# SUGAR SPECIFICITY OF ANTI-B HEMAGGLUTININ PRODUCED BY STREPTOMYCES SP.

Yoko Fujita, Kunio Oishi and Ko Aida

Institute of Applied Microbiology
University of Tokyo, Tokyo, Japan

Received June 1,1973

SUMMARY: A hemagglutinin specific for blood group B antigen has been purified to 190-fold from the culture fluid of a strain of Streptomyces sp. by conventional procedure involving ammonium sulfate fractionation and column chromatography. The molecular weight of the partially purified preparation was estimated to be approximately 5000±1000; this value is extremely small as compared with those of hemagglutinins which have been so far isolated from various sources.

Hemagglutination-inhibition tests revealed that the Streptomyces agglutinin has a specificity to combine with D-galactose and several saccharides having D-galactose residues at the non-reducing terminal, and that the special configuration of the hydroxyl groups at C-2 and C-4, particularly the hydroxyl group at C-2, is essential for binding of the sugars to the hemagglutinin.

Numerous hemagglutinins have been known to occur in the extracts of seeds of higher plants and invertebrate tissues, and have recently recieved much attention with respect to their binding ability to the specific sites on cell surface (1). However, of the agglutinins with blood group specificity, blood group A or H specific ones are predominant whereas no typical anti-B agglutinin has yet been reported despite intensive search among many sources (2, 3). For instance, some hemagglutinins, the activities of which are inhibited by D-galactose, are more strongly inhibited by N-acetylgalactosamine, the immunodominant sugar of blood group A specificity (4, 5, 6). Thus, they are preferably categorized into blood group A specific agglutinins rather than B specific ones.

This is not the case for our Streptomyces agglutinin (7) since the degree of inhibition by D-galactose is extremely higher than that by N-acetylgalactosamine or 2-deoxy-D-galactose. It is, therefore, apparant that the hydroxyl group at C-2 of D-galactose is essential for the agglutinin in binding the surface of blood group B erythrocytes.

In this communication we report the procedure of isolation and the studies on the specificity of this novel microbial hemagglutinin.

## MATERIALS AND METHODS

The blood group B substances from bullfrogs and toads were kindly supplied by Dr. S. Yamamoto, Gumma University, Japan, and Dr. Y. Hashimoto, Saitama University, Japan, respectively. Plant gums were generous gifts from Dr. O. Igarashi, Ochanomizu University, Tokyo, and Dr. Y. Hashimoto.

Crude blood group A, B and H substances were prepared from boiled human saliva by ethanol precipitation, and stored in 0.9% saline solution.

The assay of hemagglutinating activity was performed by mixing two-fold serial dilution of the agglutinin with an equal volume of 2-3% erythrocyte suspension. The activity was expressed as HA titer, the reciprocal value of the maximal dilution at which hemagglutination was observed.

Hemagglutination-inhibition test was made as follows; two-fold serial dilution of the substances to be tested were prepared on microtiter plate, to which an equal volume of the solution containing 2-4 hemagglutinating units of the Streptomyces agglutinin was added, shaken well, and incubated at room temperature. After 20-30 minutes aliquots of human B erythrocyte suspension were added to the mixture, shaken well, and the hemagglutination was examined for 90 minutes.

The amount of protein was determined by the method of Lowry et al. (8), using bovine serum albumin as standard.

Streptomyces 27S5 was cultured with shaking in 0.05M phosphate buffer (pH 6) containing 2% D-fructose, 0.5% peptone(Kyokuto), 0.1% yeast extract (Difco) and 0.05% $MgSO_4 \cdot 7H_2O$, at 27 °C. After 4-5 days, cells were removed by filtration. To the clear solution, solid ammonium sulfate was added to 75% saturation. The resulting precipitate was collected by centrifugation at 10,000 g for 30 minutes, dissolved in 0.05M phosphate buffer (pH 6), and dialyzed against the same buffer. The dialyzate was applied to a column of Sephadex G-50 equilibrated with the phosphate buffer. This treatment gave two active peaks which

partially overlapped with each other. The major peak fractions were combined and passed succesively through columns of DEAE-cellulose equilibrated with 0.01M phosphate buffer (pH 6) and 0.01M phosphate buffer (pH 7.8). Subsequent chromatography on Sephadex G-75 column at pH 6 afforded a partially purified preparation of the agglutinin.

