In the presence of excess lectin–peroxidase complexes (BL-P or SH-P) membrane-bound peroxidase activity was linearly related to the quantities of A_1 or B red-cell membranes, indicating that BL-P and SH-P can be used for quantification of A- and B-antigenic substances in red-cell membranes.

3.2. *Assay procedures of blood group A- and B-transferases*

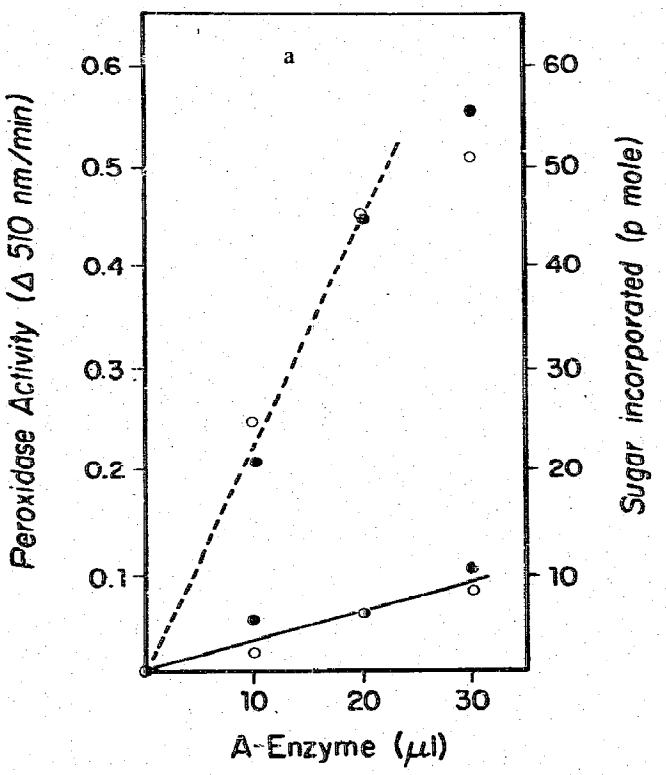
The reaction mixture (100 μl) for A-enzyme assay contained 0.5% of O red-cell membranes, 25–100 μM UDP-*N*-acetylgalactosamine, 15 mM $MnCl_2$, 0.15 M NaCl, 0.2% bovine serum albumin, 0.25% Triton X-100, 1 mM NaN_3, 40 mM cacodylate buffer (pH 6.8) and enzyme. The reaction mixture for B-enzyme assay contained 0.5% of O red-cell membranes, 25–100 μM UDPgalactose, 15 mM $MnCl_2$, 0.15 M NaCl, 0.5% bovine serum albumin, 0.25% Triton X-100, 1 mM NaN_3, 25 mM imidazole buffer (pH 6.5) and enzyme. The reaction mixture was incubated at 37°C with shaking. Cold saline–phosphate buffer was added to the reaction mixture to terminate the reaction, and the membranes were collected by centrifugation (3000 × *g* for 5 min). The washed membranes were mixed with 10 μl BL-P or SH-P solution, incubated for 6 h at 4°C, and washed 3 times with the saline–phosphate buffer by centrifugation.

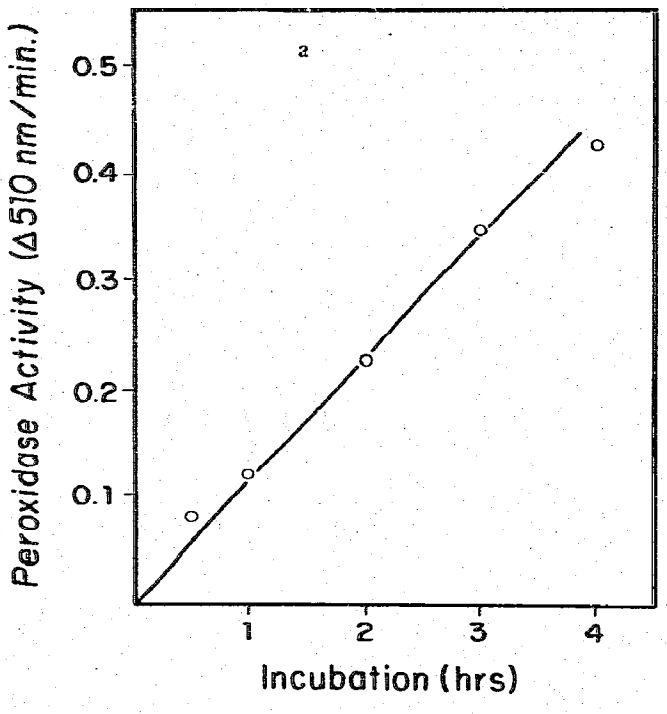
The peroxidase activity of the washed membranes was measured using 4-amino antipyrine and H_2O_2 as substrates [11]. The enzyme activity was expressed as an increase in A_{510} of the assay mixture (total 1 ml) in 1 min.

