IMMUNOCHEMICAL STUDIES ON BLOOD GROUP SPECIFICITY II. INHIBITION OF HEMAGGLUTININ OF *SOPHORA JAPONICA* BY SIMPLE SUGARS.

BY

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In 1951, Watkins and Morgan[1] described that not only hog and human H substances inhibit hemagglutination of O cells by eel anti-O (H) but also that simple sugars, for example, L-fucose and its structural homologues, would exert similar effect. It was for the first time that this fact had been presented as an example of the inhibition of normal antibody by simple sugars. Further, Morgan and Watkins[2] carried out an analogous study on the inhibition of hemagglutination of plant agglutinin by simple sugars.

In the preceding paper of this series[3], partial purification of the agglutinin from *Sophora japonica* was reported and it was revealed that our partially purified product contained anti-B, anti-C and anti-O (H) agglutinins.

This paper deals with the inhibition by simple sugars on hemagglutination of our partially purified agglutinin from *Sophora japonica* and the results obtained are discussed mainly from a standpoint of stereochemistry of sugar.

Materials and Methods

Partially purified agglutinin of *Sophora japonica* (fr-7) was prepared by the methods described in the preceding paper.

Commercial products of D-glucose, D-galactose, L-arabinose, L-fucose, D-mannose, D-xylose, D-fructose, L-rhamnose, D-ribose, D-galactosamine-HCl, N-acetyl-D-galactosamine, lactose, raffinose, saccharose, dulcitol, meso-inositol, and salicin were used.

D-Glucosamine-HCl was prepared from the dried lobster shells[4].

N-Acetyl-D-glucosamine was prepared from D-glucosamine-HCl according to Roseman and Ludowieg[5].

Melibiose was prepared from raffinose according to Hudson and Harding[6].

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D-Fucose was prepared according to Iselin and Reichstein[7].

1.6-Anhydro-D-galactose was prepared according to Hann and Hudson[17].

L-Glucose and L-mannose were prepared according to Sowden and Fischer[8].

D-Gulose was prepared according to Fischer and Stahel[9].

D-Talose was prepared according to Bosshard[10].

Methyl α-D-galactoside and methyl β-D-galactoside were prepared according to Reber and Reichstein[11].

2-O-Methyl-D-galactose was prepared according to McCreath et al.[12].

3-O-Methyl-D-galactose was prepared according to Reber and Reichstein[11].

4-O-Methyl-D-galactose was prepared according to Jeanloz[13].

6-O-Methyl-D-galactose was prepared according to Freudenberg and Smeykal[14].

1,5-Anhydro-D-dulcitol was prepared according to Fletcher and Hudson[15].

Hog A substance was prepared from pooled hog gastric mucosa according to Kabat[16]. Its minimum concentration giving inhibition against human anti-A was 0.25 μg/ml.

Cyst B substance was kindly supplied by Prof. Hiyama, University of Hirosaki. Its minimum concentration giving inhibition against human anti-B was 0.5 μg/ml.

Inhibition test with sugars was performed as follows: To each 0.1 ml of two-fold serial dilutions of the sugar solution, 0.1 ml of the agglutinin solution being diluted to have an agglutinin titer of 1 : 4 for appropriate blood cells was added, and the mixtures were incubated overnight in an ice-box. Each 0.05 ml of 4% suspension of corresponding cells were added and the results of agglutination were observed.

Results

At the concentration of 20 mg./ml, following sugars and polyols did not inhibit the hemagglutination: D-Glucose, L-glucose, D-mannose, L-fucose, D-xylose, D-fructose, saccharose, N-acetyl-D-glucosamine, D-ribose, 4-O-methyl-D-galactose, 1,6-anhydro-D-galactose, dulcitol, meso-inositol and salicin.

The inhibiting power of the sugars being active at the concentration of 20 mg./ml respectively was measured by preparing two-fold serial dilutions of them. The results were shown in Table 1.

It will be seen in the Table 1 how important role the streochemical

Table 1. Minimum concentration of substances giving inhibition, mg/ml.

