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Studies on the Landsteiner's Model

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INTRODUCTION

Landsteiner (1901) published the report on the observation of the agglutination of normal human blood. He found that there were natural antibodies in serum which agglutinated the erythrocytes of other men. Landsteiner, whose remarkable research activity has been summarised in several monographs (Speiser 1961, 1962), initially discovered three blood groups, which were first called A, B and C, but were subsequently named A, B and O. Moreover, he found serum types against three blood groups mentioned above in serum. In 1902, Landsteiner's collaborators, Decastello and Sturli found fourth serum type which contained no "isoagglutinins". This was later named AB.

On the other hand, Schiff and Adelsberger (1924) first observed that there was a similarity of carbohydrate chain between human blood group A and Forssman antigen. After that, many researchers (Finland and Curnen 1938; Beeson and Goebel 1939; Iseki 1952; Springer 1956) found blood group A, B, H substances in gram-negative and gram-positive bacteria.

Race and Sanger (1975) indicated that the following studies were most contributory to ABO groups in past 50 years: (1) biochemical studies on A, B and H substances, (2) studies on Bombay type, (3) discovery of cis-AB, (4) discovery of specific hemagglutinin from plants and some invertebrates such as _Helix pomatia_. Thus, lectins play important roles in studies of blood groups. In 1965, Prokop and his collaborators (Prokop and Rackwitz 1965; Prokop, Rackwitz and Schlesinger 1965) reported a review of opinions and observations, they described the presence of group-like (antigen like) substances in plants and some invertebrates such as _Helix pomatia_.

We have many reports on the lectins extracted from plants and in vertebrates, but there are few studies (Nagai and Prokop 1986) on interaction between blood group antigens of microorganisms and hemagglutinins secreted by them. This report presents characterization and specificity of a hemagglutinin which was secreted from a new soil bacterium in Japan, _Prokopoa nagaiensis_ (Nagai 1982).

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MATERIALS AND METHODS

1. CULTIVATION:

A medium was prepared according to the method of Nagai (1982). The growth of Prokopoa nagaiensis was continued for 7 days at 27°C. At the end of this period, the hemagglutinating activity reached a plateau of about 4 to 8 hemagglutination titer, and the culture broth was harvested by filtration with a piece of cloth.

2. ASSAYS:

Assay of hemagglutinating activity was performed with a micro-titer apparatus using a 2 % human A, B, O or AB erythrocyte suspension and a 0.15M NaCl solution as a diluent. Hemagglutination was conducted for 90 min. at room temperature. The activity was expressed as titer, the reciprocal of the highest two-fold dilution exhibiting positive hemagglutination.

For inhibition studies, purified hemagglutinin (titer, 4 units) was thoroughly mixed with varying concentrations of saccharides to be tested, and then a 2 % human B erythrocyte suspension was added to the mixture. The degree of inhibition was expressed as a final concentration of the maximum dilution at which the saccharide could inhibit hemagglutination.

3. PURIFICATION AND PHYSICOCHEMICAL ANALYSES

The procedures for the purification of the hemagglutinin from a new soil bacterium (Prokopoa nagaiensis) were previously discribed (Nagai and Prokop 1986). Physicochemical analyses such as disc electrophoresis, ultracentrifuge analysis, amino acid analysis and carbohydrate analysis for the hemagglutinin were previously discribed (Nagai and Prokop 1986).

RESULTS AND DISCUSSION

1. PURIFICATION OF THE HEMAGGLUTININ:

To 100 l of culture filtrate was added 500 ml of swollen cross-linked gum arabic gels, and this suspension was stirred 4 C overnight. The gel was collected by sedimentation and packed into a column. The column was washed successively with 0.15M NaCl, 1M NaCl and then with 0.2M D-galactose in 1M NaCl to remove contaminating proteins. The hemagglutinin was eluted by washing the column with 1M D-galactose in 1M NaCl. The active fractions were applied to a column of Sephadex G-15 to remove salts and sugars. The resulting hemagglutinin solution was lyophilized and stored at -80°C. The yield was about 270 mg, corresponding to 72 % of

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the total activity found in the original culture filtrate.

2. MOLECULAR WEIGHT DETERMINATION:

The molecular weight was calculated to be 10,400 ± 500 from sedimentation equilibrium analysis. A similar value (10,300 ± 2,000) was also estimated from the mobility-molecular weight relationship on 5 % polyacrilamide gel in the presence method was 1.8 % which corresponds to 1 ml of hexose per 11,000 daltons. The result might suggest that the hemagglutinin contains a few moles of hexose per mole of the protein. Although the hemagglutinin actually contains some sugars, it is not certain at the moment whether they are bound covalently to the protein or not.

3. AMINO ACID AND CARBOHYDRATE ANALYSES:

Amino acid analyses of the hemagglutinin revealed that the contents of glycine, alanine and valine residues was very high, but no phenylalanine residue was present. Since the amount of carbohydrates detected in the protein is very low, we consider at present that the hemagglutinin may be not a glycoprotein.

4. BLOOD GROUP SPECIFICITY:

Optimam pH of the hemagglutinin reaction with human erythrocytes was pH 7-8. The hemagglutinin strongly agglutinated all the samples of human B and AB erythrocytes tested, and its anti-B activity was much stronger than the anti-A and anti-H activity (Table 1). The hemagglutinin has no specific activity for MN and P. The anti-B activity was confirmed by the fact that the purified hemagglutinin was inhibited by salivas from secretors with blood group B, but not inhibited by those from non-secretors.