The determination of an approximate molecular weight was achieved according to the method of Andrews (9) using a Sephadex G-75 column (1x94cm). Standards were: bovine serum albumin, 67,000; ovalbumin, 45,000; $\alpha$-chymotrypsin, 25,000; lysozyme, 14,300; ribonuclease A, 13,700; bacitracin, 1,400.

## RESULTS AND DISCUSSION

An extracellular hemagglutinin of Streptomyces 27S5 was purified to a preparation with a specific activity of 237 (HA titer/protein mg/ml), which is 190-fold higher than that of the original culture fluid. The procedure of purification is summarized in Table 1.

As far as we know, this agglutinin is the first blood group specific hemagglutinin hitherto isolated from the extracellular components of microbial origin in quantity. In addition, it is of interest that this agglutinin has exceptionally smaller molecular weight (approx. 5000±1000) as compared with those of phytohemagglutinins.

With respect to serological specificity, this agglutinin is able to agglutinate specifically human blood group B erythrocytes. This specificity was further confirmed by that the hemagglutinating activity was inhibited in the presence of blood group B substances from various sources, but was unaffected by the addition of A or H substances from human saliva (Table 2). These results indicate that this hemagglutinin can be calssified as the one with blood group B specificity.

However, our hemagglutinin differs from human anti-B serum in the specificity. According to Kabat and Leskowitz (10), the degree of inhibition on the precipitin reaction with human anti-B serum by sugars was decreased in the following order: (i) raffinose, melibiose and stachyose, (ii) methyl $\alpha$-D-galactoside, (iii) D-

Table 1. Purification of the hemagglutinin 27S5

| | Total protein (mg) | Total activity (HA titer) | Specific activity* | Purificatio (-fold) |
|---|---|---|---|---|
| Culture filtrate | 11440 | 14300 | 1.25 | 1 |
| $(NH_4)_2SO_4$ fraction | 913 | 4992 | 5.47 | 4.4 |
| Sephadex G-50 fraction** | 32.4 | 1504 | 46.4 | 37.1 |
| DEAE-cellulose(pH 6) fraction | 8.8 | 1090 | 124 | 99.1 |
| DEAE-cellulose(pH 7.8) fraction | 4.2 | 600 | 143 | 114 |
| Sephadex G-75 fraction | 0.474 | 113 | 237 | 190 |

* Specific activity is defined as titer per miligram of protein.
** Major peak

Table 2. Inhibition of the hemagglutinin 27S5 by blood group active substances

| | Minimum amount(%) giving complete inhibition |
|---|---|
| Blood group A substance from human saliva | > 0.007 |
| Blood group B substance from human saliva | 0.00009 |
| Blood group H substance from human saliva | > 0.007 |
| Blood group B+H substance from bullfrog gastric mucus | 0.021 |
| Blood group A+B+H substance from toad egg jelly | 0.021 |

Table 3. Inhibition of the hemagglutinin 27S5 by D-galactose and oligosaccharides possessing terminal non-reducing galactose

| | Minimum amount (mM) giving complete inhibition |
|---|---|
| D-Galactose | 2.6 |
| Melibiose | 2.6 |
| Raffinose | 2.6 |
| Stachyose | 2.9 |
| Lactulose | 7.2 |
| Lactose | 42 |
| Phenyl $\alpha$-D-galactoside | 0.52 |
| Phenyl $\beta$-D-galactoside | 0.52 |

Table 4. Inhibition of the hemagglutinin 27S5 by various heteroglycans

| | Minimum amount ($\mu$g/ml) giving complete inhibition |
|---|---|
| Guar gum | 0.14 |
| Locust bean gum | 0.3 |
| Gum arabic | 1.6 |
| $\kappa$-Carrageenan | 27 |
| Tamarindus indica (seeds) | 53 |
| $\lambda$-Carrageenan | 103 |
| Karaya gum | > 6700 |
| Tragacanth gum | > 6700 |
| Heparin | > 67000 |

Cf. L-Rhamnose, 29 $\mu$g/ml; D-Galactose, 234 $\mu$g/ml

galactose, and (iv) methyl $\beta$-D-galactoside. In contrast, with our hemagglutinin D-galactose and $\alpha$-galactosyl oligosaccharides were effective at about equal concentrations for complete inhibition (Table 3). $\beta$-Galactosyl disaccharides examined were less inhibitory than $\alpha$-galactosides; whereas the effectiveness of phenyl $\alpha$- and $\beta$-galactosides was quite equal to each other and much higher than that of D-galactose.