Sugars and polyols	Anti-B	Anti-C*	Anti-O (H)
D-Galactose	5	2.5	2.5
L-Arabinose	10	10	10
D-Fucose	5	2.5	1.25
D-Gulose	2.5	5	>20
D-Talose	2.5	2.5	5
L-Rhamnose	20	20	>20
L-Mannose	20	20	>20
D-Glucosamine-HCl	2.5	5	>20
D-Galactosamime-HCl	2.5	5	20
N-acetyl-D-galactosamine	1	0.5	1
Lactose	1.25	1.25	1.25
Melibiose	5	2.5	5
Raffinose	10	5	10
Methyl β-D-galactoside	2.5	1.25	1.25
Methyl α-D-galactoside	5	2.5	2.5
2-0-methyl-D-galactose	5	2.5	1.25
3-0-methyl-D-galactose	2.5	2.5	1.25
4-0-methyl-D-galactose	>20	>20	>20
6-0-methyl-D-galactose	2.5	2.5	1.25
1.5-Anhydro-D-dulcitol	5	1.25	1.25
Hog A substance	0.0013	0.0013	0.0025
Cyst B substance	0.005	0.01	0.01

* tested with group A human blood cells.

configurations (isomerism and anomerism) of the sugars play in determining their inhibiting activity on hemagglutination. For example, L-galactose and L-fucose have no activity, and lactose and methyl β-D-galactoside are somewhat better inhibitor than melibiose and methyl α-D-galactoside. Inversion of the hydroxyl group on carbon 4 of D-galactose in D-glucose results in the lack of activity, but inversion of hydroxyl group on carbon 2 in D-talose has no appreciable effect. The configuration of the hydroxyl group on carbon 3 has important meaning in the case of anti-O (H) agglutinin only, since D-gulose is not inhibitory against anti-O (H) agglutinin but effective inhibitor against anti-B and anti-C agglutinins. The necessity of hydroxymethyl group at carbon 6 is investigated using D-fucose and L-arabinose. These sugars show inhibitory activity.

The blocking of hydroxyl group by methylation results almost in the same effects as the inversion of hydroxyl group, and 2-O-methyl-, 3-O-methyl- and 6-O-methyl-D-galactoses are effective inhibitor, but 4-O-methyl-D-galactose possesses no activity.

It is found that the ring oxygen of pyranose ring plays clearly important role as will be seen from the following facts, that is, both dulcitol and mesoinositol are not inhibitory but 1,5-anhydro-D-dulcitol is effective inhibitor.

1,6-Anhydro-D-galactose is devoid of inhibitory activity. This may be due to conformational effect.

In the cases of amino sugars, although both N-acetyl-D-galactosamine and D-galactosamine-HCl act always as a strong inhibitor, D-glucosamine-HCl has no inhibitory effect only against anti-O (H) agglutinin.

Discussions

The mechanism of inhibition of hemagglutination may be reasonably explained by the assumption that the sugar, the stereochemical structure of which resembles most closely to the one of specific sugar groups resiting at the active surface site of red cell as a receptor, is capable of linking to the active site of agglutinin being specific to that red cell. Thus, the information from this phenomenon give us a clue to the study on the chemical nature of blood group specificity.

In 1953, Morgan and Watkins[2] found that the hemagglutination by the crude extract from the seeds of *Sophora japonica* was inhibited by N-acetyl-D-glucosamine, D-galactose, lactose and melibiose. More recently, almost similar results were given by Krüpe[18] and Matsuyama[19]. They considered that the configurations of hydroxyl groups at carbon 3 and carbon 4 were probably essential factor for inhibiting power of sugars.

In our experiments using partially purified agglutinin, the active sugars (D-galactose, D-galactosamine-HCl, N-acetyl-D-galactosamine, D-fucose, L-arabinose, D-talose and D-gulose) have stereochemically common structures as follows:

(1) Every sugar exists in general in Cl-conformation.

(2) They have an axial hydroxyl group on carbon 4.

The inversion of hydroxyl group on carbon 4 of D-galactose in D-

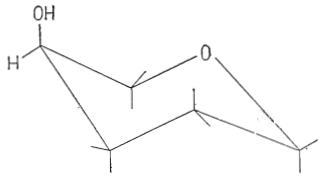

Fig. 1. The common structure of the sugars, which are inhibitory against *Sophoria Japonica* agglutinin.