Numerous hemagglutinins have been purified to homogeneity, but typical anti-B hemagglutinins have not been obtained from plants. In 1974, however, Hayes and Goldstein purified and characterized from _Bandeiraea simplicifolia_ seeds a so-called anti-B lectin, the existence of which had been known through its anti-B activity (Mäkelä and Mäkelä 1956). The lectin is specific for α- galactpyranosyl residues, but agglutinates both human blood group A_1 and B erythrocytes. The hemagglutinating activity for A_1 cells is one-quarter of that for B cells.

In contrast, the purified _Prokopoa nagaiensis_ agglutinin agglutinated human B erythrocytes 256 times as strongly as A or O erythrocytes. Thus, as far as serological activity is concerned, the _Prokopoa nagaiensis_ agglutinin is more suitable to be called an anti-B hemagglutinin than the _Bandeiraea simplicifolia_ lectin.

5. HAPTEN INHIBITION STUDIES:

The sugar specificity of purified hemagglutinin was examined by hemagglutination-inhibition tests. The hemagglutination was inhibited by D-galactose and its derivatives with the pyranose form,

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Table 1. Hemagglutination of human erythrocytes by the *Prokopoa nagaiensis* hemagglutinin

	Blood group			
	A	B	O	AB
Hemagglutination titer	2	512	2	128

Protein concentration : 1 mg/ml

Table 2. Landsteiner's phenomena

	Bacterium[1]	Erythrocyte[2]
Surface antigen	A-like antigen	A-antigen
Hemagglutinin or Normal antibody	Anti-B	Anti-B (ß)

1: *Prokopoa nagaiensis* NAGAI 1981
2: Blood group A (Human)

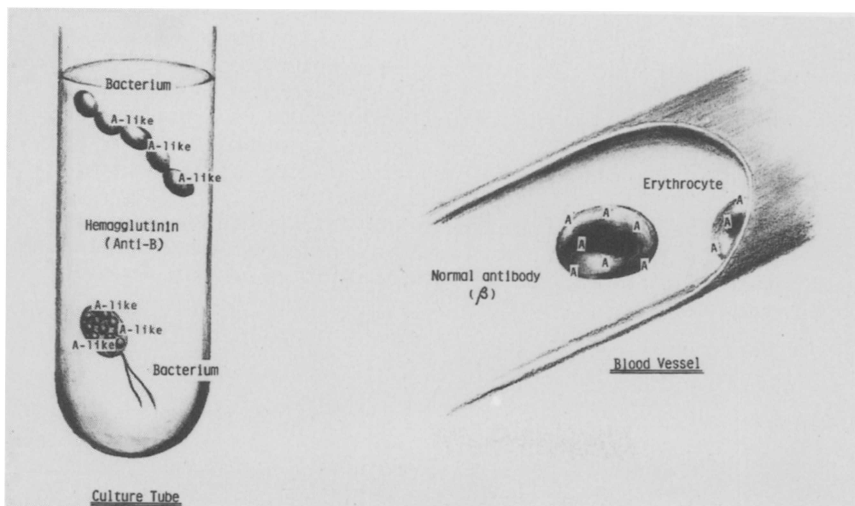

Fig. 1. Landsteiner's phenomena

but not by the derivatives with the lactone ring. ß-D-galactosides were more inhibitory than α-D-galactosides. L-rhamnose was the potent inhibitor among the monosaccharides tested, and its inhibitory effect was 10 times stronger than D-galactose. These results are the same as previous results which were obtained with purified samples.

6. HEMAGGLUTINATION IN OTHER BACTERIA:

We have many reports about blood group substances of bacteria; these reports are deeply concerned with blood group antigens. But there are few studies (Nagai and Prokop 1986) on interaction between blood group antigens of microorganisms and hemagglutinins

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secreted by them.

The hemagglutination reaction between bacteria and erythrocytes are different from those between lectins and erythrocytes because the former is caused by bacterial elements and the latter is caused by soluble proteins. Many gram-negative bacteria such as enteric bacteria and Pseudomonas, and Corynebacterium have fimbriae (adhesive pili) on their surface as agglutinative organs. The hemagglutination reaction between fimbriae and erythrocytes are a non-reversible one because the reaction is inhibited by D-mannose. Little information is available on the study of soluble hemagglutinin. Hemagglutinin of B.pertussis is also fimbriae which was separated from the surface of the bacterium. However, the hemagglutinin of C. botulinus is secreted in culture medium is inhibited by D-galactose.

7. LANDSTENER'S PHENOMENA IN THE FIELD OF MICROORGANISM

In this study, blood group of the bacterium (Prokopoa nagaiensis) were examined. We performed absorption test by using the bacterium, and it was found that the bacterium had specific blood group antigen which is A-like antigen (Table 2, Fig. 1). Based on the concept of the "horror autotoxicus", that is, blood group A contains anti-B serum (Aß) and blood group B contains anti-A serum (Bα), these results obtained above are quite similar to "Landsteiner's phenomena" of blood group system. Therefore, we found the "Landsteiner's phenomena" in the field of microorganisms.

On the other hand, the bacterium infected with a bacteriophage could not produce the hemagglutinin. This phenomen is quite similar to situation of the human blood group system which is controled by regulator gene.

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