In addition, among various heteroglycans galactomannans, in which the galactose residues attach to mannan backbone through $\alpha$-(1-6) linkage, exhibited remarkable inhibition upon the hemagglutination (Table 4).

Besides D-galactose and the galactosyl sugars, L-rhamnose, D-fucose and L-arabinose were also effective in the inhibition of this hemagglutination, as shown in Table 5. Among these sugars, L-rhamnose was found to be the most powerful inhibitor; its activity was about 8 times as high as that of D-galactose. Table 5 shows that sugars being active in binding the Streptomyces agglutinin have a common configuration of the hydroxyl groups at C-2 and C-4 of pyranose form. These structural feature does not coincide with Mäkelä's proposition (2) that explains well the specificity of phytohemagglutinins by means of the configuration of the hydroxyl groups at C-3 and C-4 of inhibitory sugars.

Table 5. Inhibition of the hemagglutinin 27S5 by various sugars

| | Minimum amount (mM) giving complete inhibition |
|---|---|
| L-Rhamnose | 0.16 |
| D-Galactose | 1.3 |
| D-Fucose | 3.2 |
| L-Arabinose | 5.2 |
| D-Glucose | > 67 |
| N-Acetylglucosamine | > 67 |
| Methyl $\alpha$ -D-glucoside | > 67 |
| Methyl $\beta$ -D-glucoside | > 67 |
| D-Mannose | > 67 |
| Mannitol | > 67 |
| D-Fructose | > 67 |
| L-Sorbose | >167 |
| L-Fucose | > 8.3 |
| D-Arabinose | > 83 |
| D-Xylose | > 67 |
| D-Ribose | > 67 |
| Sucrose | > 67 |
| Cellobiose | > 13 |
| Streptomycin | > 67 |

Table 6. Inhibition of the hemagglutinin 27S5 by [illegible]actose derivatives

| | Minimum amount (mM) giving complete inhibition |
|---|---|
| D-Galactose | 1.3 |
| 2-Deoxy-D-galactose | 33.3 |
| Galactosamine | 66.7 |
| N-Acetylgalactosamine | 83.3 |

Furthermore, substitution of the hydroxyl group at C-2 of D-galactose with hydrogen, amino group, or acetamido group resulted in marked decrease in inhibitory activity (Table 6). It is obvious, therefore, that the requirement of the hydroxyl group at C-2 of D-galactose for binding ability is critical and that this

requirement supports the blood group B specificity of this hemagglutinin.

Studies are in progress for further characterization of this hemagglutinin and to elucidate the molecular basis of the specific binding site to the cell surfaces.

## REFERENCES

1. Sharon, N. and Lis, H., Science, 177, 949 (1972)
2. Mäkelä, O., Ann. Med. Exp. Biol. Fenn., 35, suppl. 11 (1957)
3. Khalap, S., Thompson, T. E. and Gold, E. R., Vox Sang., 18, 501 (1970)
4. Hammarström, S. and Kabat, E. A., Biochemistry, 8, 2696 (1969)
5. Etzler, M. E. and Kabat, E. A., Biochemistry, 9, 869 (1970)
6. Galbraith, W. and Goldstein, I. J., Biochemistry, 11, 3976 (1972)
7. Fujita, Y., Oishi, K. and Aida, K., J. Gen. Appl. Microbiol., 18, 73 (1972)
8. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., J. Biol. Chem., 193, 265 (1951)
9. Andrews, P., Biochem. J., 91, 222 (1964)
10. Kabat, E. A. and Leskowitz, S., J. Amer. Chem. Soc., 77, 5159 (1955)