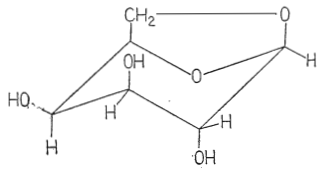

Fig. 2. β-1.6-anhydro-D-galactose.

glucose or the blocking of this hydroxyl group by methylation resulted in the complete loss of activity. The finding leads us to an assumption that the free axial hydroxyl group on carbon 4 is essential for the hydrogen bonding. This assumption is also supported by the finding that 1,6-anhydro-D-galactose, which exists in 1C-conformation as Fig. 2, lacks inhibitory activity.

Mekäla[20] found the important role of α- and β-anomerism of sugar on the inhibition of hemagglutinations of *Pisum sativum* and *Cytisus sessilifolius agglutinins,* and he claimed that these agglutinins were useful in assignment of α- or β-anomer of unknown sugars. In our case, α- and β-anomerism seems to have some important meaning as will be seen from the experiments using lactose, melibiose, methyl β-D-galactoside and methyl α-D-galactoside, even though the differences of activity between α- and β-anomers are less definite than those between D- and L-isomers.

The fact that 1,5-anhydro-D-dulcitol is an effective inhibitor, although dulcitol and meso-inositol are not inhibitory, shows the important meaning of ring oxygen of pyranose ring. The lone pairs of the ring oxygen atom would be also essential for hydrogen bonding.

The marked differences of behavior between anti-B, anti-C and anti-O (H) agglutinins of our partially purified product were found in the inhibition test using D-gulose and D-glucosamine-HCl. Although D-gulose, which differs from D-galactose only in the configuration of hydroxyl group on carbon 3, is inactive against anti-O (H) agglutinin, 3-O-methyl-D-galactose is an effective inhibitor against anti-O (H) agglutinin. These findings seem to imply that an equatorially oriented oxygen atom on carbon 3 is required for the anti-O (H) agglutinin in addition to an axial hydroxyl group on carbon 4 and ring oxygen atom.

On these differences of specificities between anti-B, anti-C and anti-O(H) agglutinins, further researches are indicated in connection with the detailed investigation on the combining sites of these agglutinins.

The data that L-rhamnose and L-mannose having 1C-conformation as Fig. 3 (a) possess distinct but weak activities seem to be incompatible

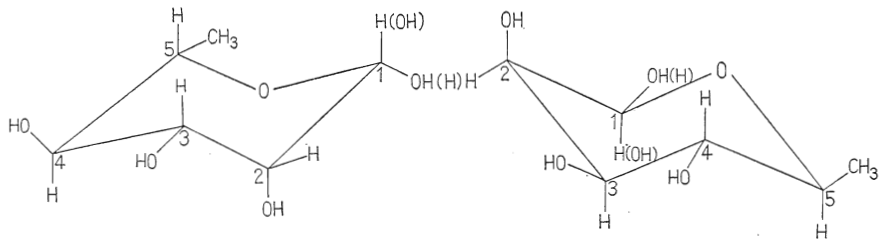

Fig. 3 (a) L-Rhamnose Fig. 3 (b)

with the foregoing discussion. However, when they are rewritten as Fig. 3 (b), they have stereochemically common structure concerning the position of their axial hydroxyl group and ring oxygen atom with D-galactose.

From the foregoing series of experimental data and discussion, it may be assumed that the inhibitor against the agglutinin of *Sophora japonica* is essentially required to have an axial hydroxyl group and ring oxygen atom in the relationship as shown in Fig. 4 presumably for the hydrogen bonding with agglutinin.

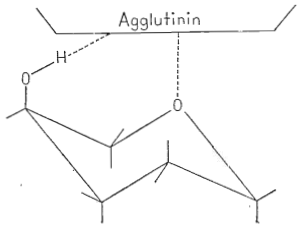

Fig. 4

Summary

Thirty-three kinds of sugars and polyols were tested on their inhibiting activity on the hemagglutination of the partially purified agglutinin from *Sophora japonica*. The results obtained were discussed mainly from the standpoint of stereochemical structure of sugars, and it was revealed that the existence of the both axial hydroxyl group on carbon 4 and ring oxygen atom in the same relationship as in D-galactose were essential for the inhibiting activity.

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