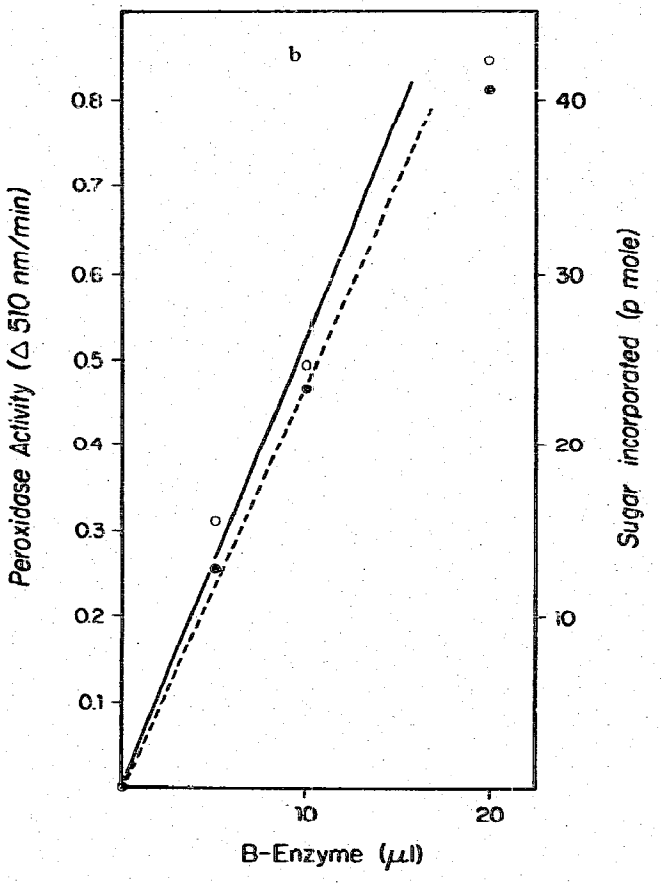
3.3. *Assay results of blood-group A- and B-transferases*

Typical assay results are shown in fig.1,2. These results indicated a linear enzyme-dose response and a linear initial time course which fulfilled two basic requirements for enzyme assay.

In order to convert transferase activity expressed in absorbancy units into molar quantities of sugars transferred into membranes, the enzyme activity was also assayed by measuring incorporation of isotope-labeled sugars into membranes. Since highly purified A- and B-enzymes (100 000-fold, and 1000-fold,







respectively) were used for the assay, the interference of non-blood-group glycosyltransferases existing in crude plasma was not expected to be serious. In fact, in all these measurements, the transferase activities assayed by the two methods agreed very well (fig.1).

In the assay of A-enzyme, 1 *A* unit obtained by the lectin–peroxidase method corresponds to an incorporation of $\sim 6 \times 10^{-10}$ mol *N*-acetylgalactosamine into O red-cell membranes; in the assay of B-enzyme, 1 *A* unit of lectin–peroxidase method corresponds to an incorporation of $\sim 5 \times 10^{-11}$ mol galactose (fig.1).

Fig.1. Assay of blood-group A- and B-transferases. The reaction mixture was incubated for 16 h (for A-enzyme) or 5 h (for B-enzyme) at 37°C with shaking, and the amount of newly synthesized A- (or B-) antigenic substance on red-cell membranes was assayed by utilizing the lectin–peroxidase as described in the text (——). Radioactivity incorporated into red-cell membranes was also measured (– – –). (a) Assay of A-enzyme in the presence of: 48 μM (–●–, – –●– –); 36 μM (–○–); 88 μM (– –○– –) UDPGalNAc. (b) Assay of B-enzyme in the presence of 71 μM UDPGal.

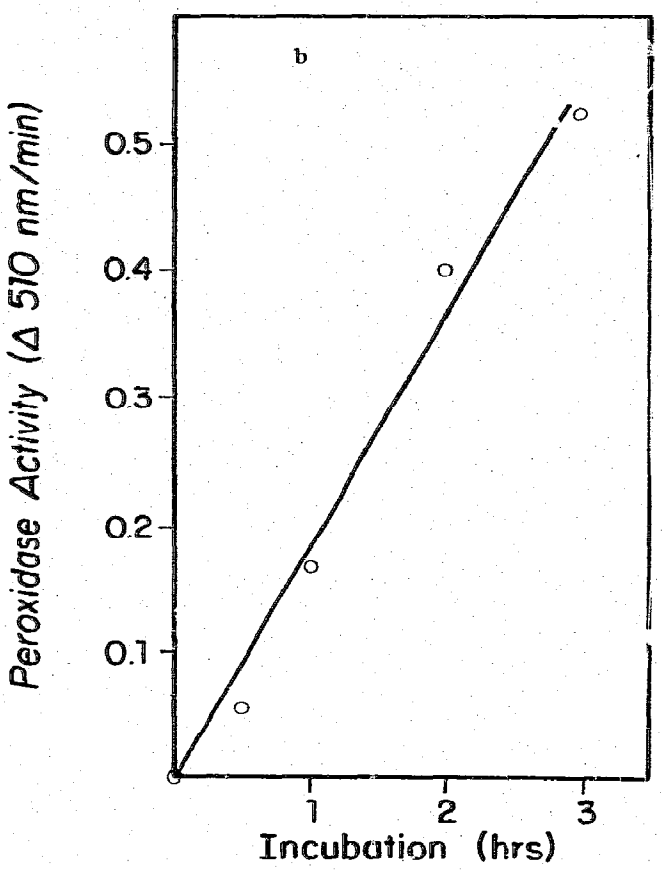


Fig.2. Time course of synthesis of blood group A- and B-substances by the A- and B-transferases. The amount of A- and B-substance was assayed by utilizing the lectin–peroxidase described in the text. (a) Synthesis of A-substance in the presence of 0.1 mM UDPGal*N*Ac and partially-purified A-enzyme. (b) Synthesis of B-substance in the presence of 0.1 mM UDPGal and partially-purified B-enzyme.

4. Discussion

The present assay method utilizing lectin–peroxidase for blood-group A-enzyme and B-enzyme has the following merits:

(1) O red-cell membranes, not an artificial oligosaccharide, are used for the assay;

(2) Quantities of A-substance and B-substance synthesized by the transferases, not simple incorporation of radioisotope labeled sugars into acceptors, are measured.

Thus, the method excludes the possible interference caused by non-blood-group glycosyltransferases contaminating the crude enzyme samples, and measures a real biological activity of the blood-group transferases. Accuracy of this assay method is better than ± 6%.

Although substantially purified enzyme preparations were used for establishing the assay methods, these methods can be used for assay of blood-group transferase activity of crude A_1 and B plasma which have 2–5% of the activity of the enzyme solutions used in the present study.

Besides the common A_1 and B types, several unusual blood types, such as A_2, Am, Ax, B_2, B_3 and Cis-AB, have been found in man. Genetic mechanisms for the expression of these rare blood types are not well understood, although it has been speculated that defective A- and B-enzymes might be involved in these abnormalities [12,13]. Comparison of kinetic properties of the transferases from normal and unusual subjects using the present assay method may contribute to an understanding of the origin of these unusual blood types.

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